An Annotated Bibliography of Microarrays and Related Technologies for Detection of Waterborne Pathogens

Introduction

The U.S. Environmental Protection Agency (EPA) held an expert workshop in Cincinnati, Ohio on March 22-23, 2005 entitled **"Workshop on the Feasibility of Using DNA/RNA Microarrays and Related Technologies for High Through-Put Detection of Waterborne Pathogens"**. The goal of this workshop was to explore the feasibility of using microarray technology for detecting waterborne pathogens.

Prior to the microarray workshop, an extensive literature search was conducted to identify areas of microarray technology that could potentially be applied to EPA water programs. This document presents the results of the literature search, including references and abstracts, categorized by the following: microarray technology; clinical applications; food applications; environmental applications; and review articles. In addition, there is a section entitled "Speaker Suggested Articles", that contains the list of articles suggested by the expert speakers invited to present at the workshop.

Please note that the references and abstracts were taken from a variety of search engines, each using a slightly different format. The exact text and format from each of these sources are retained in this document, therefore references and abstracts vary in format, and they contain some spelling and grammatical errors derived from their original source. The references and abstracts are presented in alphabetical order by author, within each category. It is possible that a reference will appear in more than one category. Hyperlinks to each categorical section, and to different points in the alphabet have been included to facilitate searching.

For more information regarding the workshop, please contact Dr. Keya Sen at: sen.keya@epa.gov.

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Microarray Technology

A-H

Abecassis, V., L. Jaffrelo, et al. (2003). "Microarray-based method for combinatorial library sequence mapping and characterization." Biotechniques **34**(6): 1272-9.

Here we describe a DNA-chip-based method for high-throughput sequence mapping. This involves competitive hybridization between short and differentially labeled fluorescent oligonucleotide probes and glass-supported PCR products. Competition between an excess of oligonucleotide probes targeting the same sequence segment improves sequence discrimination and reduces sensitivity to experimental conditions such as probe concentrations, hybridization, and washing temperatures and durations. The method was found to be particularly adapted to sequence mapping of combinatorial libraries obtained by DNA shuffling between members of a gene family. We present an application of this technique for the characterization of recombination biases in combinatorial libraries used in directed evolution.

Adamczyk, J., M. Hesselsoe, et al. (2003). "The isotope array, a new tool that employs substrate-mediated labeling of rRNA for determination of microbial community structure and function." Appl Environ Microbiol **69**(11): 6875-87.

A new microarray method, the isotope array approach, for identifying microorganisms which consume a (14)C-labeled substrate within complex microbial communities was developed. Experiments were performed with a small microarray consisting of oligonucleotide probes targeting the 16S rRNA of ammonia-oxidizing bacteria (AOB). Total RNA was extracted from a pure culture of Nitrosomonas eutropha grown in the presence of [(14)C]bicarbonate. After fluorescence labeling of the RNA and microarray hybridization, scanning of all probe spots for fluorescence and radioactivity revealed that specific signals were obtained and that the incorporation of (14)C into rRNA could be detected unambiguously. Subsequently, we were able to demonstrate the suitability of the isotope array approach for monitoring community composition and CO(2) fixation activity of AOB in two nitrifying activated-sludge samples which were incubated with [(14)C]bicarbonate for up to 26 h. AOB community structure in the activated-sludge samples, as predicted by the microarray hybridization pattern, was confirmed by quantitative fluorescence in situ hybridization (FISH) and comparative amoA sequence analyses. CO(2) fixation activities of the AOB populations within the complex activated-sludge communities were detectable on the microarray by (14)C incorporation and were confirmed independently by combining FISH and microautoradiography. AOB rRNA from activated sludge incubated with radioactive bicarbonate in the presence of allylthiourea as an inhibitor of AOB activity showed no incorporation of (14)C and thus was not detectable on the radioactivity scans of the microarray. These results suggest that the isotope array can be used in a PCR-independent manner to exploit the high parallelism and discriminatory power of microarrays for the direct identification of microorganisms which consume a specific substrate in the environment.

Adey, N. B., M. Lei, et al. (2002). "Gains in sensitivity with a device that mixes microarray hybridization solution in a 25-microm-thick chamber." Anal Chem **74**(24): 6413-7.

A microarray hybridization system that allows mixing in volumes comparable to those used by glass coverslips is presented. This system is composed of a disposable flexible lid that binds to 1 in. x 3 in. glass slides via an adhesive gasket, forming a uniform 25-microm-thick hybridization chamber. This chamber rests on a base unit for temperature control. The lid contains two air-driven bladders that continuously mix the hybridization fluid. Mixing enhances sensitivity from a typical microarray experiment 2-3-fold. Mixing is particularly effective at high spotted probe and low labeled target concentrations and overcoming local target depletion that occurs when homologous probes are spotted in close proximity. Mixing appears to be compatible with most hybridization conditions; however, mix

versus no-mix control experiments should be performed. Also covered are a number of microfluidic issues related to manufacturing, filling, mixing, and packaging.

Akagi, K., M. Kanai, et al. (2001). "A novel tetracycline-dependent transactivator with E2F4 transcriptional activation domain." Nucleic Acids Res **29**(4): E23.

A tetracycline-controlled gene expression system provides a powerful tool to dissect the functions of gene products. However, it often appears difficult to establish cell lines or transgenic animals stably expressing tetracycline-dependent transactivators, possibly as a result of toxicity of the transactivator domains used. In order to overcome this problem, we developed a novel tetracycline-dependent transactivator that works efficiently in mammalian cells. This transactivator is a fusion of the tet reverse repressor mutant and the transcriptional activating domain of human E2F4, which is ubiquitously expressed in vivo. We demonstrate here that this tetracycline-regulated gene expression system provides a two log transcriptional activation in mammalian cells as assessed by northern blot and luciferase analyses. Combining this system with green fluorescent protein reporter systems or microarray gene expression profiling will facilitate the study of gene function.

Alexandre, I., S. Hamels, et al. (2001). "Colorimetric silver detection of DNA microarrays." Anal Biochem **295**(1): 1-8.

Development of microarrays has revolutionized gene expression analysis and molecular diagnosis through miniaturization and the multiparametric features. Critical factors affecting detection efficiency of targets hybridization on microarray are the design of capture probes, the way they are attached to the support, and the sensitivity of the detection method. Microarrays are currently detected in fluorescence using a sophisticated confocal laser-based scanner. In this work, we present a new colorimetric detection method which is intented to make the use of microarray a powerful procedure and a low-cost tool in research and clinical settings. The signal generated with this method results from the precipitation of silver onto nanogold particles bound to streptavidin, the latter being used for detecting biotinylated DNA. This colorimetric method has been compared to the Cy-3 fluorescence method. The detection limit of both methods was equivalent and corresponds to 1 amol of biotinylated DNA attached on an array. Scanning and data analysis of the array were obtained with a colorimetric-based workstation.

Alexandre, I., Y. Houbion, et al. (2002). "Compact disc with both numeric and genomic information as DNA microarray platform." Biotechniques **33**(2): 435-6, 438-9.

The compact disc (CD) is an ideal toolfor reading, writing, and storing numeric information. It was used in this work as a support for constructing DNA microarrays suited for genomic analysis. The CD was divided into two functional areas: the external ring of the CD was used for multiparametric DNA analysis on arrays, and the inner portion was usedfor storing numeric information. Because polycarbonate and CD resins autofluoresce, a colorimetric method for DNA microarray detection was used that is well adaptedfor the fast detection necessary when using a CD reader. A double-sided CD reader was developed for the simultaneous analysis of both array and numeric data. The numeric data are engraved as pits in the CD tracks and result in the succession of 0/1, which results from the modulation of the laser reflection when one reads the edges of the pits. Another diffraction-based laser was placed above the CD for the detection of the DNA targets on the microarrays. Both readersfit easily in a PC tower. Both numeric and genomic information data were simultaneously acquired, and each array was reconstituted, analyzed, and processed for quantification by the appropriate software.

Al-Khaldi, S. F., D. Villanueva, et al. (2004). "Identification and characterization of Clostridium perfringens using single target DNA microarray chip." Int J Food Microbiol **91**(3): 289-96.

A DNA microarray method was developed to identify the presence of toxin genes: encoding beta toxin (cpb), epsilon toxin (etx), enterotoxin (cpe), alpha toxin (cpa), and iota toxin (iA) in Clostridium perfringens. To build the DNA chip, each gene sequence was represented by one approximately 22-bp

amino-modified oligonucleotide printed twice on aldehyde-coated slides. Multiplex PCR with Cy3 and Cy5-dCTP derivatized fluorescent nucleotides was used to label five genes and fluorescent probes were prepared. The PCR probes were denatured and single-strand-labeled DNAs were separated and purified using magnetic beads. The presence of toxin genes in C. perfringens was detected by hybridization of amplified ssDNA probes to oligonucleotides on the chip representing one target sequence of each toxin gene. The DNA chip was able to identify eight strains of C. perfringens.

Allakhverdiev, S. I., Y. Nishiyama, et al. (2002). "Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in synechocystis." Plant Physiol **130**(3): 1443-53.

Light stress and salt stress are major environmental factors that limit the efficiency of photosynthesis. However, we have found that the effects of light and salt stress on photosystem II (PSII) in the cyanobacterium Synechocystis sp. PCC 6803 are completely different. Strong light induced photodamage to PSII, whereas salt stress inhibited the repair of the photodamaged PSII and did not accelerate damage to PSII directly. The combination of light and salt stress appeared to inactivate PSII very rapidly as a consequence of their synergistic effects. Radioactive labeling of cells revealed that salt stress inhibited the synthesis of proteins de novo and, in particular, the synthesis of the D1 protein. Northern- and western-blotting analyses demonstrated that salt stress inhibited the transcription and the translation of psbA genes, which encode D1 protein. DNA microarray analysis indicated that the light-induced expression of various genes was suppressed by salt stress. Thus, our results suggest that salt stress inhibits the repair of PSII via suppression of the activities of the transcriptional and translational machinery.

Allen, T. D., A. L. Dawe, et al. (2003). "Use of cDNA microarrays to monitor transcriptional responses of the chestnut blight fungus Cryphonectria parasitica to infection by virulence-attenuating hypoviruses." Eukaryot Cell **2**(6): 1253-65.

Hypoviruses are a family of cytoplasmically replicating RNA viruses of the chestnut blight fungus Cryphonectria parasitica. Members of this mycovirus family persistently alter virulence (hypovirulence) and related fungal developmental processes, including asexual and sexual sporulation. In order to gain a better understanding of the molecular basis for these changes, we have developed a C. parasitica cDNA microarray to monitor global transcriptional responses to hypovirus infection. In this report, a spotted DNA microarray representing approximately 2,200 C. parasitica genes was used to monitor changes in the transcriptional profile after infection by the prototypic hypovirus CHV1-EP713. Altered transcript abundance was identified for 295 clones (13.4% of the 2,200 unique cDNAs) as a result of CHV1-EP713 infection-132 up-regulated and 163 down-regulated. In comparison, less than 20 specific C. parasitica genes were previously identified by Northern analysis and mRNA differential display as being responsive to hypovirus infection. A 93% validation rate was achieved between real-time reverse transcription-PCR results and microarray predictions. Differentially expressed genes represented a broad spectrum of biological functions, including stress responses, carbon metabolism, and transcriptional regulation. These findings are consistent with the view that infection by a 12.7-kbp hypovirus RNA results in a persistent reprogramming of a significant portion of the C. parasitica transcriptome. The potential impact of microarray studies on current and future efforts to establish links between hypovirus-mediated changes in cellular gene expression and phenotypes is discussed.

Allen, T. D. and D. L. Nuss (2004). "Linkage between Mitochondrial Hypovirulence and Viral Hypovirulence in the Chestnut Blight Fungus Revealed by cDNA Microarray Analysis." Eukaryot Cell **3**(5): 1227-32.

The phenomenon of transmissible hypovirulence (virulence attenuation) associated with biological control of natural populations of the chestnut blight fungus Cryphonectria parasitica can be experimentally reproduced by infection with hypovirus cDNA clones (viral hypovirulence) or by

mutation of mitochondrial DNA (mtDNA) in the absence of virus infection (mitochondrial hypovirulence). We now report the use of an established C. parasitica cDNA microarray to monitor nuclear transcriptional responses to an mtDNA mutation of C. parasitica strain EP155, designated EP155/mit2, which was previously shown to induce elevated alternative oxidase activity and hypovirulence (C. B. Monterio-Vitorello, J. A. Bell, D. W. Fulbright, and H. A. Bertrand, Proc. Natl. Acad. Sci. USA 92:5935-5939, 1995). Approximately 10% of the 2,200 genes represented on the microarray exhibited altered transcript accumulation as a result of the mit2 mtDNA mutation. While genes involved in mitochondrial function were clearly represented in the EP155/mit2-responsive gene list, direct parallels to the well-characterized Saccharomyces cerevisiae retrograde response to mitochondrial dysfunction were not observed. Remarkably, 47% of the genes that were differentially expressed following the infection of strain EP155 by the prototypic hypovirus CHV1-EP713 had similarly changed transcript accumulation in the virus-free EP155/mit2 mutant. These results establish a linkage between viral and mitochondrial hypovirulence and raise questions regarding the relationship between hypovirus infection and mitochondrial dysfunction. The combined set of transcriptional profile data provides a foundation for future studies on mitochondrion-to-nucleus communications in the context of hypovirus infection and senescence associated with mitochondrial dysfunction in filamentous fungi.

Allen, T. D. and D. L. Nuss (2004). "Specific and common alterations in host gene transcript accumulation following infection of the chestnut blight fungus by mild and severe hypoviruses." J Virol **78**(8): 4145-55.

We report the use of a cDNA microarray to monitor global transcriptional responses of the chestnut blight fungus, Cryphonectria parasitica, to infection by mild and severe isolates of virulence-attenuating hypoviruses that share 87 to 93% and 90 to 98% identity at the nucleotide and amino acid levels, respectively. Infection by the mild hypovirus isolate CHV1-Euro7 resulted in differential expression of 166 of the ca. 2,200 genes represented on the microarray (90 upregulated and 76 downregulated). This is roughly half the number of genes scored as differentially expressed after infection by the severe isolate, CHV1-EP713 (295 genes; 132 upregulated and 163 downregulated). Comparison of the lists of genes responsive to infection by the two hypovirus isolates revealed 80 virus-common responsive genes. Infection by CHV1-EP713 also caused changes in gene transcript accumulation that were, in general, of greater magnitude than those observed with CHV1-Euro7 infections. Thus, the host transcriptional response to infection by severe hypovirus CHV1-EP713 appears to be considerably more dynamic than the response to infection by the mild isolate CHV1-Euro7. Real-time reverse transcription-PCR was performed on 39 different clones, with false-positive rates of 3 and 8% observed for the microarray-predicted list of genes responsive to CHV1-EP713 and CHV1-Euro7 infections, respectively. This analysis has allowed an initial assignment for ca. 2,200 unique C. parasitica-expressed genes as being unresponsive to hypovirus infection, selectively responsive to a specific hypovirus, or generally responsive to hypovirus infection.

Ang, S., C. Z. Lee, et al. (2001). "Acid-induced gene expression in Helicobacter pylori: study in genomic scale by microarray." Infect Immun **69**(3): 1679-86.

To understand the RNA expression in response to acid stress of Helicobacter pylori in genomic scale, a microarray membrane containing 1,534 open reading frames (ORFs) from strain 26695 was used. Total RNAs of H. pylori under growth conditions of pH 7.2 and 5.5 were extracted, reverse transcribed into cDNA, and labeled with biotin. Each microarray membrane was hybridized with cDNA probe from the same strain under two different pH conditions and developed by a catalyzed reporter deposition method. Gene expression of all ORFs was measured by densitometry. Among the 1,534 ORFs, 53 ORFs were highly expressed (> or = 30% of rRNA control in densitometry ratios). There were 445 ORFs which were stably expressed (<30% of rRNA in densitometry) under both pH conditions without significant variation. A total of 80 ORFs had significantly increased expression levels at low pH, while expressions of 4 ORFs were suppressed under acidic condition. The remaining 952 ORFs were not detectable under

either pH condition. These data were highly reproducible and comparable to those obtained by the RNA slot blot method. Our results suggest that microarray can be used in monitoring prokaryotic gene expression in genomic scale.

Araki, T., M. Tsujioka, et al. (2003). "A STAT-regulated, stress-induced signalling pathway in Dictyostelium." J Cell Sci **116**(Pt 14): 2907-15.

The Dictyostelium stalk cell inducer differentiation-inducing factor (DIF) directs tyrosine phosphorylation and nuclear accumulation of the STAT (signal transducer and activator of transcription) protein Dd-STATc. We show that hyperosmotic stress, heat shock and oxidative stress also activate Dd-STATc. Hyperosmotic stress is known to elevate intracellular cGMP and cAMP levels, and the membrane-permeant analogue 8-bromo-cGMP rapidly activates Dd-STATc, whereas 8-bromo-cAMP is a much less effective inducer. Surprisingly, however, Dd-STATc remains stress activatable in null mutants for components of the known cGMP-mediated and cAMP-mediated stress-response pathways and in a double mutant affecting both pathways. Also, Dd-STATc null cells are not abnormally sensitive to hyperosmotic stress. Microarray analysis identified two genes, gapA and rtoA, that are induced by hyperosmotic stress. Osmotic stress induction of gapA and rtoA is entirely dependent on Dd-STATc. Neither gene is inducible by DIF but both are rapidly inducible with 8-bromo-cGMP. Again, 8-bromo-cAMP is a much less potent inducer than 8-bromo-cGMP. These data show that Dd-STATc functions as a transcriptional activator in a stress-response pathway and the pharmacological evidence, at least, is consistent with cGMP acting as a second messenger.

Arfin, S. M., A. D. Long, et al. (2000). "Global gene expression profiling in Escherichia coli K12. The effects of integration host factor." J Biol Chem **275**(38): 29672-84.

We have used nylon membranes spotted in duplicate with full-length polymerase chain reaction-generated products of each of the 4,290 predicted Escherichia coli K12 open reading frames (ORFs) to measure the gene expression profiles in otherwise isogenic integration host factor IHF(+) and IHF(-) strains. Our results demonstrate that random hexamer rather than 3' ORF-specific priming of cDNA probe synthesis is required for accurate measurement of gene expression levels in bacteria. This is explained by the fact that the currently available set of 4,290 unique 3' ORF-specific primers do not hybridize to each ORF with equal efficiency and by the fact that widely differing degradation rates (steady-state levels) are observed for the 25-base pair region of each message complementary to each ORF-specific primer. To evaluate the DNA microarray data reported here, we used a linear analysis of variance (ANOVA) model appropriate for our experimental design. These statistical methods allowed us to identify and appropriately correct for experimental variables that affect the reproducibility and accuracy of DNA microarray measurements and allowed us to determine the statistical significance of gene expression differences between our IHF(+) and IHF(-) strains. Our results demonstrate that small differences in gene expression levels can be accurately measured and that the significance of differential gene expression measurements cannot be assessed simply by the magnitude of the fold difference. Our statistical criteria, supported by excellent agreement between previously determined effects of IHF on gene expression and the results reported here, have allowed us to identify new genes regulated by IHF with a high degree of confidence.

Arora, S. K., M. C. Wolfgang, et al. (2004). "Sequence polymorphism in the glycosylation island and flagellins of Pseudomonas aeruginosa." J Bacteriol **186**(7): 2115-22.

A genomic island consisting of 14 open reading frames, orfA to orfN was previously identified in Pseudomonas aeruginosa strain PAK and shown to be essential for glycosylation of flagellin. DNA microarray hybridization analysis of a number of P. aeruginosa strains from diverse origins showed that this island is polymorphic. PCR and sequence analysis confirmed that many P. aeruginosa strains carry an abbreviated version of the island (short island) in which orfD, -E and -H are polymorphic and orfI, -J, -K, -L, and -M are absent. To ascertain whether there was a relationship between the inheritance of the short

island and specific flagellin sequence variants, complete or partial nucleotide sequences of flagellin genes from 24 a-type P. aeruginosa strains were determined. Two distinct flagellin subtypes, designated A1 and A2, were apparent. Strains with the complete 14-gene island (long island) were almost exclusively of the A1 type, whereas strains carrying the short island were associated with both A1- and A2-type flagellins. These findings indicate that P. aeruginosa possesses a relatively low number of distinct flagellin types and probably has the capacity to further diversify this antigenic surface protein by glycosylation.

Asai, K., H. Yamaguchi, et al. (2003). "DNA microarray analysis of Bacillus subtilis sigma factors of extracytoplasmic function family." FEMS Microbiol Lett **220**(1): 155-60.

Target gene candidates of the seven extracytoplasmic function (ECF) sigma factors of Bacillus subtilis have been surveyed using DNA microarray analysis of mRNA extracted from cells grown in Luria-Bertani broth, in which an ECF sigma factor gene was placed under the control of the spac promoter on multicopy plasmid pDG148 and overexpressed. The number of target candidates for each of the sigma factors varied greatly, and a total of 278 genes were selected. Interestingly, the above target gene candidates shared only one gene out of 94 target genes of the general stress sigma B that have been reported in the literature thus far. Furthermore, lacZ-fusion experiments based on the results of DNA microarray analysis indicated that each ECF sigma factor directs transcription of its own operon, with the exception of sigZ. The DNA microarray data collected in this study are available at the KEGG Expression Database web site (http://www.genome.ad.jp/kegg/expression/).

Asha, H., I. Nagy, et al. (2003). "Analysis of Ras-induced overproliferation in Drosophila hemocytes." Genetics **163**(1): 203-15.

We use the Drosophila melanogaster larval hematopoietic system as an in vivo model for the genetic and functional genomic analysis of oncogenic cell overproliferation. Ras regulates cell proliferation and differentiation in multicellular eukaryotes. To further elucidate the role of activated Ras in cell overproliferation, we generated a collagen promoter-Gal4 strain to overexpress Ras(V12) in Drosophila hemocytes. Activated Ras causes a dramatic increase in the number of circulating larval hemocytes (blood cells), which is caused by cellular overproliferation. This phenotype is mediated by the Raf/MAPK pathway. The mutant hemocytes retain the ability to phagocytose bacteria as well as to differentiate into lamellocytes. Microarray analysis of hemocytes overexpressing Ras(V12) vs. Ras(+) identified 279 transcripts that are differentially expressed threefold or more in hemocytes expressing activated Ras. This work demonstrates that it will be feasible to combine genetic and functional genomic approaches in the Drosophila hematopoietic system to systematically identify oncogene-specific downstream targets.

Bahi, A., F. Boyer, et al. (2004). "CD81-induced behavioural changes during chronic cocaine administration: in vivo gene delivery with regulatable lentivirus." Eur J Neurosci **19**(6): 1621-33.

CD81, a tetraspanin transmembrane protein involved in cell adhesion, is up-regulated in the mesolimbic dopaminergic pathway 24 h following acute administration of high doses of cocaine [Brenz-Verca et al., (2001) Mol. Cell. Neurosci., 17, 303-316]. Further evidence consecutive with this observation and based on microarray analysis are presented here. In addition, a regulatable lentivirus was developed bearing the rat CD81 gene under the control of a tetracycline inducible system. This lentivirus vector was stereotaxically injected into the ventral tegmental area (VTA) of two groups of animals, one fed water (expressing CD81) and the other Doxycycline solution (which down-regulates CD81 expression) and locomotor activity after chronic cocaine administration (10 mg/kg daily) was monitored. After 2 weeks, the groups were inverted, animals receiving water were placed on Doxycycline and the second group was placed on water. In all cases highly a significant increase (3.2-fold) in locomotor activity was observed in animals expressing CD81 in the VTA vs. animals placed on Doxycycline. Similar studies where CD81 was delivered into the nucleus accumbens (NAcc) resulted in significantly higher effects (30%), in accordance with microarray data and our previous reports, yielding a 4.2-fold

increase in locomotor activity. No change was observed under similar conditions in control animals, which were injected a regulatable lentivirus expressing GFP. These findings suggest that CD81 expression in the mesolimbic dopaminergic pathway contributes to behavioural changes associated with cocaine sensitization. This study provides a powerful approach for evaluating a gene function in vivo in a single animal under various paradigms, even on gene candidates, which display small changes of expression.

Bao, K. and S. N. Cohen (2003). "Recruitment of terminal protein to the ends of Streptomyces linear plasmids and chromosomes by a novel telomere-binding protein essential for linear DNA replication." Genes Dev **17**(6): 774-85.

Bidirectional replication of Streptomyces linear plasmids and chromosomes from a central origin produces unpaired 3'-leading-strand overhangs at the telomeres of replication intermediates. Filling in of these overhangs leaves a terminal protein attached covalently to the 5' DNA ends of mature replicons. We report here the essential role of a novel 80-kD DNA-binding protein (telomere-associated protein, Tap) in this process. Biochemical studies, yeast two-hybrid analysis, and immunoprecipitation/immunodepletion experiments indicate that Tap binds tightly to specific sequences in 3' overhangs and also interacts with Tpg, bringing Tpg to telomere termini. Using DNA microarrays to analyze the chromosomes of tap mutant bacteria, we demonstrate that survivors of Tap ablation undergo telomere deletion, chromosome circularization, and amplification of subtelomeric DNA. Microarray-based chromosome mapping at single-ORF resolution revealed common endpoints for independent deletions, identified amplified chromosomal ORFs adjacent to these endpoints, and quantified the copy number of these ORFs. Sequence analysis confirmed chromosome circularization and revealed the insertion of adventitious DNA between joined chromosome ends. Our results show that Tap is required for linear DNA replication in Streptomyces and suggest that it functions to recruit and position Tpg at the telomeres of replication intermediates. They also identify hotspots for the telomeric deletions and subtelomeric DNA amplifications that accompany chromosome circularization.

Barloy-Hubler, F., A. Cheron, et al. (2004). "Smc01944, a secreted peroxidase induced by oxidative stresses in Sinorhizobium meliloti 1021." Microbiology **150**(Pt 3): 657-64.

Sequencing of the Sinorhizobium meliloti strain 1021 genome led to the detection of 6204 open reading frames, 41 % of which have no hypothetical function. To help annotate this genome, a transcriptome analysis was carried out with a dedicated microarray consisting of 146 genes belonging to three different classes: (i) no hypothetical function; (ii) potentially involved in oxidative stress responses; (iii) known to participate in oxidative stress responses (e.g. catalase and superoxide dismutase genes). This transcriptome analysis, together with biological experiments and in silico investigations, identified new genes induced by exogenous H(2)O(2). The smc01944 gene was the most strongly induced: quantitative PCR showed that the amount of smc01944 mRNA increased 50-fold following the addition of 10 mM H(2)O(2), whereas the amount of katA mRNA (encoding a catalase) only increased 10-fold. Smc01944 is a non-haem chloroperoxidase (Cpo). The only member of this family to have been so far characterized is encoded by prxC of Pseudomonas fluorescens. Unexpectedly, the NH(2)-terminus of Smc01944 includes a signal peptide and Smc01944 is secreted into the supernatant. Interestingly, smc01944 is preceded by smc01945, encoding an OhrR-like regulator (MarR family). Thus, Smc01944 is the first exported Cpo encoded by a gene possibly regulated by an OhrR regulator. It was also shown that smc01944 is induced by t-butyl and cumene hydroperoxides but only slightly by menadione. The study of Smc01944 described in this work showed that the oxidative stress response of S. meliloti seems to differ from that of other bacteria characterized to date.

Bavykin, S. G., J. P. Akowski, et al. (2001). "Portable system for microbial sample preparation and oligonucleotide microarray analysis." Appl Environ Microbiol **67**(2): 922-8.

We have developed a three-component system for microbial identification that consists of (i) a

universal syringe-operated silica minicolumn for successive DNA and RNA isolation, fractionation, fragmentation, fluorescent labeling, and removal of excess free label and short oligonucleotides; (ii) microarrays of immobilized oligonucleotide probes for 16S rRNA identification; and (iii) a portable battery-powered device for imaging the hybridization of fluorescently labeled RNA fragments with the arrays. The minicolumn combines a guanidine thiocyanate method of nucleic acid isolation with a newly developed hydroxyl radical-based technique for DNA and RNA labeling and fragmentation. DNA and RNA can also be fractionated through differential binding of double- and single-stranded forms of nucleic acids to the silica. The procedure involves sequential washing of the column with different solutions. No vacuum filtration steps, phenol extraction, or centrifugation is required. After hybridization, the overall fluorescence pattern is captured as a digital image or as a Polaroid photo. This three-component system was used to discriminate Escherichia coli, Bacillus subtilis, Bacillus thuringiensis, and human HL60 cells. The procedure is rapid: beginning with whole cells, it takes approximately 25 min to obtain labeled DNA and RNA samples and an additional 25 min to hybridize and acquire the microarray image using a stationary image analysis system or the portable imager.

Bekal, S., R. Brousseau, et al. (2003). "Rapid identification of Escherichia coli pathotypes by virulence gene detection with DNA microarrays." J Clin Microbiol **41**(5): 2113-25.

One approach to the accurate determination of the pathogenic potential (pathotype) of isolated Escherichia coli strains would be through a complete assessment of each strain for the presence of all known E. coli virulence factors. To accomplish this, an E. coli virulence factor DNA microarray composed of 105 DNA PCR amplicons printed on glass slides and arranged in eight subarrays corresponding to different E. coli pathotypes was developed. Fluorescently labeled genomic DNAs from E. coli strains representing known pathotypes were initially hybridized to the virulence gene microarrays for both chip optimization and validation. Hybridization pattern analysis with clinical isolates permitted a rapid assessment of their virulence attributes and determination of the pathogenic group to which they belonged. Virulence factors belonging to two different pathotypes were detected in one human E. coli isolate (strain H87-5406). The microarray was also tested for its ability to distinguish among phylogenetic groups of genes by using gene probes derived from the attaching-and-effacing locus (espA, espB, tir). After hybridization with these probes, we were able to distinguish E. coli strains harboring espA, espB, and tir sequences closely related to the gene sequences of an enterohemorrhagic strain (EDL933), a human enteropathogenic strain (E2348/69), or an animal enteropathogenic strain (RDEC-1). Our results show that the virulence factor microarray is a powerful tool for diagnosis-based studies and that the concept is useful for both gene quantitation and subtyping. Additionally, the multitude of virulence genes present on the microarray should greatly facilitate the detection of virulence genes acquired by horizontal transfer and the identification of emerging pathotypes.

Beliaev, A. S., D. K. Thompson, et al. (2002). "Microarray transcription profiling of a Shewanella oneidensis etrA mutant." J Bacteriol **184**(16): 4612-6.

DNA microarrays were used to examine the effect of an insertional mutation in the Shewanella oneidensis etrA (electron transport regulator) locus on gene expression under anaerobic conditions. The mRNA levels of 69 genes with documented functions in energy and carbon metabolism, regulation, transport, and other cellular processes displayed significant alterations in transcript abundance in an etrA-mutant genetic background. This is the first microarray study indicating a possible involvement of EtrA in the regulation of gene expression in S. oneidensis MR -1.

Beliaev, A. S., D. K. Thompson, et al. (2002). "Gene and protein expression profiles of Shewanella oneidensis during anaerobic growth with different electron acceptors." Omics **6**(1): 39-60.

Changes in mRNA and protein expression profiles of Shewanella oneidenesis MR-1 during switch from aerobic to fumarate-, Fe(III)-, or nitrate-reducing conditions were examined using DNA microarrays and two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). In response to

changes in growth conditions, 121 of the 691 arrayed genes displayed at least a two-fold difference in transcript abundance as determined by microarray analysis. Genes involved in aerobic respiration encoding cytochrome c and d oxidases and TCA cycle enzymes were repressed under anaerobic conditions. Genes induced during anaerobic respiration included those involved in cofactor biosynthesis and assembly (moaACE, ccmHF, nosD, cysG), substrate transport (cysUP, cysTWA, dcuB), and anaerobic energy metabolism (dmsAB, psrC, pshA, hyaABC, hydA). Transcription of genes encoding a periplasmic nitrate reductase (napBHGA), cytochrome c552, and prismane was elevated 8- to 56-fold in response to the presence of nitrate, while cymA, ifcA, and frdA were specifically induced three- to eightfold under fumarate-reducing conditions. The mRNA levels for two oxidoreductase-like genes of unknown function and several cell envelope genes involved in multidrug resistance increased two- to fivefold specifically under Fe(III)-reducing conditions. Analysis of protein expression profiles under aerobic and anaerobic conditions revealed 14 protein spots that showed significant differences in abundance on 2-D gels. Protein identification by mass spectrometry indicated that the expression of prismane, dihydrolipoamide succinyltransferase, and alcaligin siderophore biosynthesis protein correlated with the microarray data.

Berka, R. M., J. Hahn, et al. (2002). "Microarray analysis of the Bacillus subtilis K-state: genome-wide expression changes dependent on ComK." Mol Microbiol **43**(5): 1331-45.

In Bacillus subtilis, the competence transcription factor ComK activates its own transcription as well as the transcription of genes that encode DNA transport proteins. ComK is expressed in about 10% of the cells in a culture grown to competence. Using DNA microarrays representing approximately 95% of the protein-coding open reading frames in B. subtilis, we compared the expression profiles of wild-type and comK strains, as well as of a mecA mutant (which produces active ComK in all the cells of the population) and a comK mecA double mutant. In these comparisons, we identified at least 165 genes that are upregulated by ComK and relatively few that are downregulated. The use of reporter fusions has confirmed these results for several genes. Many of the ComK-regulated genes are organized in clusters or operons, and 23 of these clusters are preceded by apparent ComK-box promoter motifs. In addition to those required for DNA uptake, other genes that are upregulated in the presence of ComK are probably involved in DNA repair and in the uptake and utilization of nutritional sources. From this and previous work, we conclude that the ComK regulon defines a growth-arrested state, distinct from sporulation, of which competence for genetic transformation is but one notable feature. We suggest that this is a unique adaptation to stress and that it be termed the 'K-state'.

Bernstein, J. A., A. B. Khodursky, et al. (2002). "Global analysis of mRNA decay and abundance in Escherichia coli at single-gene resolution using two-color fluorescent DNA microarrays." Proc Natl Acad Sci U S A **99**(15): 9697-702.

Much of the information available about factors that affect mRNA decay in Escherichia coli, and by inference in other bacteria, has been gleaned from study of less than 25 of the approximately 4,300 predicted E. coli messages. To investigate these factors more broadly, we examined the half-lives and steady-state abundance of known and predicted E. coli mRNAs at single-gene resolution by using two-color fluorescent DNA microarrays. An rRNA-based strategy for normalization of microarray data was developed to permit quantitation of mRNA decay after transcriptional arrest by rifampicin. We found that globally, mRNA half-lives were similar in nutrient-rich media and defined media in which the generation time was approximately tripled. A wide range of stabilities was observed for individual mRNAs of E. coli, although approximately 80% of all mRNAs had half-lives between 3 and 8 min. Genes having biologically related metabolic functions were commonly observed to have similar stabilities. Whereas the half-lives of a limited number of mRNAs correlated positively with their abundance, we found that overall, increased mRNA stability is not predictive of increased abundance. Neither the density of putative sites of cleavage by RNase E, which is believed to initiate mRNA decay in E. coli, nor the free energy of folding of 5' or 3' untranslated region sequences was predictive of mRNA half-life. Our results

identify previously unsuspected features of mRNA decay at a global level and also indicate that generalizations about decay derived from the study of individual gene transcripts may have limited applicability.

Bialek, K., A. Swistowski, et al. (2003). "Epitope-targeted proteome analysis: towards a large-scale automated protein-protein-interaction mapping utilizing synthetic peptide arrays." Anal Bioanal Chem **376**(7): 1006-13.

We describe the development of a process for the genome-wide mapping of interactions between protein domains and peptide ligands entirely based on high-throughput biochip technologies. A phage library displaying protein domains from a randomly fragmented and cloned cDNA library will be "panned" on an array of synthetic peptide ligands. After multiplexed affinity enrichment, peptide-specific phage populations will be automatically eluted, propagated, labelled and identified by hybridisation to a DNA microarray. Peptide arrays are synthesized in situ by SPOT synthesis on a planar substrate. By utilizing a commercially available library of human brain cDNA plus a set of distinct model domains cloned into T7-phage, we could show that a single panning round on an array of known peptide ligands for these model domains synthesized on a cellulose membrane can yield an enrichment of better than a factor of 1,000. This is sufficient to detect peptide-specific enrichment of Cy3(post-panning) against Cy5(pre-panning)-labelled phage DNA inserts on a cDNA microarray. Thus, the proof-of-principle of our approach could be successfully demonstrated and first interaction data are being collected

Bjorkbacka, H., K. A. Fitzgerald, et al. (2004). "The induction of macrophage gene expression by LPS predominantly utilizes MyD88-independent signaling cascades." Physiol Genomics.

Myeloid differentiation protein-88 (MyD88) is a signal adaptor protein required for cytokine production following engagement of Toll-Like receptors (TLRs) by their cognate ligands. Activation of both TLR-3 and TLR-4, however, can engage signaling events independent of MyD88 expression. The relative importance of these MyD88-dependent and -independent signaling pathways in the macrophage response to lipopolysaccharide (LPS) is unknown. Here we define these events using microarray expression profiling of LPS stimulated macrophages taken from MyD88-null and wild type mice. Of the 1055 genes found to be LPS responsive, only 21.5% were dependent on MyD88 expression, with MyD88-independent genes constituting 74.7% of the genetic response. This MyD88-independent gene expression was predominantly transcriptionally regulated, as it was unaffected by cycloheximide blockade of new protein synthesis. A previously undescribed group of LPS-regulated genes (3.8%), whose induction or repression was significantly greater in the absence of MyD88, was also identified by these studies. The regulation of these genes suggested that MyD88 could serve as a molecular brake, constraining gene activity in a subset of LPS responsive genes. The findings generated with LPS stimulation were recapitulated by exposure of macrophages to live E. coli. These expression-profiling studies redefine the current dogma of TLR4 signaling and establish that MyD88, although essential for some of the best-characterized macrophage responses to LPS, is not required for the regulation of the majority of genes engaged by macrophage exposure to endotoxin or live bacteria.

Bjorkholm, B., A. Lundin, et al. (2001). "Comparison of genetic divergence and fitness between two subclones of Helicobacter pylori." Infect Immun **69**(12): 7832-8.

Helicobacter pylori has a very plastic genome, reflecting its high rate of recombination and point mutation. This plasticity promotes divergence of the population by the development of subclones and presumably enhances adaptation to host niches. We have investigated the genotypic and phenotypic characteristics of two such subclones isolated from one patient as well as the genetic evolution of these isolates during experimental infection. Whole-genome genotyping of the isolates using DNA microarrays revealed that they were more similar to each other than to a panel of other genotyped strains recovered from different hosts. Nonetheless, they still showed significant differences. For example, one isolate (67:21) contained the entire Cag pathogenicity island (PAI), whereas the other (67:20) had excised the

PAI. Phenotypic studies disclosed that both isolates expressed adhesins that recognized human histo-blood group Lewis(b) glycan receptors produced by gastric pit and surface mucus cells. In addition, both isolates were able to colonize, to equivalent density and with similar efficiency, germ-free transgenic mice genetically engineered to synthesize Lewis(b) glycans in their pit cells (12 to 14 mice/isolate). Remarkably, the Cag PAI-negative isolate was unable to colonize conventionally raised Lewis(b) transgenic mice harboring a normal gastric microflora, whereas the Cag PAI-positive isolate colonized 74% of the animals (39 to 40 mice/isolate). The genomic evolution of both isolates during the infection of conventionally raised and germ-free mice was monitored over the course of 3 months. The Cag PAI-positive isolate was also surveyed after a 10 month colonization of conventionally raised transgenic animals (n = 9 mice). Microarray analysis of the Cag PAI and sequence analysis of the cagA, recA, and 16S rRNA genes disclosed no changes in recovered isolates. Together, these results reveal that the H. pylori population infecting one individual can undergo significant divergence, creating stable subclones with substantial genotypic and phenotypic differences.

Black, M. A. and R. W. Doerge (2002). "Calculation of the minimum number of replicate spots required for detection of significant gene expression fold change in microarray experiments." Bioinformatics **18**(12): 1609-16.

MOTIVATION: We present statistical methods for determining the number of per gene replicate spots required in microarray experiments. The purpose of these methods is to obtain an estimate of the sampling variability present in microarray data, and to determine the number of replicate spots required to achieve a high probability of detecting a significant fold change in gene expression, while maintaining a low error rate. Our approach is based on data from control microarrays, and involves the use of standard statistical estimation techniques. RESULTS: After analyzing two experimental data sets containing control array data, we were able to determine the statistical power available for the detection of significant differential expression given differing levels of replication. The inclusion of replicate spots on microarrays not only allows more accurate estimation of the variability present in an experiment, but more importantly increases the probability of detecting genes undergoing significant fold changes in expression, while substantially decreasing the probability of observing fold changes due to chance rather than true differential expression.

Boonham, N., K. Walsh, et al. (2003). "Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis." J Virol Methods **108**(2): 181-7.

Currently, most diagnostic methodology is geared towards detection of a very specific target species and often a number of assays need to be run in parallel to reach a result. The generic methods that are available for virus testing tends to give identification to the genus level only. The method described in this paper addresses this problem by exploiting a technology that has potential to test for a large number of targets in a single assay. Using the array constructed, the method was able to detect several common potato viruses (PVY, PVX, PVA, PVS) in single and mixed infections. The method was shown to be able to discriminate sequences with less than 80% sequence identity but was able to detect sequence variants with greater than 90% sequence identity. Thus the method should be useful for discriminating at the species level, but able to cope well with the intrinsic variability found within the genomes of RNA viruses. The sensitivity of the assay was found to be comparable with ELISA. The paper illustrates a significant step forward in the development of diagnostic methodologies by presenting for the first time a method that could theoretically be used not just for viruses, but for all the plant pathogens and pests that a modern diagnostic laboratory would want to test for, in a single completely generic and highly parallel format.

Britton, R. A., P. Eichenberger, et al. (2002). "Genome-wide analysis of the stationary-phase sigma factor (sigma-H) regulon of Bacillus subtilis." J Bacteriol **184**(17): 4881-90.

Sigma-H is an alternative RNA polymerase sigma factor that directs the transcription of many

genes that function at the transition from exponential growth to stationary phase in Bacillus subtilis. Twenty-three promoters, which drive transcription of 33 genes, are known to be recognized by sigma-H-containing RNA polymerase. To identify additional genes under the control of sigma-H on a genome-wide basis, we carried out transcriptional profiling experiments using a DNA microarray containing >99% of the annotated B. subtilis open reading frames. In addition, we used a bioinformatics-based approach aimed at the identification of promoters recognized by RNA polymerase containing sigma-H. This combination of approaches was successful in confirming most of the previously described sigma-H-controlled genes. In addition, we identified 26 putative promoters that drive expression of 54 genes not previously known to be under the direct control of sigma-H. Based on the known or inferred function of most of these genes, we conclude that, in addition to its previously known roles in sporulation and competence, sigma-H controls genes involved in many physiological processes associated with the transition to stationary phase, including cytochrome biogenesis, generation of potential nutrient sources, transport, and cell wall metabolism.

Brokx, S. J., M. Ellison, et al. (2004). "Genome-wide analysis of lipoprotein expression in Escherichia coli MG1655." J Bacteriol **186**(10): 3254-8.

To gain insight into the cell envelope of Escherichia coli grown under aerobic and anaerobic conditions, lipoproteins were examined by using functional genomics. The mRNA expression levels of each of these genes under three growth conditions--aerobic, anaerobic, and anaerobic with nitrate--were examined by using both Affymetrix GeneChip E. coli antisense genome arrays and real-time PCR (RT-PCR). Many genes showed significant changes in expression level. The RT-PCR results were in very good agreement with the microarray data. The results of this study represent the first insights into the possible roles of unknown lipoprotein genes and broaden our understanding of the composition of the cell envelope under different environmental conditions. Additionally, these data serve as a test set for the refinement of high-throughput bioinformatic and global gene expression methods.

Brum, L. M., M. C. Lopez, et al. (2003). "Microarray analysis of A549 cells infected with rabbitpox virus (RPV): a comparison of wild-type RPV and RPV deleted for the host range gene, SPI-1." Virology **315**(2): 322-34.

A documented consequence of poxvirus infections is global inhibition of host protein synthesis and reduction in mRNA levels. We examined this mRNA decrease by infecting A549 cells, derived from a human lung carcinoma, with rabbitpox virus (RPV), or RPV deleted for the serine protease inhibitor SPI-1 (RPVDeltaSPI-1), which exhibits a growth defect on A549 cells. At various times postinfection, mRNA profiles were analyzed using Affymetrix U95AV2 microarrays. There was a decline in overall cellular mRNA levels beginning at 2.5 hpi, and by 5 hpi, mRNA levels were drastically reduced for the majority of genes. However, several mRNAs increased, including those of heat-shock genes. Finally, a comparison of host mRNA profiles of RPV- to RPVDeltaSPI-1-infected cells revealed subtle differences in mRNA levels at 5 and 12 hpi. In summary, while there was a global decrease of host mRNA levels, the induction of selected mRNAs may be required for a successful poxvirus infection.

Brun, Y. V. (2001). "Global analysis of a bacterial cell cycle: tracking down necessary functions and their regulators." Trends Microbiol **9**(9): 405-7.

New, post-genomic analyses are increasing the rate at which information about highly complex processes such as bacterial growth and development can be acquired. The recent use of DNA-microarray and proteomic analysis to study the differentiating bacterium Caulobacter crescentus has provided the first global view of the requirements of a bacterium as it progresses through its cell cycle. Potential regulators of cell cycle progression have been identified, and it has been suggested that proteolysis could have a global role in regulating the bacterial cell cycle.

Bucca, G., A. M. Brassington, et al. (2003). "Negative feedback regulation of dnaK, clpB and lon

expression by the DnaK chaperone machine in Streptomyces coelicolor, identified by transcriptome and in vivo DnaK-depletion analysis." Mol Microbiol **50**(1): 153-66.

The dnaK operon of Streptomyces coelicolor encodes the DnaK chaperone machine and the negative autoregulator HspR, which confers repression of the operon by binding to several inverted repeat sequences in the promoter region, dnaKp. Previous in vitro studies demonstrated that DnaK forms a specific complex with HspR bound to its operator sequences in dnaKp, and a model was proposed in which DnaK functions as a corepressor of the dnaK operon (Bucca, G., Brassington, A., Schonfeld, H.J., and Smith, C.P. (2000) Mol Microbiol 38: 1093-1103). Here we report in vivo DnaK depletion experiments which demonstrate that DnaK is a negative regulator of the dnaK operon. Cellular depletion of the DnaK chaperone leads to high-level transcription from dnaKp at the normal growth temperature. DNA microarray-based analysis of gene expression in wild-type and hspR-disruption mutant strains has identified a core cluster of genes regulated by HspR: the dnaK and clpB-SCO3660 operons and lon. These three transcription units are considered to be the direct targets of HspR. Significantly, analysis of the entire genome sequence revealed that the promoter regions of dnaK, clpB and lon are the only sequences that contain the HspR consensus binding sequence 5'-TTGAGY-N7-ACTCAA. S1 nuclease mapping confirmed that transcription of both clpB and lon is substantially enhanced at ambient temperature in strains depleted of DnaK, providing further evidence that these genes are members of the DnaK-HspR regulon. From transcriptome analysis, 17 genes were shown to be upregulated more than twofold in an hspR disruption mutant. This included the seven genes encoded by the dnaK, clpB and lon transcription units. Significantly, the other 10 genes are not heat-shock inducible in the wild type and their upregulation in the hspR mutant is considered to be an indirect consequence of enhanced synthesis of one or more components of the HspR regulon (the DnaK chaperone machine, ClpB and Lon protease).

Bunai, K., M. Ariga, et al. (2004). "Profiling and comprehensive expression analysis of ABC transporter solute-binding proteins of Bacillus subtilis membrane based on a proteomic approach." Electrophoresis **25**(1): 141-55.

We analyzed ABC transporter solute-binding proteins (SBPs) of the Bacillus subtilis membrane using a proteomic approach. We prepared a washed cell membrane fraction that was insoluble in 134 mM nondetergent sulfobetaine and then extracted proteins using mixtures of detergents in a stepwise manner. The membrane proteins were resolved by three two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) or two one-dimensional (1-D) PAGE procedures, electroblotted, and digested in the presence of 5% or 80% acetonitrile. Thereafter, matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF MS) identified 637 proteins corresponding to 15.9% of the total cellular proteins. We predicted that among these, 256 were membrane proteins, 101 were lipoproteins or secretory proteins and 280 were soluble proteins containing peripheral proteins that function in both the cytoplasm and the cell membrane such as SecA and FtsY. Among the 637 proteins, we identified 30 SBPs among 38 importers predicted by a bioinformatic search of the genome. We confirmed expression of the genes for the 30 SBPs using DNA microarray analysis. We compared the 2-D gel separation profiles of submembrane fractions solubilized by 1% n-dodecyl-beta-D-maltoside from cells cultured on Luria Bertani (LB), S7, and S7 medium without glutamate as well as DNA microarray data on LB and S7. The results suggested that YcdH, YtmK and YurO are binding proteins for Mn(++), glutamate and glucose, respectively, and that YqiX and YxeM are binding proteins for amino acids (tryptophan in S7 medium).

Busti, E., R. Bordoni, et al. (2002). "Bacterial discrimination by means of a universal array approach mediated by LDR (ligase detection reaction)." BMC Microbiol **2**(1): 27.

BACKGROUND: PCR amplification of bacterial 16S rRNA genes provides the most comprehensive and flexible means of sampling bacterial communities. Sequence analysis of these cloned fragments can provide a qualitative and quantitative insight of the microbial population under scrutiny although this approach is not suited to large-scale screenings. Other methods, such as denaturing gradient gel electrophoresis, heteroduplex or terminal restriction fragment analysis are rapid and therefore

amenable to field-scale experiments. A very recent addition to these analytical tools is represented by microarray technology. RESULTS: Here we present our results using a Universal DNA Microarray approach as an analytical tool for bacterial discrimination. The proposed procedure is based on the properties of the DNA ligation reaction and requires the design of two probes specific for each target sequence. One oligo carries a fluorescent label and the other a unique sequence (cZipCode or complementary ZipCode) which identifies a ligation product. Ligated fragments, obtained in presence of a proper template (a PCR amplified fragment of the 16s rRNA gene) contain either the fluorescent label or the unique sequence and therefore are addressed to the location on the microarray where the ZipCode sequence has been spotted. Such an array is therefore "Universal" being unrelated to a specific molecular analysis. Here we present the design of probes specific for some groups of bacteria and their application to bacterial diagnostics. CONCLUSIONS: The combined use of selective probes, ligation reaction and the Universal Array approach yielded an analytical procedure with a good power of discrimination among bacteria.

Buttitta, L., T. S. Tanaka, et al. (2003). "Microarray analysis of somitogenesis reveals novel targets of different WNT signaling pathways in the somitic mesoderm." Dev Biol **258**(1): 91-104.

WNT signaling plays a major role in patterning the dermomyotome of the somitic mesoderm. However, knowledge of downstream target genes and their regulation is limited. To identify new genes involved in the development and early patterning of the somite, we performed a comparison of gene expression by microarray between the presomitic mesoderm and the 5 most recently formed somites of the mouse at embryonic day 9.5. We identified 207 genes upregulated and 120 genes downregulated in somite formation. Expression analysis and functional categorization of these genes demonstrate this to be a diverse pool that provides a valuable resource for studying somite development. Thus far, we have found three genes expressed in the dermomyotome of the early somite. Consistent with their expression patterns, these genes are transcriptional targets of WNT signals, but display differential activation by different WNTs. We further demonstrate that 1 of these genes, Troy, is a direct target of canonical WNT signaling, while the other 2 genes, Selp and Arl4, are not. Thus, our microarray study using microdissected tissues not only provides global information on gene expression during somite development, it also provides novel targets to study the inductive signaling pathways that direct somite patterning.

Bystricka, D., O. Lenz, et al. (2003). "DNA microarray: parallel detection of potato viruses." Acta Virol **47**(1): 41-4.

DNA microarray assay has become a useful tool for gene expression studies. Less frequent is its application to detection of viruses or diagnostics of virus diseases. Here we show design of a microscope slide-based microarray assay for simultaneous identification of several potato viruses. Different primer pairs were designed or adopted to obtain specific amplicons from six potato viruses: Potato virus A (PVA), Potato virus S (PVS), Potato virus X (PVX), Potato virus Y (PVY), Potato mop-top virus (PMTV) and Potato leaf-roll virus (PLRV). Purified viral DNA probes were spotted on a microscope slide coated with poly-L-lysine. The same primers were used for preparation of fluorochrome-labeled targets. The latter were denatured and hybridized on the microarray slide (chip). An example of simultaneous assay of two pathogens is given and possibilities of practical application of this type of assay are discussed.

Cahir-McFarland, E. D., K. Carter, et al. (2004). "Role of NF-kappa B in cell survival and transcription of latent membrane protein 1-expressing or Epstein-Barr virus latency III-infected cells." J Virol **78**(8): 4108-19.

Epstein-Barr virus (EBV) latency III infection converts B lymphocytes into lymphoblastoid cell lines (LCLs) by expressing EBV nuclear and membrane proteins, EBNAs, and latent membrane proteins (LMPs), which regulate transcription through Notch and tumor necrosis factor receptor pathways. The

role of NF-kappa B in LMP1 and overall EBV latency III transcriptional effects was investigated by treating LCLs with BAY11-7082 (BAY11). BAY11 rapidly and irreversibly inhibited NF-kappa B, decreased mitochondrial membrane potential, induced apoptosis, and altered LCL gene expression. BAY11 effects were similar to those of an NF-kappa B inhibitor, Delta N-I kappa B alpha, in effecting decreased JNK1 expression and in microarray analyses. More than 80% of array elements that decreased with Delta N-I kappa B alpha expression decreased with BAY11 treatment. Newly identified NF-kappa B-induced, LMP1-induced, and EBV-induced genes included pleckstrin, Jun-B, c-FLIP, CIP4, and I kappa B epsilon. Of 776 significantly changed array elements, 134 were fourfold upregulated in EBV latency III, and 74 were fourfold upregulated with LMP1 expression alone, whereas only 28 were more than fourfold downregulated by EBV latency III. EBV latency III-regulated gene products mediate cell migration (EBI2, CCR7, RGS1, RANTES, MIP1 alpha, MIP1 beta, CXCR5, and RGS13), antigen presentation (major histocompatibility complex proteins and JAW1), mitogen-activated protein kinase pathway (DUSP5 and p62Dok), and interferon (IFN) signaling (IFN-gamma R alpha, IRF-4, and STAT1). Comparison of EBV latency III LCL gene expression to immunoglobulin M (IgM)-stimulated B cells, germinal-center B cells, and germinal-center-derived lymphomas clustered LCLs with IgM-stimulated B cells separately from germinal-center cells or germinal-center lymphoma cells. Expression of IRF-2, AIM1, ASK1, SNF2L2, and components of IFN signaling pathways further distinguished EBV latency III-infected B cells from IgM-stimulated or germinal-center B cells.

Cai, H. Y., M. Archambault, et al. (2003). "Molecular genetic methods in the veterinary clinical bacteriology laboratory: current usage and future applications." Anim Health Res Rev **4**(2): 73-93.

In the last 5 years, numerous molecular methods have been published for the detection and characterization of bacteria in the field of veterinary medicine. PCR has been the most commonly used technology. Although not currently used for clinical veterinary diagnosis, new technologies such as liquid-phase hybridization, real-time PCR, pathogen load determination and DNA/protein microarray have been described and have many possible applications in the clinical bacteriology laboratory because of their sensitivity and efficiency. This review describes the basic principles and application of recently published DNA-based molecular techniques for the purpose of veterinary clinical bacteriological diagnosis. It covers advances in probe hybridization technology, DNA/RNA amplification techniques and other molecular detection methods, including 16S rRNA analysis for bacterial characterization and DNA microarrays for bacterial detection. The review briefly summarizes the application of molecular methods for the diagnosis of specific important bacterial infections of animals, and for other animal pathogens that are slow or difficult to isolate in the clinical bacteriology laboratory. In addition, the molecular detection of antimicrobial resistance genes and of bovine mastitis pathogens is briefly described and current commercially available tests are listed.

Cai, Y., Y. Liu, et al. (2003). "Down-regulation of transcription of the proapoptotic gene BNip3 in cultured astrocytes by murine coronavirus infection." Virology **316**(1): 104-15.

Murine coronavirus mouse hepatitis virus (MHV) causes encephalitis and demyelination in the central nervous system of susceptible rodents. Astrocytes are the major target for MHV persistence. However, the mechanisms by which astrocytes survive MHV infection and permit viral persistence are not known. Here we performed DNA microarray analysis on differential gene expression in astrocyte DBT cells by MHV infection and found that the mRNA of the proapoptotic gene BNip3 was significantly decreased following MHV infection. This finding was further confirmed by quantitative reverse transcription-polymerase chain reaction, Western blot analysis, and BNip3-promoter-luciferase reporter system. Interestingly, infection with live and ultraviolet light-inactivated viruses equally repressed BNip3 expression, indicating that the down-regulation of BNip3 expression does not require virus replication and is mediated during cell entry. Furthermore, treatment of cells with chloroquine, which blocks the acidification of endosomes, significantly inhibited the repression of the BNip3 promoter activity induced by the acidic pH-dependent MHV mutant OBLV60, which enters cells via endocytosis, indicating that the

down-regulation of BNip3 expression is mediated by fusion between viral envelope and cell membranes during entry. Deletion analysis showed that the sequence between nucleotides 262 and 550 of the 588-base-pair BNip3 promoter is necessary and sufficient for driving the BNip3 expression and that it contains signals that are responsible for MHV-induced down-regulation of BNip3 expression in DBT cells. These results may provide insights into the mechanisms by which MHV evades host antiviral defense and promotes cell survival, thereby allowing its persistence in the host astrocytes.

Calevro, F., H. Charles, et al. (2004). "Assessment of 35mer amino-modified oligonucleotide based microarray with bacterial samples." J Microbiol Methods **57**(2): 207-18.

Parallel quantification of a large number of messenger RNA transcripts, using microarray technology, promises to provide unsuspected information about many cellular processes. Although experimental protocols on microarray applications are available, only limited methodological information on glass-slide manufacturing and signal interpretation has been published. The aim of this paper is to provide new insights into the practical aspects of the construction and hybridization of oligonucleotide-based microarrays. The intracellular symbiotic bacterium of aphids, Buchnera aphidicola, is used here as a model organism. The first part of the work is devoted to the optimization of procedures for printing slides, labeling of cDNA targets and hybridization. In the second part, based on a statistical analysis of the results, we discuss the influence of the probe attachment chemistry, of the labeling method, of the oligonucleotide position and of the concentration of a spotted oligonucleotide on signal intensity. The problem of signal specificity is also addressed, based on the calculation of the fluorescent ratio for each probe to its corresponding mismatch control probe. Lastly, the selection of internal spiked RNAs appropriate to our bacterial samples and useful for the data normalization step is presented.

Call, D. R., M. K. Bakko, et al. (2003). "Identifying antimicrobial resistance genes with DNA microarrays." Antimicrob Agents Chemother **47**(10): 3290-5.

We developed and tested a glass-based microarray suitable for detecting multiple tetracycline (tet) resistance genes. Microarray probes for 17 tet genes, the beta-lactamase bla(TEM-1) gene, and a 16S ribosomal DNA gene (Escherichia coli) were generated from known controls by PCR. The resulting products (ca. 550 bp) were applied as spots onto epoxy-silane-derivatized, Teflon-masked slides by using a robotic spotter. DNA was extracted from test strains, biotinylated, hybridized overnight to individual microarrays at 65 degrees C, and detected with Tyramide Signal Amplification, Alexa Fluor 546, and a microarray scanner. Using a detection threshold of 3x the standard deviation, we correctly identified tet genes carried by 39 test strains. Nine additional strains were not known to harbor any genes represented on the microarray, and these strains were negative for all 17 tet probes as expected. We verified that R741a, which was originally thought to carry a novel tet gene, tet(I), actually harbored a tet(G) gene. Microarray technology has the potential for screening a large number of different antibiotic resistance genes by the relatively low-cost methods outlined in this paper.

Campbell, E. J., P. M. Schenk, et al. (2003). "Pathogen-responsive expression of a putative ATP-binding cassette transporter gene conferring resistance to the diterpenoid sclareol is regulated by multiple defense signaling pathways in Arabidopsis." Plant Physiol **133**(3): 1272-84.

The ATP-binding cassette (ABC) transporters are encoded by large gene families in plants. Although these proteins are potentially involved in a number of diverse plant processes, currently, very little is known about their actual functions. In this paper, through a cDNA microarray screening of anonymous cDNA clones from a subtractive library, we identified an Arabidopsis gene (AtPDR12) putatively encoding a member of the pleiotropic drug resistance (PDR) subfamily of ABC transporters. AtPDR12 displayed distinct induction profiles after inoculation of plants with compatible and incompatible fungal pathogens and treatments with salicylic acid, ethylene, or methyl jasmonate. Analysis of AtPDR12 expression in a number of Arabidopsis defense signaling mutants further revealed that salicylic acid accumulation, NPR1 function, and sensitivity to jasmonates and ethylene were all required

for pathogen-responsive expression of AtPDR12. Germination assays using seeds from an AtPDR12 insertion line in the presence of sclareol resulted in lower germination rates and much stronger inhibition of root elongation in the AtPDR12 insertion line than in wild-type plants. These results suggest that AtPDR12 may be functionally related to the previously identified ABC transporters SpTUR2 and NpABC1, which transport sclareol. Our data also point to a potential role for terpenoids in the Arabidopsis defensive armory.

Campbell, J. W., R. M. Morgan-Kiss, et al. (2003). "A new Escherichia coli metabolic competency: growth on fatty acids by a novel anaerobic beta-oxidation pathway." Mol Microbiol **47**(3): 793-805.

Escherichia coli uses fatty acids as a sole carbon and energy source during aerobic growth by means of the enzymes encoded by the fad regulon. We report that this bacterium can also grow on fatty acids under anaerobic conditions provided that a terminal respiratory electron acceptor such as nitrate is available. This anaerobic utilization pathway is distinct from the well-studied aerobic pathway in that (i). it proceeds normally in mutant strains lacking various enzymes of the aerobic pathway; (ii). it functions with fatty acids (octanoate and decanoate) that cannot be used by wild-type E. coli strains under aerobic conditions; and (iii). super-repressor mutants of the fadR regulatory locus that block aerobic growth on fatty acids fail to block the anaerobic pathway. We have identified homologues of the FadA, FadB and FadD proteins required for aerobic fatty acid utilization called YfcY, YfcX and YdiD, respectively, which are involved in anaerobic growth on fatty acids. A strong FadR binding site was detected upstream of the yfcY gene consistent with microarray analyses, indicating that yfcYX expression is negatively regulated by FadR under aerobic growth conditions. In contrast, transcriptional regulation of ydiD appears to be independent of FadR, and anaerobic growth on fatty acids is not under FadR control. These three genes are conserved in the available genome sequences of pathogenic E. coli, Shigella and Salmonella strains.

Canny, G., O. Levy, et al. (2002). "Lipid mediator-induced expression of bactericidal/ permeability-increasing protein (BPI) in human mucosal epithelia." Proc Natl Acad Sci U S A **99**(6): 3902-7.

Epithelial cells which line mucosal surfaces are the first line of defense against bacterial invasion and infection. Recent studies have also indicated that epithelial cells contribute significantly to the orchestration of ongoing inflammatory processes. Here, we demonstrate that human epithelial cells express bactericidal/permeability-increasing protein (BPI), an antibacterial and endotoxin-neutralizing molecule previously associated with neutrophils. Moreover, we demonstrate that such BPI expression is transcriptionally regulated by analogs of endogenously occurring anti-inflammatory eicosanoids (aspirin-triggered lipoxins, ATLa). Initial studies to verify microarray analysis revealed that epithelial cells of wide origin (oral, pulmonary, and gastrointestinal mucosa) express BPI and each is similarly regulated by aspirin-triggered lipoxins. Studies aimed at localization of BPI revealed that such expression occurs on the cell surface of cultured epithelial cell lines and dominantly localizes to epithelia in human mucosal tissue. Functional studies employing a BPI-neutralizing anti-serum revealed that surface BPI blocks endotoxin-mediated signaling in epithelia and kills Salmonella typhimurium. These studies identify a previously unappreciated "molecular shield" for protection of mucosal surfaces against Gram-negative bacteria and their endotoxin.

Cao, Z., B. S. McAdory, et al. (2003). "The chemorepellent semaphorin is expressed in the horseshoe crab, Limulus polyphemus." Cell Mol Biol (Noisy-le-grand) **49**(8): 1261-7.

Semaphorins are a family of soluble and membrane-bound proteins that play a critical role in axonal guidance and other processes of neuronal development. Currently, more than twenty semaphorins have been identified, all of which share a conserved 500 amino acid domain near the amino terminus. Semaphorins are divided into eight classes according to species of origin and structural similarities. Classes 1 and 2 are found in invertebrates, classes 3 through 7 are present in vertebrates and viruses encode class V semaphorin. Microarray analysis of Limulus CNS RNA revealed the presence of a semaphorin-like gene in Limulus polyphemus. Based on these data, we aligned 31 different sequences

and designed degenerate primers for the consensus domains (WTT/SFLKA) and (DPY/VCA/GW). RT-PCR products were generated using 6 forward primers and 4 reverse primers. The expected size PCR products (750 bp) was obtained and then ligated with pCR II TOPO vector and transferred into E. coli Top 10. Five partial semaphorin cDNAs were found in Limulus: semaphorins 1a, 1b, 2a, 2b and F (now known as 5) were partially cloned. Subsequent Northern blot analyses using these Limulus specific-probes revealed hybridization with total RNAs purified from six different tissues.

Cardozo, A. K., H. Heimberg, et al. (2001). "A comprehensive analysis of cytokine-induced and nuclear factor-kappa B-dependent genes in primary rat pancreatic beta-cells." J Biol Chem **276**(52): 48879-86.

Type 1 diabetes mellitus results from an autoimmune destruction of pancreatic beta-cells. Cytokines, such as interleukin-1 beta and interferon-gamma, are putative mediators of immune-induced beta-cell death and, under in vitro conditions, cause beta-cell apoptosis. We have recently shown that interleukin-1 beta + interferon-gamma modifies the expression of >200 genes in beta-cells. Several of these genes are putative targets for the transcription factor nuclear factor-kappa B (NF-kappa B), and in subsequent experiments we showed that NF-kappa B activation is mostly pro-apoptotic in beta-cells. To identify cytokine-induced and NF-kappa B-regulated genes in primary rat beta-cells, we presently combined two experimental approaches: 1) blocking of NF-kappa B activation in cytokine-exposed beta-cells by a recombinant adenovirus (AdI kappa B((SA)2)) containing an inhibitor of NF-kappa B alpha (I kappa Bac) super-repressor (S32A/S36A) and 2) study of gene expression by microarray analysis. We identified 66 cytokine-modified and NF-kappa B-regulated genes in beta-cells. Cytokine-induced NF-kappa B activation decreased Pdx-1 and increased c-Myc expression. This, together with NF-kappa B-dependent inhibition of Glut-2, pro-hormone convertase-1, and Isl-1 expression, probably contributes to the loss of differentiated beta-cell functions. NF-kappa B also regulates several genes encoding for chemokines and cytokines in beta-cells. The present data suggest that NF-kappa B is a key "switch regulator" of transcription factors and gene networks controlling cytokine-induced beta-cell dysfunction and death.

Carlyon, J. A., W. T. Chan, et al. (2002). "Repression of rac2 mRNA expression by Anaplasma phagocytophila is essential to the inhibition of superoxide production and bacterial proliferation." J Immunol **169**(12): 7009-18.

Anaplasma phagocytophila, the etiologic agent of human granulocytic ehrlichiosis, is an emerging bacterial pathogen that invades neutrophils and can be cultivated in HL-60 cells. Infected neutrophils and HL-60 cells fail to produce superoxide anion (O(2)(-)), which is partially attributable to the fact that A. phagocytophila inhibits transcription of gp91(phox), an integral component of NADPH oxidase. cDNA microarray and RT-PCR analyses demonstrated that transcription of the gene encoding Rac2, a key component in NADPH oxidase activation, was down-regulated in infected HL-60 cells. Quantitative RT-PCR demonstrated that rac2 mRNA expression was reduced 7-fold in retinoic acid-differentiated HL-60 cells and 50-fold in neutrophils following A. phagocytophila infection. Rac2 protein expression was absent in infected HL-60 cells. Rac1 and Rac2 are interchangeable in their abilities to activate NADPH oxidase. HL-60 cells transfected to express myc-tagged rac1 and gp91(phox) from the CMV immediate early promoter maintained the ability to generate O(2)(-) 120 h postinfection. A. phagocytophila proliferation was severely inhibited in these cells. These results directly attribute the inhibition of rac2 and gp91(phox) transcription to the loss of NADPH oxidase activity in A. phagocytophila-infected cells and demonstrate its importance to bacterial intracellular survival.

Carpentier, A. S., A. Riva, et al. (2004). "The operons, a criterion to compare the reliability of transcriptome analysis tools: ICA is more reliable than ANOVA, PLS and PCA." Comput Biol Chem **28**(1): 3-10.

The number of statistical tools used to analyze transcriptome data is continuously increasing and no one, definitive method has so far emerged. There is a need for comparison and a number of different

approaches has been taken to evaluate the effectiveness of the different statistical tools available for microarray analyses. In this paper, we describe a simple and efficient protocol to compare the reliability of different statistical tools available for microarray analyses. It exploits the fact that genes within an operon exhibit the same expression patterns. In order to compare the tools, the genes are ranked according to the most relevant criterion for each tool; for each tool we look at the number of different operons represented within the first twenty genes detected. We then look at the size of the interval within which we find the most significant genes belonging to each operon in question. This allows us to define and estimate the sensitivity and accuracy of each statistical tool. We have compared four statistical tools using Bacillus subtilis expression data: the analysis of variance (ANOVA), the principal component analysis (PCA), the independent component analysis (ICA) and the partial least square regression (PLS). Our results show ICA to be the most sensitive and accurate of the tools tested. In this article, we have used the protocol to compare statistical tools applied to the analysis of differential gene expression. However, it can also be applied without modification to compare the statistical tools developed for other types of transcriptome analyses, like the study of gene co-expression.

Carter, K. L., E. Cahir-McFarland, et al. (2002). "Epstein-barr virus-induced changes in B-lymphocyte gene expression." J Virol **76**(20): 10427-36.

To elucidate the mechanisms by which Epstein-Barr virus (EBV) latency III gene expression transforms primary B lymphocytes to lymphoblastoid cell lines (LCLs), the associated alterations in cell gene expression were assessed by using 4,146 cellular cDNAs arrayed on nitrocellulose filters and real-time reverse transcription-PCR (RT-PCR). A total of 1,405 of the 4,146 cDNAs were detected using cDNA probes from poly(A)(+) RNA of IB4 LCLs, a non-EBV-infected Burkitt's lymphoma (BL) cell line, BL41, or EBV latency III-converted BL41 cells (BL41EBV). Thirty-eight RNAs were consistently twofold more abundant in the IB4 LCL and BL41EBV than in BL41 by microarray analysis. Ten of these are known to be EBV induced. A total of 23 of 28 newly identified EBV-induced genes were confirmed by real-time RT-PCR. In addition, nine newly identified genes and CD10 were EBV repressed. These EBV-regulated genes encode proteins involved in signal transduction, transcription, protein biosynthesis and degradation, and cell motility, shape, or adhesion. Seven of seven newly identified EBV-induced RNAs were more abundant in newly established LCLs than in resting B lymphocytes. Surveys of eight promoters of newly identified genes implicate NF-kappaB or PU.1 as potentially important mediators of EBV-induced effects through LMP1 or EBNA2, respectively. Thus, examination of the transcriptional effects of EBV infection can elucidate the molecular mechanisms by which EBV latency III alters B lymphocytes.

Chan, V. L. (2003). "Bacterial genomes and infectious diseases." Pediatr Res **54**(1): 1-7.

The genome sequencing approach has proved to be highly effective and invaluable for gaining an insight on structure of bacteria genomes and the biology and evolution of bacteria. The diversity of bacteria genomes is beyond expectation. Gaining a full understanding of the biology and pathogenic mechanisms of these pathogens will be a major task because on an average only approximately 69% of the encoded proteins in each genome have known functions. Genome sequence analyses have identified novel putative virulence genes, vaccine candidates, targets for antibacterial drugs, and specific diagnostic probes. Microarray technology that makes use of the genomic sequences of human and bacterial pathogens will be a major tool for gaining full understanding of the complexity of host-pathogen interactions and mechanisms of pathogenesis.

Chandler, D. P., G. J. Newton, et al. (2003). "Sequence versus structure for the direct detection of 16S rRNA on planar oligonucleotide microarrays." Appl Environ Microbiol **69**(5): 2950-8.

A two-probe proximal chaperone detection system consisting of a species-specific capture probe for the microarray and a labeled, proximal chaperone probe for detection was recently described for direct detection of intact rRNAs from environmental samples on oligonucleotide arrays. In this study, we

investigated the physical spacing and nucleotide mismatch tolerance between capture and proximal chaperone detector probes that are required to achieve species-specific 16S rRNA detection for the dissimilatory metal and sulfate reducer 16S rRNAs. Microarray specificity was deduced by analyzing signal intensities across replicate microarrays with a statistical analysis-of-variance model that accommodates well-to-well and slide-to-slide variations in microarray signal intensity. Chaperone detector probes located in immediate proximity to the capture probe resulted in detectable, nonspecific binding of nontarget rRNA, presumably due to base-stacking effects. Species-specific rRNA detection was achieved by using a 22-nt capture probe and a 15-nt detector probe separated by 10 to 14 nt along the primary sequence. Chaperone detector probes with up to three mismatched nucleotides still resulted in species-specific capture of 16S rRNAs. There was no obvious relationship between position or number of mismatches and within- or between-genus hybridization specificity. From these results, we conclude that relieving secondary structure is of principal concern for the successful capture and detection of 16S rRNAs on planar surfaces but that the sequence of the capture probe is more important than relieving secondary structure for achieving specific hybridization.

Chang, K. C., Y. C. Yeh, et al. (2001). "Identification of genes associated with natural competence in Helicobacter pylori by transposon shuttle random mutagenesis." Biochem Biophys Res Commun **288**(4): 961-8.

To identify genes involved in DNA transformation, we generated 1500 insertion mutants of a Helicobacter pylori strain by transposon shuttle mutagenesis. All mutant strains were screened for their frequency of natural transformation. A total of 20 mutant strains were found to exhibit a significantly decreased transformation frequency. DNA sequencing revealed seven genetic loci, including the reported comB locus, HP0017 (a putative virB4 homologue) and five loci without database match (HP0015, HP1089, HP1326, HP1424, and HP1473) from the 20 mutants. Reknockout of HP1326 revealed no impairment in natural transformation, while the other 5 mutants showed the same defective in natural transformation. Mutation of HP0017 severely impaired natural transformation both chromosome and plasmid DNA. Slot blot analysis revealed that some noncompetent strains had decreased virB4 RNA expression levels compared with competent strains. Nineteen ORFs had decreased expression levels in virB4 knockout mutant by microarray. Therefore, our data indicate that HP0017 is a virB4 homologue and is essential in the natural competence of H. pylori. HP0015, HP1089, HP1424, and HP1473 genes could be also involved in natural transformation.

Chang, L. K., T. T. Wei, et al. (2003). "Inhibition of Epstein-Barr virus lytic cycle by (-)-epigallocatechin gallate." Biochem Biophys Res Commun **301**(4): 1062-8.

(-)-Epigallocatechin gallate (EGCG), abundant in green tea, is a potent anti-microbial and anti-tumor compound. This investigation used immunoblot, flow cytometry, microarray, and indirect immunofluorescence analyses to show that at concentrations exceeding 50 microM, EGCG inhibits the expression of Epstein-Barr virus (EBV) lytic proteins, including Rta, Zta, and EA-D, but does not affect the expression of EBNA-1. Moreover, DNA microarray and transient transfection analyses demonstrated that EGCG blocks EBV lytic cycle by inhibiting the transcription of immediate-early genes, thus inhibiting the initiation of EBV lytic cascade.

Chang, Y. E. and L. A. Laimins (2000). "Microarray analysis identifies interferon-inducible genes and Stat-1 as major transcriptional targets of human papillomavirus type 31." J Virol **74**(9): 4174-82.

Human papillomaviruses (HPVs) infect keratinocytes and induce proliferative lesions. In infected cells, viral gene products alter the activities of cellular proteins, such as Rb and p53, resulting in altered cell cycle response. It is likely that HPV gene products also alter expression of cellular genes. In this study we used microarray analysis to examine the global changes in gene expression induced by high-risk HPV type 31 (HPV31). Among 7,075 known genes and ESTs (expressed sequence tags) tested, we found that 178 were upregulated and 150 were downregulated twofold or more in HPV31 cells compared to

normal human keratinocytes. While no specific pattern could be deduced from the list of genes that were upregulated, downregulated genes could be classified to three groups: genes that are involved in the regulation of cell growth, genes that are specifically expressed in keratinocytes, and genes whose expression is increased in response to interferon stimulation. The basal level of expression of several interferon-responsive genes was found to be downregulated in HPV31 cells by both microarray analysis and Northern blot analysis in different HPV31 cell lines. When cells were treated with alpha or gamma interferon, expression of interferon-inducible genes was impaired. At high doses of interferon, the effects were less pronounced. Among the genes repressed by HPV31 was the signal transducer and activator of transcription (Stat-1), which plays a major role in mediating the interferon response. Suppression of Stat-1 expression may contribute to a suppressed response to interferon as well as immune evasion.

Chao, S. H., J. R. Walker, et al. (2003). "Identification of homeodomain proteins, PBX1 and PREP1, involved in the transcription of murine leukemia virus." Mol Cell Biol **23**(3): 831-41.

Cyclin-dependent kinase inhibitors (CDKIs) have been shown to block human immunodeficiency virus and herpes simplex virus. It is hypothesized that CDKIs block viral replication by inhibiting transcription of specific cellular genes. Here we find that three CDKIs, flavopiridol, purvalanol A, and methoxy-roscovitine, block Moloney murine leukemia virus (MLV) transcription events. Using gene expression microarray technology to examine the inhibitory effects of CDKIs, we observed a cellular gene, the pre-B-cell leukemia transcription factor 1 (Pbx1) gene, down-regulated by CDKI treatment. The PBX consensus element (PCE), TGATTGAC, is conserved in the long terminal repeats of several murine retroviruses, including Moloney MLV. Mutations in the PCE completely inhibited viral transcription whereas overexpression of PBX1 and a PBX1-associated protein, PREP1, enhanced viral transcription. The interaction between the PCE and PBX1-PREP1 proteins was confirmed by gel shift experiments. Blocking PBX1 protein synthesis resulted in a significant decrease in viral transcription. Collectively, our results represent the first work demonstrating that the homeodomain proteins PBX1 and PREP1 are cellular factors involved in Moloney MLV transcription regulation.

Chao, T. C., A. Becker, et al. (2004). "The Sinorhizobium meliloti fur gene regulates, with dependence on Mn(II), transcription of the sitABCD operon, encoding a metal-type transporter." J Bacteriol **186**(11): 3609-20.

Sinorhizobium meliloti is an alpha-proteobacterium able to induce nitrogen-fixing nodules on roots of specific legumes. In order to propagate in the soil and for successful symbiotic interaction the bacterium needs to sequester metals like iron and manganese from its environment. The metal uptake has to be in turn tightly regulated to avoid toxic effects. In this report we describe the characterization of a chromosomal region of S. meliloti encoding the sitABCD operon and the putative regulatory fur gene. It is generally assumed that the sitABCD operon encodes a metal-type transporter and that the fur gene is involved in iron ion uptake regulation. A constructed S. meliloti sitA deletion mutant was found to be growth dependent on Mn(II) and to a lesser degree on Fe(II). The sitA promoter was strongly repressed by Mn(II), with dependence on Fur, and moderately by Fe(II). Applying a genome-wide S. meliloti microarray it was shown that in the fur deletion mutant 23 genes were up-regulated and 10 genes were down-regulated when compared to the wild-type strain. Among the up-regulated genes only the sitABCD operon could be associated with metal uptake. On the other hand, the complete rhbABCDEF operon, which is involved in siderophore synthesis, was identified among the down-regulated genes. Thus, in S. meliloti Fur is not a global repressor of iron uptake. Under symbiotic conditions the sitA promoter was strongly expressed and the S. meliloti sitA mutant exhibited an attenuated nitrogen fixation activity resulting in a decreased fresh weight of the host plant Medicago sativa.

Chatterjee, A. and S. J. Roux (2000). "Ceratopteris richardii: a productive model for revealing secrets of signaling and development." J Plant Growth Regul **19**(3): 284-9.

Ceratopteris richardii is an aquatic fern grown in tropical and subtropical regions of the world. It

is proven to be a productive model system for studies in the genetics, biochemistry, and cell biology of basic biologic processes that occur in early gametophytic development. It provides several advantages to biologists, especially those interested in gravitational biology, polarity development, and in the genetics of sexual development. It is easy to culture, has a relatively short life cycle, and offers an array of attractive features that facilitate genetic studies. The germination and early development of large populations of genetically identical spores are easy to synchronize, and both the direction of polarity development and cell-level gravity responses can be measured and readily manipulated within the first 24 h of spore development. Although there is no reliable transformation system available yet in Ceratopteris, recent studies suggest that the technique of RNA interference can be used to block translation of specific genes in a related fern, Marsilea, and current studies will soon reveal the applicability of this approach, as well as of other transformation approaches, in Ceratopteris. A recently completed expressed sequence tag (EST) sequencing project makes available the partial sequence of more than 2000 cDNAs, representing a significant percentage of the genes being expressed during the first 24 h of spore germination, when many developmentally interesting processes are occurring. A microarray of these ESTs is being constructed, so especially for those scientists interested in basic cellular phenomena that occur early in spore germination, the availability of the ESTs and of the microarray will make Ceratopteris an even more attractive model system.

Chen, H., J. Liu, et al. (2001). "Genetic events associated with arsenic-induced malignant transformation: applications of cDNA microarray technology." Mol Carcinog **30**(2): 79-87.

Arsenic is a human carcinogen. Our recent work showed that chronic (>18 wk), low-level (125-500 nM) arsenite exposure induces malignant transformation in normal rat liver cell line TRL1215. In these arsenic-transformed cells, the cellular S-adenosylmethionine pool was depleted from arsenic metabolism, resulting in global DNA hypomethylation. DNA methylation status in turn may affect the expression of a variety of genes. This study examined the aberrant gene expression associated with arsenic-induced transformation with the use of Atlas Rat cDNA Expression microarrays. Poly(A(+)) RNA was prepared from arsenic-transformed cells and passage-matched control cells, and (32)P-labeled cDNA probes were synthesized with Clontech Rat cDNA Synthesis primers and moloney murine leukemia virus reverse transcriptase. The hybrid intensity was analyzed with AtlasImage software and normalized with the sum of the four housekeeping genes. Four hybridizations from separate cell preparations were performed, and mean and SEM for the expression of each gene were calculated for statistical analysis. Among the 588 genes, approximately 80 genes (approximately 13%) were aberrantly expressed. These included genes involved in cell-cycle regulation, signal transduction, stress response, apoptosis, cytokine production and growth-factor and hormone-receptor production and various oncogenes. These initial gene expression analyses for the first time showed potentially important aberrant gene expression patterns associated with arsenic-induced malignant transformation and set the stage for numerous further studies. Mol. Carcinog. 30:79-87, 2001. Published 2001 Wiley-Liss, Inc.

Chen, W., N. J. Provart, et al. (2002). "Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses." Plant Cell **14**(3): 559-74.

Numerous studies have shown that transcription factors are important in regulating plant responses to environmental stress. However, specific functions for most of the genes encoding transcription factors are unclear. In this study, we used mRNA profiles generated from microarray experiments to deduce the functions of genes encoding known and putative Arabidopsis transcription factors. The mRNA levels of 402 distinct transcription factor genes were examined at different developmental stages and under various stress conditions. Transcription factors potentially controlling downstream gene expression in stress signal transduction pathways were identified by observed activation and repression of the genes after certain stress treatments. The mRNA levels of a number of previously characterized transcription factor genes were changed significantly in connection with other regulatory pathways, suggesting their multifunctional nature. The expression of 74 transcription factor genes

responsive to bacterial pathogen infection was reduced or abolished in mutants that have defects in salicylic acid, jasmonic acid, or ethylene signaling. This observation indicates that the regulation of these genes is mediated at least partly by these plant hormones and suggests that the transcription factor genes are involved in the regulation of additional downstream responses mediated by these hormones. Among the 43 transcription factor genes that are induced during senescence, 28 of them also are induced by stress treatment, suggesting extensive overlap responses to these stresses. Statistical analysis of the promoter regions of the genes responsive to cold stress indicated unambiguous enrichment of known conserved transcription factor binding sites for the responses. A highly conserved novel promoter motif was identified in genes responding to a broad set of pathogen infection treatments. This observation strongly suggests that the corresponding transcription factors play general and crucial roles in the coordinated regulation of these specific regulons. Although further validation is needed, these correlative results provide a vast amount of information that can guide hypothesis-driven research to elucidate the molecular mechanisms involved in transcriptional regulation and signaling networks in plants.

Chen, W. V., J. Delrow, et al. (2004). "Identification and validation of PDGF transcriptional targets by microarray-coupled gene-trap mutagenesis." Nat Genet **36**(3): 304-12.

We developed a versatile, high-throughput genetic screening strategy by coupling gene mutagenesis and expression profiling technologies. Using a retroviral gene-trap vector optimized for efficient mutagenesis and cloning, we randomly disrupted genes in mouse embryonic stem (ES) cells and amplified them to construct a cDNA microarray. With this gene-trap array, we show that transcriptional target genes of platelet-derived growth factor (PDGF) can be efficiently and reliably identified in physiologically relevant cells and are immediately accessible to genetic studies to determine their in vivo roles and relative contributions to PDGF-regulated developmental processes. The same platform can be used to search for genes of specific biological relevance in a broad array of experimental settings, providing a fast track from gene identification to functional validation.

Chennathukuzhi, V., J. M. Stein, et al. (2003). "Mice deficient for testis-brain RNA-binding protein exhibit a coordinate loss of TRAX, reduced fertility, altered gene expression in the brain, and behavioral changes." Mol Cell Biol **23**(18): 6419-34.

Testis-brain RNA-binding protein (TB-RBP), the mouse orthologue of the human protein Translin, is a widely expressed and highly conserved protein with proposed functions in chromosomal translocations, mitotic cell division, and mRNA transport and storage. To better define the biological roles of TB-RBP, we generated mice lacking TB-RBP. Matings between heterozygotes gave rise to viable, apparently normal homozygous mutant mice at a normal Mendelian ratio. The TB-RBP-related and -interacting protein Translin-associated factor X was reduced to 50% normal levels in heterozygotes and was absent in TB-RBP-null animals. The null mice were 10 to 30% smaller than their wild-type or heterozygote littermates at birth and remained so to about 6 to 9 months of age, showed normal B- and T-cell development, and accumulated visceral fat. TB-RBP-null male mice were fertile and sired offspring but had abnormal seminiferous tubules and reduced sperm counts. Null female mice were subfertile and had reduced litter sizes. Microarray analysis of total brain RNA from null and wild-type mice revealed an altered gene expression profile with the up-regulation of 14 genes and the down-regulation of 217 genes out of 12,473 probe sets. Numerous neurotransmitter receptors and ion channels, including gamma-aminobutyric acid A receptor alpha1 and glutamate receptor alpha3, were strongly down-regulated. Behavioral abnormalities were also seen. Compared to littermates, the TB-RBP-null mice appeared docile and exhibited reduced Rota-Rod performance.

Cherkasova, E., M. Laassri, et al. (2003). "Microarray analysis of evolution of RNA viruses: evidence of circulation of virulent highly divergent vaccine-derived polioviruses." Proc Natl Acad Sci U S A **100**(16): 9398-403.

Two approaches based on hybridization of viral probes with oligonucleotide microarrays were

developed for rapid analysis of genetic variations during microevolution of RNA viruses. Microarray analysis of viral recombination and microarray for resequencing and heterogeneity analysis were able to generate instant genetic maps of vaccine-derived polioviruses (VDPVs) and reveal the degree of their evolutionary divergence. Unlike conventional methods based on cDNA sequencing and restriction fragment length polymorphism, the microarray approaches are better suited for analysis of heterogeneous populations and mixtures of different strains. The microarray hybridization profile is very sensitive to the cumulative presence of small quantities of different mutations, including those that cannot be revealed by sequencing, making this approach useful for characterization of profiles of nucleotide sequence diversity in viral populations. By using these methods, we identified a type-3 VDPV isolated from a healthy person and missed by conventional methods of screening. The mutational profile of the polio strain was consistent with >1 yr of circulation in human population and was highly virulent in transgenic mice, confirming the ability of VDPV to persist in communities despite high levels of immunity. The proposed methods for fine genotyping of heterogeneous viral populations can also have utility for a variety of other applications in studies of genetic changes in viruses, bacteria, and genes of higher organisms.

Cheung, K. J., V. Badarinarayana, et al. (2003). "A microarray-based antibiotic screen identifies a regulatory role for supercoiling in the osmotic stress response of Escherichia coli." Genome Res **13**(2): 206-15.

Changes in DNA supercoiling are induced by a wide range of environmental stresses in Escherichia coli, but the physiological significance of these responses remains unclear. We now demonstrate that an increase in negative supercoiling is necessary for transcriptional activation of a large subset of osmotic stress-response genes. Using a microarray-based approach, we have characterized supercoiling-dependent gene transcription by expression profiling under conditions of high salt, in conjunction with the microbial antibiotics novobiocin, pefloxacin, and chloramphenicol. Algorithmic clustering and statistical measures for gauging cellular function show that this subset is enriched for genes critical in osmoprotectant transport/synthesis and rpoS-driven stationary phase adaptation. Transcription factor binding site analysis also supports regulation by the global stress sigma factor rpoS. In addition, these studies implicate 60 uncharacterized genes in the osmotic stress regulon, and offer evidence for a broader role for supercoiling in the control of stress-induced transcription

Chhabra, S. R., K. R. Shockley, et al. (2003). "Carbohydrate-induced differential gene expression patterns in the hyperthermophilic bacterium Thermotoga maritima." J Biol Chem **278**(9): 7540-52.

The hyperthermophilic bacterium Thermotoga maritima MSB8 was grown on a variety of carbohydrates to determine the influence of carbon and energy source on differential gene expression. Despite the fact that T. maritima has been phylogenetically characterized as a primitive microorganism from an evolutionary perspective, results here suggest that it has versatile and discriminating mechanisms for regulating and effecting complex carbohydrate utilization. Growth of T. maritima on monosaccharides was found to be slower than growth on polysaccharides, although growth to cell densities of 10(8) to 10(9) cells/ml was observed on all carbohydrates tested. Differential expression of genes encoding carbohydrate-active proteins encoded in the T. maritima genome was followed using a targeted cDNA microarray in conjunction with mixed model statistical analysis. Coordinated regulation of genes responding to specific carbohydrates was noted. Although glucose generally repressed expression of all glycoside hydrolase genes, other sugars induced or repressed these genes to varying extents. Expression profiles of most endo-acting glycoside hydrolase genes correlated well with their reported biochemical properties, although exo-acting glycoside hydrolase genes displayed less specific expression patterns. Genes encoding selected putative ABC sugar transporters were found to respond to specific carbohydrates, and in some cases putative oligopeptide transporter genes were also found to respond to specific sugar substrates. Several genes encoding putative transcriptional regulators were expressed during growth on specific sugars, thus suggesting functional assignments. The transcriptional response of T. maritima to specific carbohydrate growth substrates indicated that sugar backbone- and linkage-

specific regulatory networks are operational in this organism during the uptake and utilization of carbohydrate substrates. Furthermore, the wide ranging collection of such networks in T. maritima suggests that this organism is capable of adapting to a variety of growth environments containing carbohydrate growth substrates.

Chitko-McKown, C. G., J. M. Fox, et al. (2004). "Gene expression profiling of bovine macrophages in response to Escherichia coli O157:H7 lipopolysaccharide." Dev Comp Immunol **28**(6): 635-45.

The aim of this study was to identify changes in bovine macrophage gene expression in response to treatment with Escherichia coli 0157:H7 lipopolysaccharide (LPS), utilizing a human gene microarray. Bovine cDNA from control and LPS-treated primary macrophages hybridized to greater than 5644 (79.8%) of the non-control gene targets on a commercially available microarray containing greater than 7075 targets (Incyte Genomics, St. Louis, MO). Of these target sequences, 44 were differentially expressed upon exposure to LPS, including 18 genes not previously reported to exist in cattle. These included a pentaxin-related gene, CASP8, TNF-induced genes, interferon-induced genes, and inhibitors of apoptosis. Using the human microarray, cDNA from bovine LPS-treated and control macrophages consistently hybridized to targets known to be expressed constitutively by macrophages, as expected given the predicted cDNA sequence homology. That this human system was accurately estimating levels of bovine transcripts was further verified by real-time quantitative reverse transcriptase polymerase chain reaction (RTQ-PCR) using bovine-specific primers. This first report of bovine-human cross-species expression profiling by microarray hybridization demonstrates the utility of this technique in bovine gene expression and discovery.

Chizhikov, V., A. Rasooly, et al. (2001). "Microarray analysis of microbial virulence factors." Appl Environ Microbiol **67**(7): 3258-63.

Hybridization with oligonucleotide microchips (microarrays) was used for discrimination among strains of Escherichia coli and other pathogenic enteric bacteria harboring various virulence factors. Oligonucleotide microchips are miniature arrays of gene-specific oligonucleotide probes immobilized on a glass surface. The combination of this technique with the amplification of genetic material by PCR is a powerful tool for the detection of and simultaneous discrimination among food-borne human pathogens. The presence of six genes (eaeA, slt-I, slt-II, fliC, rfbE, and ipaH) encoding bacterial antigenic determinants and virulence factors of bacterial strains was monitored by multiplex PCR followed by hybridization of the denatured PCR product to the gene-specific oligonucleotides on the microchip. The assay was able to detect these virulence factors in 15 Salmonella, Shigella, and E. coli strains. The results of the chip analysis were confirmed by hybridization of radiolabeled gene-specific probes to genomic DNA from bacterial colonies. In contrast, gel electrophoretic analysis of the multiplex PCR products used for the microarray analysis produced ambiguous results due to the presence of unexpected and uncharacterized bands. Our results suggest that microarray analysis of microbial virulence factors might be very useful for automated identification and characterization of bacterial pathogens.

Cho, J. C. and J. M. Tiedje (2001). "Bacterial species determination from DNA-DNA hybridization by using genome fragments and DNA microarrays." Appl Environ Microbiol **67**(8): 3677-82.

Whole genomic DNA-DNA hybridization has been a cornerstone of bacterial species determination but is not widely used because it is not easily implemented. We have developed a method based on random genome fragments and DNA microarray technology that overcomes the disadvantages of whole-genome DNA-DNA hybridization. Reference genomes of four fluorescent Pseudomonas species were fragmented, and 60 to 96 genome fragments of approximately 1 kb from each strain were spotted on microarrays. Genomes from 12 well-characterized fluorescent Pseudomonas strains were labeled with Cy dyes and hybridized to the arrays. Cluster analysis of the hybridization profiles revealed taxonomic relationships between bacterial strains tested at species to strain level resolution, suggesting that this approach is useful for the identification of bacteria as well as determining the genetic distance among

bacteria. Since arrays can contain thousands of DNA spots, a single array has the potential for broad identification capacity. In addition, the method does not require laborious cross-hybridizations and can provide an open database of hybridization profiles, avoiding the limitations of traditional DNA-DNA hybridization.

Chou, C. C., C. H. Chen, et al. (2004). "Optimization of probe length and the number of probes per gene for optimal microarray analysis of gene expression." Nucleic Acids Res **32**(12): e99.

Gene-specific oligonucleotide probes are currently used in microarrays to avoid cross-hybridization of highly similar sequences. We developed an approach to determine the optimal number and length of gene-specific probes for accurate transcriptional profiling studies. The study surveyed probe lengths from 25 to 1000 nt. Long probes yield better signal intensity than short probes. The signal intensity of short probes can be improved by addition of spacers or using higher probe concentration for spotting. We also found that accurate gene expression measurement can be achieved with multiple probes per gene and fewer probes are needed if longer probes rather than shorter probes are used. Based on theoretical considerations that were confirmed experimentally, our results showed that 150mer is the optimal probe length for expression measurement. Gene-specific probes can be identified using a computational approach for 150mer probes and they can be treated like long cDNA probes in terms of the hybridization reaction for high sensitivity detection. Our experimental data also show that probes which do not generate good signal intensity give erroneous expression ratio measurement results. To use microarray probes without experimental validation, gene-specific probes approximately 150mer in length are necessary. However, shorter oligonucleotide probes also work well in gene expression analysis if the probes are validated by experimental selection or if multiple probes per gene are used for expression measurement.

Clarke, L. L., L. R. Gawenis, et al. (2004). "Abnormal Paneth cell granule dissolution and compromised resistance to bacterial colonization in the intestine of CF mice." Am J Physiol Gastrointest Liver Physiol **286**(6): G1050-8.

Paneth cells of intestinal crypts contribute to host defense by producing antimicrobial peptides that are packaged as granules for secretion into the crypt lumen. Here, we provide evidence using light and electron microscopy that postsecretory Paneth cell granules undergo limited dissolution and accumulate within the intestinal crypts of cystic fibrosis (CF) mice. On the basis of this finding, we evaluated bacterial colonization and expression of two major constituents of Paneth cells, i.e., alpha-defensins (cryptdins) and lysozyme, in CF murine intestine. Paneth cell granules accumulated in intestinal crypt lumens in both untreated CF mice with impending intestinal obstruction and in CF mice treated with an osmotic laxative that prevented overt clinical symptoms and mucus accretion. Ultrastructure studies indicated little change in granule morphology within mucus casts, whereas granules in laxative-treated mice appear to undergo limited dissolution. Protein extracts from CF intestine had increased levels of processed cryptdins compared with those from wild-type (WT) littermates. Nonetheless, colonization with aerobic bacteria species was not diminished in the CF intestine and oral challenge with a cryptdin-sensitive enteric pathogen, Salmonella typhimurium, resulted in greater colonization of CF compared with WT intestine. Modest downregulation of cryptdin and lysozyme mRNA in CF intestine was shown by microarray analysis, real-time quantitative PCR, and Northern blot analysis. Based on these findings, we conclude that antimicrobial peptide activity in CF mouse intestine is compromised by inadequate dissolution of Paneth cell granules within the crypt lumens.

Cleary, M. D., U. Singh, et al. (2002). "Toxoplasma gondii asexual development: identification of developmentally regulated genes and distinct patterns of gene expression." Eukaryot Cell **1**(3): 329-40.

Asexual development in Toxoplasma gondii is a vital aspect of the parasite's life cycle, allowing transmission and avoidance of the host immune response. Differentiation of rapidly dividing tachyzoites into slowly growing, encysted bradyzoites involves significant changes in both physiology and

morphology. We generated microarrays of approximately 4,400 Toxoplasma cDNAs, representing a minimum of approximately 600 genes (based on partial sequencing), and used these microarrays to study changes in transcript levels during tachyzoite-to-bradyzoite differentiation. This approach has allowed us to (i) determine expression profiles of previously described developmentally regulated genes, (ii) identify novel developmentally regulated genes, and (iii) identify distinct classes of genes based on the timing and magnitude of changes in transcript levels. Whereas microarray analysis typically involves comparisons of mRNA levels at different time points, we have developed a method to measure relative transcript abundance between genes at a given time point. This method was used to determine transcript levels in parasites prior to differentiation and to further classify bradyzoite-induced genes, thus allowing a more comprehensive view of changes in gene expression than is provided by standard expression profiles. Newly identified developmentally regulated genes include putative surface proteins (a SAG1-related protein, SRS9, and a mucin-domain containing protein), regulatory and metabolic enzymes (methionine aminopeptidase, oligopeptidase, aminotransferase, and glucose-6-phosphate dehydrogenase homologues), and a subset of genes encoding secretory organelle proteins (MIC1, ROP1, ROP2, ROP4, GRA1, GRA5, and GRA8). This analysis permits the first in-depth look at changes in gene expression during development of this complex protozoan parasite.

Conacci-Sorrell, M. E., T. Ben-Yedidia, et al. (2002). "Nr-CAM is a target gene of the beta-catenin/LEF-1 pathway in melanoma and colon cancer and its expression enhances motility and confers tumorigenesis." Genes Dev **16**(16): 2058-72.

beta-catenin and plakoglobin (gamma-catenin) are homologous molecules involved in cell adhesion, linking cadherin receptors to the cytoskeleton. beta-catenin is also a key component of the Wnt pathway by being a coactivator of LEF/TCF transcription factors. To identify novel target genes induced by beta-catenin and/or plakoglobin, DNA microarray analysis was carried out with RNA from cells overexpressing either protein. This analysis revealed that Nr-CAM is the gene most extensively induced by both catenins. Overexpression of either beta-catenin or plakoglobin induced Nr-CAM in a variety of cell types and the LEF/TCF binding sites in the Nr-CAM promoter were required for its activation by catenins. Retroviral transduction of Nr-CAM into NIH3T3 cells stimulated cell growth, enhanced motility, induced transformation, and produced rapidly growing tumors in nude mice. Nr-CAM and LEF-1 expression was elevated in human colon cancer tissue and cell lines and in human malignant melanoma cell lines but not in melanocytes or normal colon tissue. Dominant negative LEF-1 decreased Nr-CAM expression and antibodies to Nr-CAM inhibited the motility of B16 melanoma cells. The results indicate that induction of Nr-CAM transcription by beta-catenin or plakoglobin plays a role in melanoma and colon cancer tumorigenesis, probably by promoting cell growth and motility.

Conway, T. and G. K. Schoolnik (2003). "Microarray expression profiling: capturing a genome-wide portrait of the transcriptome." Mol Microbiol **47**(4): 879-89.

The bacterial transcriptome is a dynamic entity that reflects the organism's immediate, ongoing and genome-wide response to its environment. Microarray expression profiling provides a comprehensive portrait of the transcriptional world enabling us to view the organism as a 'system' that is more than the sum of its parts. The vigilance of microorganisms to environmental change, the alacrity of the transcriptional response, the short half-life of bacterial mRNA and the genome-scale nature of the investigation collectively explain the power of this method. These same features pose the most significant experimental design and execution issues which, unless surmounted, predictably generate a distorted image of the transcriptome. Conversely, the expression profile of a properly conceived and conducted microarray experiment can be used for hypothesis testing: disclosure of the metabolic and biosynthetic pathways that underlie adaptation of the organism to chang-ing conditions of growth; the identification of co-ordinately regulated genes; the regulatory circuits and signal transduction systems that mediate the adaptive response; and temporal features of developmental programmes. The study of bacterial pathogenesis by microarray expression profiling poses special challenges and opportunities. Although the

technical hurdles are many, obtaining expression profiles of an organism growing in tissue will probably reveal strategies for growth and survival in the host's microenvironment. Identifying these colonization strategies and their cognate expression patterns involves a 'deconstruction' process that combines bioinformatics analysis and in vitro DNA array experimentation.

Cook, H. L., H. E. Mischo, et al. (2004). "The Herpesvirus saimiri small nuclear RNAs recruit AU-rich element-binding proteins but do not alter host AU-rich element-containing mRNA levels in virally transformed T cells." Mol Cell Biol **24**(10): 4522-33.

Herpesvirus saimiri (HVS) encodes seven Sm-class small nuclear RNAs, called HSURs (for Herpesvirus saimiri U RNAs), that are abundantly expressed in HVS-transformed, latently infected marmoset T cells but are of unknown function. HSURs 1, 2, and 5 have highly conserved 5'-end sequences containing the AUUUA pentamer characteristic of AU-rich elements (AREs) that regulate the stability of many host mRNAs, including those encoding most proto-oncogenes and cytokines. To test whether the ARE-containing HSURs act to sequester host proteins that regulate the decay of these mRNAs, we demonstrate their in vivo interaction with the ARE-binding proteins hnRNP D and HuR in HVS-transformed T cells using a new cross-linking assay. Comprehensive Northern and microarray analyses revealed, however, that the levels of endogenous ARE-containing mRNAs are not altered in T cells latently infected with HVS mutants lacking HSURs 1 and 2. HSUR 1 binds the destabilizing ARE-binding protein tristetraprolin induced following activation of HVS-transformed T cells, but even in such stimulated cells, the levels of host ARE-containing mRNAs are not altered by deletion of HSURs 1 and 2. Instead, HSUR 1 itself is degraded by an ARE-dependent pathway in HVS-transformed T cells, suggesting that HVS may take advantage of the host ARE-mediated mRNA decay pathway to regulate HSUR expression. This is the first example of posttranscriptional regulation of the expression of an Sm small nuclear RNA.

Cook, S. A., T. Matsui, et al. (2002). "Transcriptional effects of chronic Akt activation in the heart." J Biol Chem **277**(25): 22528-33.

Akt activation reduces cardiomyocyte death and induces cardiac hypertrophy. To help identify effector mechanisms, gene expression profiles in hearts from transgenic mice with cardiac-specific expression of activated Akt (myr-Akt) were compared with littermate controls. 40 genes were identified as differentially expressed. Quantitative reverse transcription-PCR confirmed qualitative results of transcript profiling for 9 of 10 genes examined, however, there were notable quantitative discrepancies between the quantitative reverse transcription-PCR and microarray data sets. Interestingly Akt induced significant up-regulation of insulin-like growth factor-binding protein-5 (IGFBP-5), which could contribute to its anti-apoptotic effects in the heart. In addition, Akt-mediated down-regulation of peroxisome proliferator-activated receptor (PPAR) gamma coactivator-1 (PGC-1) and PPAR-alpha may shift myocytes toward glycolytic metabolism shown to preserve cardiomyocyte function and survival during transient ischemia. IGFBP-5 transcripts also increased after adenoviral gene transfer of myr-Akt to cultured cardiomyocytes, suggesting that this represents a direct effect of Akt activation. In contrast, substantial induction of growth differentiation factor-8 (GDF-8), a highly conserved inhibitor of skeletal muscle growth, was observed in transgenic hearts but not after acute Akt activation in vitro, suggesting that GDF-8 induction may represent a secondary effect perhaps related to the cardiac hypertrophy seen in these mice. Thus, microarray analysis reveals previously unappreciated Akt regulation of genes that could contribute to the effects of Akt on cardiomyocyte survival, metabolism, and growth.

Cooper, K. L. and R. V. Goering (2003). "Development of a universal probe for electronic microarray and its application in characterization of the Staphylococcus aureus polC gene." J Mol Diagn **5**(1): 28-33.

Electronic microarray technology is an exceptionally accurate and effective technique for detecting and defining single nucleotide polymorphisms (SNPs) in DNA sequences. Target oligonucleotides are electronically addressed to a gel matrix containing streptavidin to which biotinylated

polymerase chain reaction (PCR) amplicons are bound. Typically, a fluorescent-labeled reporter oligonucleotide specific for each locus of interest is hybridized and reported. We detail the development of a universal reporter system to replace the standard method that is used to detect many different sequences accurately. The universal reporter eliminates the need to synthesize specific labeled reporters for each SNP sequence thereby dramatically reducing the cost and time required for assay development. The feasibility of this approach was demonstrated by successfully analyzing eight SNPs distributed within a highly variable 1-kb region of the polC gene from six isolates of Staphylococcus aureus.

Costa de Oliveira, R., G. M. Yanai, et al. (2002). "Competitive hybridization on spotted microarrays as a tool to conduct comparative genomic analyses of Xylella fastidiosa strains." FEMS Microbiol Lett **216**(1): 15-21.

Xylella fastidiosa strains are responsible for several plant diseases and since such isolates display a broad host range and complex biological behavior, genomic comparisons employing microarray hybridizations may provide an effective method to compare them. Thus, we performed a thorough validation of this type of approach using two recently sequenced strains of this phytopathogen. By matching microarray hybridization results to direct sequence comparisons, we were able to establish precise cutoff ratios for common and exclusive sequences, allowing the identification of exclusive genes involved in important biological traits. This validation will enable the use of microarray-based comparisons across a wide variety of microorganisms

Coussens, P. M., C. J. Colvin, et al. (2002). "Gene expression profiling of peripheral blood mononuclear cells from cattle infected with Mycobacterium paratuberculosis." Infect Immun **70**(10): 5494-502.

A bovine-specific cDNA microarray system containing 721 unique leukocyte expressed sequence tags (ESTs) and amplicons representing known genes was used to compare gene expression profiles of peripheral blood mononuclear cells (PBMCs) from clinical and subclinical Johne's disease-positive Holstein cows (n = 2 per group). Stimulation of PBMCs from clinically infected cows with Mycobacterium paratuberculosis tended to decrease expression of 83 genes (fold change, >1.5). Of these 83 genes, 16 displayed significant down regulation across both clinical cows ($P < 0.1$), including genes encoding microspherule protein 1, fibroblast growth factor, and the Lyn B protein kinase. Only eight genes from PBMCs of clinically infected cows exhibited a modest up regulation following stimulation with M. paratuberculosis, including those encoding bovine CD40L, gamma interferon, interleukin-10 (IL-10), and tissue inhibitor of matrix metalloproteinases (TIMP) 4. In contrast, stimulation of PBMCs from subclinically infected cows with M. paratuberculosis tended to up regulate expression of 71 genes representing 68 unique transcripts. Of these, 11 genes showed significant up regulation (fold change, >1.5; $P < 0.1$) across both animals, including those encoding bovine CD40L, several matrix metalloproteinases, and SPARC (secreted protein, acidic and rich in cystine). Repression of gene expression was also observed in PBMCs from the subclinical cows, with 16 genes being significantly down regulated (fold change, >1.5; $P < 0.1$) across both animals, including those encoding the bovine orthologs of cytochrome oxidase subunit III, IL-1 receptor type I, and fibrinogen-like 2 protein. Only one clone, representing an unknown bovine EST, was similarly down regulated in PBMCs from both the clinical and subclinical cows. Thus, the most prominent change induced by exposure of PBMCs from clinical cows to M. paratuberculosis in vitro tended to be repression of gene expression, while changes in similarly treated PBMCs from subclinical cows was balanced between gene activation and repression. Comparison of gene expression profiles between PBMCs from clinical and uninfected (control) cows stimulated with the general mitogen concanavalin A were highly similar (overall $r = 0.84$), suggesting that M. paratuberculosis-induced gene repression in clinically infected cow PBMCs was not due to a general failure of the immune response in these animals.

Cummings, C. A., M. M. Brinig, et al. (2004). "Bordetella species are distinguished by patterns of substantial gene loss and host adaptation." J Bacteriol **186**(5): 1484-92.

Pathogens of the bacterial genus Bordetella cause respiratory disease in humans and animals. Although virulence and host specificity vary across the genus, the genetic determinants of this diversity remain unidentified. To identify genes that may underlie key phenotypic differences between these species and clarify their evolutionary relationships, we performed a comparative analysis of genome content in 42 Bordetella strains by hybridization of genomic DNA to a microarray representing the genomes of three Bordetella species and by subtractive hybridization. Here we show that B. pertussis and B. parapertussis are predominantly differentiated from B. bronchiseptica by large, species-specific regions of difference, many of which encode or direct synthesis of surface structures, including lipopolysaccharide O antigen, which may be important determinants of host specificity. The species also exhibit sequence diversity at a number of surface protein-encoding loci, including the fimbrial major subunit gene, fim2. Gene loss, rather than gene acquisition, accompanied by the proliferation of transposons, has played a fundamental role in the evolution of the pathogenic bordetellae and may represent a conserved evolutionary mechanism among other groups of microbial pathogens.

Dagkessamanskaia, A., M. Moscoso, et al. (2004). "Interconnection of competence, stress and CiaR regulons in Streptococcus pneumoniae: competence triggers stationary phase autolysis of ciaR mutant cells." Mol Microbiol **51**(4): 1071-86.

Of the 13 two-component signal transduction systems (TCS) identified in Streptococcus pneumoniae, two, ComDE and CiaRH, are known to affect competence for natural genetic transformation. ComD and ComE act together with the comC-encoded competence-stimulating peptide (CSP) and with ComAB, the CSP-dedicated exporter, to co-ordinate activation of genes required for differentiation to competence. Several lines of evidence suggest that the CiaRH TCS and competence regulation are interconnected, including the observation that inactivation of the CiaR response regulator derepresses competence. However, the nature of the interconnection remains poorly understood. Interpretation of previous transcriptome analyses of ciaR mutants was complicated by competence derepression in the mutants. To circumvent this problem, we have used microarray analysis to investigate the transition from non-competence to competence in a comC-null wild-type strain and its ciaR derivative after the addition of CSP. This study increased the number of known CSP-induced genes from approximately 47 to 105 and revealed approximately 42 genes with reduced expression in competent cells. Induction of the CiaR regulon, as well as the entire HrcA and part of the CtsR stress response regulons, was observed in wild-type competent cells. Enhanced induction of stress response genes was detected in ciaR competent cells. In line with these observations, CSP was demonstrated to trigger growth arrest and stationary phase autolysis in ciaR cells. Taken together, these data strongly suggest that differentiation to competence imposes a temporary stress on cells, and that the CiaRH TCS is required for the cells to exit normally from the competent state.

Dahan, S., S. Knutton, et al. (2004). "Transcriptome of enterohemorrhagic Escherichia coli O157 adhering to eukaryotic plasma membranes." Infect Immun **72**(9): 5452-9.

Using a DNA microarray, we determined changes in enterohemorrhagic Escherichia coli O157:H7 gene expression during binding to plasma membranes. Analysis of the complete transcriptomes of the bound bacteria revealed increased levels of stress-associated mRNAs and decreased levels of mRNA encoding proteins involved in translation and type III secretion.

Dahl, J. L., C. N. Kraus, et al. (2003). "The role of RelMtb-mediated adaptation to stationary phase in long-term persistence of Mycobacterium tuberculosis in mice." Proc Natl Acad Sci U S A **100**(17): 10026-31.

Long-term survival of nonreplicating Mycobacterium tuberculosis (Mtb) is ensured by the coordinated shutdown of active metabolism through a broad transcriptional program called the stringent response. In Mtb, this response is initiated by the enzymatic action of RelMtb and deletion of relMtb produces a strain (H37RvDeltarelMtb) severely compromised in the maintenance of long-term viability.

Although aerosol inoculation of mice with H37RvDeltarelMtb results in normal initial bacterial growth and containment, the ability of this strain to sustain chronic infection is severely impaired. Significant histopathologic differences were noted in lungs and spleens of mice infected with H37RvDeltarelMtb compared with controls throughout the course of the infection. Microarray analysis revealed that H37RvDeltarelMtb suffers from a generalized alteration of the transcriptional apparatus, as well as specific changes in the expression of virulence factors, cell-wall biosynthetic enzymes, heat shock proteins, and secreted antigens that may alter immune recognition of the recombinant organism. Thus, RelMtb is critical for the successful establishment of persistent infection in mice by altering the expression of antigenic and enzymatic factors that may contribute to successful latent infection.

Dasgupta, N., M. C. Wolfgang, et al. (2003). "A four-tiered transcriptional regulatory circuit controls flagellar biogenesis in Pseudomonas aeruginosa." Mol Microbiol **50**(3): 809-24.

The single polar flagellum of Pseudomonas aeruginosa is an important virulence and colonization factor of this opportunistic pathogen. In this study, the annotation of the genes belonging to the fla regulon was updated and their organization was analysed in strains PAK and PAO1, representative type-a and type-b strains of P. aeruginosa respectively. The flagellar genes are clustered in three non-contiguous regions of the chromosome. A polymorphic locus flanked by flgJ and fleQ in Region I contains a glycosylation island in PAK. The expression and ordered assembly of the complex multicomponent flagellum is intricately regulated. Dedicated flagellar genes fleQ, fleS, fleR, fliA, flgM and fleN encode proteins that participate in the regulation of the flagellar transcriptional circuit. In addition, expression of the flagellum is coordinately regulated with other P. aeruginosa virulence factors by the alternative sigma factor sigma54, encoded by rpoN. In order to gain insight into the hierarchical regulation of flagellar genes, deletion mutations were constructed in fleQ, fleR, fliA and rpoN. The transcriptional impact of these mutations was examined by transcriptional profiling using a P. aeruginosa whole genome microarray. Analysis of the transcriptomes generated for each of these mutants indicates a four-tiered (Classes I-IV) hierarchy of transcriptional regulation. Class I genes are constitutively expressed and include the transcriptional regulator fleQ and the alternative sigma factor fliA (sigma28). Class II genes including fleSR, encoding a two-component regulatory system require FleQ and RpoN (sigma54) for their transcriptional activation. Class III genes are positively regulated by the activated response regulator FleR in concert with RpoN. The transcription of Class IV genes is dependent on the availability of free FliA following the export of the FliA specific antisigma factor FlgM through the basal body rod-hook structure (assembled from Class II and III gene products). Two previously uncharacterized genes, which are coordinately regulated with known flagellar genes have been identified by genome-wide analysis and their role in flagellar biogenesis was analysed.

David, C. A., T. Middleton, et al. (2002). "Microarray compound screening (microARCS) to identify inhibitors of HIV integrase." J Biomol Screen **7**(3): 259-66.

A novel high-throughput strand transfer assay has been developed, using Microarray Compound Screening (microARCS) technology, to identify inhibitors of human immunodeficiency virus (HIV) integrase. This technology utilizes agarose matrices to introduce a majority of the reagents throughout the assay. Integration of biotinylated donor DNA with fluorescein isothiocyanate (FITC)-labeled target DNA occurs on a SAM membrane in the presence of integrase. An anti-FITC antibody conjugated to alkaline phosphatase (AP) was used to do an enzyme-linked immunosorbent assay with the SAM. An agarose gel containing AttoPhos, a substrate of AP, was used for detection of the integrase reactions on the SAM. For detection, the AttoPhos gel was separated from the SAM after incubation and then the gel was imaged using an Eagle Eye II closed-circuit device camera system. Potential integrase inhibitors appear as dark spots on the gel image. A library of approximately 250,000 compounds was screened using this HIV integrase strand transfer assay in microARCS format. Compounds from different structural classes were identified in this assay as novel integrase inhibitors.

Dawe, A. L., V. C. McMains, et al. (2003). "An ordered collection of expressed sequences from Cryphonectria parasitica and evidence of genomic microsynteny with Neurospora crassa and Magnaporthe grisea." Microbiology **149**(Pt 9): 2373-84.

Cryphonectria parasitica, the causative agent of chestnut blight, has proven to be a tractable experimental system for studying fungal pathogenesis. Moreover, the development of infectious cDNA clones of C. parasitica hypoviruses, capable of attenuating fungal virulence, has provided the opportunity to examine molecular aspects of fungal plant pathogenesis in the context of biological control. In order to establish a genomic base for future studies of C. parasitica, the authors have analysed a collection of expressed sequences. A mixed cDNA library was prepared from RNA isolated from wild-type (virus-free) and hypovirus-infected C. parasitica strains. Plasmid DNA was recovered from individual transformants and sequenced from the 5' end of the insert. Contig analysis of the collected sequences revealed that they represented approximately 2200 individual ORFs. An assessment of functional diversity present in this collection was achieved by using the BLAST software utilities and the NCBI protein database. Candidate genes were identified with significant potential relevance to C. parasitica growth, development, pathogenesis and vegetative incompatibility. Additional investigations of a 12.9 kbp genomic region revealed microsynteny between C. parasitica and both Neurospora crassa and Magnaporthe grisea, two closely related fungi. These data represent the largest collection of sequence information currently available for C. parasitica and are now forming the basis of further studies using microarray analyses to determine global changes in transcription that occur in response to hypovirus infection.

De Hoon, M. J., S. Imoto, et al. (2002). "Statistical analysis of a small set of time-ordered gene expression data using linear splines." Bioinformatics **18**(11): 1477-85.

MOTIVATION: Recently, the temporal response of genes to changes in their environment has been investigated using cDNA microarray technology by measuring the gene expression levels at a small number of time points. Conventional techniques for time series analysis are not suitable for such a short series of time-ordered data. The analysis of gene expression data has therefore usually been limited to a fold-change analysis, instead of a systematic statistical approach. METHODS: We use the maximum likelihood method together with Akaike's Information Criterion to fit linear splines to a small set of time-ordered gene expression data in order to infer statistically meaningful information from the measurements. The significance of measured gene expression data is assessed using Student's t-test. RESULTS: Previous gene expression measurements of the cyanobacterium Synechocystis sp. PCC6803 were reanalyzed using linear splines. The temporal response was identified of many genes that had been missed by a fold-change analysis. Based on our statistical analysis, we found that about four gene expression measurements or more are needed at each time point.

de la Fuente, C., F. Santiago, et al. (2002). "Gene expression profile of HIV-1 Tat expressing cells: a close interplay between proliferative and differentiation signals." BMC Biochem **3**(1): 14.

BACKGROUND: Expression profiling holds great promise for rapid host genome functional analysis. It is plausible that host expression profiling in an infection could serve as a universal phenotype in virally infected cells. Here, we describe the effect of one of the most critical viral activators, Tat, in HIV-1 infected and Tat expressing cells. We utilized microarray analysis from uninfected, latently HIV-1 infected cells, as well as cells that express Tat, to decipher some of the cellular changes associated with this viral activator. RESULTS: Utilizing uninfected, HIV-1 latently infected cells, and Tat expressing cells, we observed that most of the cellular host genes in Tat expressing cells were down-regulated. The down-regulation in Tat expressing cells is most apparent on cellular receptors that have intrinsic receptor tyrosine kinase (RTK) activity and signal transduction members that mediate RTK function, including Ras-Raf-MEK pathway. Co-activators of transcription, such as p300/CBP and SRC-1, which mediate gene expression related to hormone receptor genes, were also found to be down-regulated. Down-regulation of receptors may allow latent HIV-1 infected cells to either hide from the immune system or avoid extracellular differentiation signals. Some of the genes that were up-regulated included co-receptors

for HIV-1 entry, translation machinery, and cell cycle regulatory proteins. CONCLUSIONS: We have demonstrated, through a microarray approach, that HIV-1 Tat is able to regulate many cellular genes that are involved in cell signaling, translation and ultimately control the host proliferative and differentiation signals.

de la Fuente, C., L. Wang, et al. (2003). "Paradoxical effects of a stress signal on pro- and anti-apoptotic machinery in HTLV-1 Tax expressing cells." Mol Cell Biochem **245**(1-2): 99-113.

Adult T-cell leukemia (ATL) and HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) are associated with Human T-cell lymphotropic virus type 1 (HTLV-1) infection. The viral transactivator, Tax is able to mediate the cell cycle progression by targeting key regulators of the cell cycle such as p21/waf1, p16/ink4a, p53, cyclins D1-3/cdk complexes, and the mitotic spindle checkpoint MAD apparatus, thereby deregulating cellular DNA damage and checkpoint control. Genome expression profiling of infected cells exemplified by the development of DNA microarrays represents a major advance in genome-wide functional analysis. Utilizing cDNA microarray analysis, we have observed an apparent opposing and paradoxical regulatory network of host cell gene expression upon the introduction of DNA damage stress signal. We find the apparent induction of cell cycle inhibitors, and pro- as well as anti-apoptotic gene expression is directly linked to whether cells are at either G1, S, or G2/M phases of the cell cycle. Specifically, a G1/S block is induced by p21/waf1 and p16/ink4a, while pro-apoptotic expression at S, and G2/M is associated with caspase activation, and anti-apoptotic gene expression is associated with up regulation of Bcl-2 family member, namely bfl-1 gene. Therefore, the microarray results indicating expression of both pro- and anti-apoptotic genes could easily be explained by the particular stage of the cell cycle. Mechanism and the functional outcome of induction for both pathways are discussed.

de Saizieu, A., C. Gardes, et al. (2000). "Microarray-based identification of a novel Streptococcus pneumoniae regulon controlled by an autoinduced peptide." J Bacteriol **182**(17): 4696-703.

We have identified in the Streptococcus pneumoniae genome sequence a two-component system (TCS13, Blp [bacteriocin-like peptide]) which is closely related to quorum-sensing systems regulating cell density-dependent phenotypes such as the development of genetic competence or the production of antimicrobial peptides in lactic acid bacteria. In this study we present evidence that TCS13 is a peptide-sensing system that controls a regulon including genes encoding Blps. Downstream of the Blp TCS (BlpH R) we identified open reading frames (blpAB) that have the potential to encode an ABC transporter that is homologous to the ComA/B export system for the competence-stimulating peptide ComC. The putative translation product of blpC, a small gene located downstream of blpAB, has a leader peptide with a Gly-Gly motif. This leader peptide is typical of precursors processed by this family of transporters. Microarray-based expression profiling showed that a synthetic oligopeptide corresponding to the processed form of BlpC (BlpC*) induces a distinct set of 16 genes. The changes in the expression profile elicited by synthetic BlpC* depend on BlpH since insertional inactivation of its corresponding gene abolishes differential gene induction. Comparison of the promoter regions of the blp genes disclosed a conserved sequence element formed by two imperfect direct repeats upstream of extended -10 promoter elements. We propose that BlpH is the sensor for BlpC* and the conserved sequence element is a recognition sequence for the BlpR response regulator.

de Souza, A. A., M. A. Takita, et al. (2003). "Analysis of gene expression in two growth states of Xylella fastidiosa and its relationship with pathogenicity." Mol Plant Microbe Interact **16**(10): 867-75.

Xylella fastidiosa is a plant pathogen responsible for diseases of economically important crops. Although there is considerable disagreement about its mechanism of pathogenicity, blockage of the vessels is one of the most accepted hypotheses. Loss of virulence by this bacterium was observed after serial passages in axenic culture. To confirm the loss of pathogenicity of X. fastidiosa, the causing agent of citrus variegated chlorosis (CVC), freshly-isolated bacteria (first passage [FP] condition) as well as

bacteria obtained after 46 passages in axenic culture (several passage [SP] condition) were inoculated into sweet orange and periwinkle plants. Using real time quantitative polymerase chain reaction, we verified that the colonization of FP cells was more efficient for both hosts. The sequence of the complete X. fastidiosa genome allowed the construction of a DNA microarray that was used to investigate the total changes in gene expression associated with the FP condition. Most genes found to be induced in the FP condition were associated with adhesion and probably with adaptation to the host environment. This report represents the first study of the transcriptome of this pathogen, which has recently gained more importance, since the genome of several strains has been either partially or entirely sequenced.

de Veer, M. J., M. Holko, et al. (2001). "Functional classification of interferon-stimulated genes identified using microarrays." J Leukoc Biol **69**(6): 912-20.

Interferons (IFNs) are a family of multifunctional cytokines that activate transcription of subsets of genes. The gene products induced by IFNs are responsible for IFN antiviral, antiproliferative, and immunomodulatory properties. To obtain a more comprehensive list and a better understanding of the genes regulated by IFNs, we compiled data from many experiments, using two different microarray formats. The combined data sets identified >300 IFN-stimulated genes (ISGs). To provide new insight into IFN-induced cellular phenotypes, we assigned these ISGs to functional categories. The data are accessible on the World Wide Web at http://www.lerner.ccf.org/labs/williams/, including functional categories and individual genes listed in a searchable database. The entries are linked to GenBank and Unigene sequence information and other resources. The goal is to eventually compile a comprehensive list of all ISGs. Recognition of the functions of the ISGs and their specific roles in the biological effects of IFNs is leading to a greater appreciation of the many facets of these intriguing and essential cytokines. This review focuses on the functions of the ISGs identified by analyzing the microarray data and focuses particularly on new insights into the protein kinase RNA-regulated (PRKR) protein, which have been made possible with the availability of PRKR-null mice.

Degrave, W. M., S. Melville, et al. (2001). "Parasite genome initiatives." Int J Parasitol **31**(5-6): 532-6.

During 1993-1994, scientists from developing and developed countries planned and initiated a number of parasite genome projects and several consortiums for the mapping and sequencing of these medium-sized genomes were established, often based on already ongoing scientific collaborations. Financial and other support came from WHO/TDR, Wellcome Trust and other funding agencies. Thus, the genomes of Plasmodium falciparum, Schistosoma mansoni, Trypanosoma cruzi, Leishmania major, Trypanosoma brucei, Brugia malayi and other pathogenic nematodes are now under study. From an initial phase of network formation, mapping efforts and resource building (EST, GSS, phage, cosmid, BAC and YAC library constructions), sequencing was initiated in gene discovery projects but soon also on a small chromosome, and now on a fully fledged genome scale. Proteomics, functional analysis, genetic manipulation and microarray analysis are ongoing to different degrees in the respective genome initiatives, and as the funding for the whole genome sequencing becomes secured, most of the participating laboratories, apart from larger sequencing centres, become oriented to post-genomics. Bioinformatics networks are being expanded, including in developing countries, for data mining, annotation and in-depth analysis.

Del Monte, F., R. Dalal, et al. (2004). "Transcriptional changes following restoration of SERCA2a levels in failing rat hearts." Faseb J **18**(12): 1474-6.

Heart failure is characterized at the cellular level by impaired contractility and abnormal Ca2+ homeostasis. We have previously shown that restoration of a key enzyme that controls intracellular Ca(2+) handling, the sarcoplasmic reticulum Ca2+ ATPase (SERCA2a), induces functional improvement in heart failure. We used high-density oligonucleotide arrays to explore the effects of gene transfer of SERCA2a on genetic reprogramming in a model of heart failure. A total of 1,300 transcripts were identified to be unmodified by the effect of virus alone. Of those, 251 transcripts were found to be up- or

down-regulated upon failure. A total of 51 transcripts which were either up--(27) or down--(24) regulated in heart failure were normalized to the nonfailing levels by the restoration of SERCA2a by gene transfer. The microarray analysis identified new genes following SERCA2a restoration in heart failure, which will give us insights into their role in the normalization of multiple pathways within the failing cell.

DeLisa, M. P., C. F. Wu, et al. (2001). "DNA microarray-based identification of genes controlled by autoinducer 2-stimulated quorum sensing in Escherichia coli." J Bacteriol **183**(18): 5239-47.

Bacterial cell-to-cell communication facilitates coordinated expression of specific genes in a growth rate-II and cell density-dependent manner, a process known as quorum sensing. While the discovery of a diffusible Escherichia coli signaling pheromone, termed autoinducer 2 (AI-2), has been made along with several quorum sensing genes, the overall number and coordination of genes controlled by quorum sensing through the AI-2 signal has not been studied systematically. We investigated global changes in mRNA abundance elicited by the AI-2 signaling molecule through the use of a luxS mutant that was unable to synthesize AI-2. Remarkably, 242 genes, comprising ca. 5.6% of the E. coli genome, exhibited significant transcriptional changes (either induction or repression) in response to a 300-fold AI-2 signaling differential, with many of the identified genes displaying high induction levels (more than fivefold). Significant induction of ygeV, a putative sigma(54)-dependent transcriptional activator, and yhbH, a sigma(54) modulating protein, suggests sigma(54) may be involved in E. coli quorum sensing.

Denef, V. J., J. Park, et al. (2004). "Biphenyl and benzoate metabolism in a genomic context: outlining genome-wide metabolic networks in Burkholderia xenovorans LB400." Appl Environ Microbiol **70**(8): 4961-70.

We designed and successfully implemented the use of in situ-synthesized 45-mer oligonucleotide DNA microarrays (XeoChips) for genome-wide expression profiling of Burkholderia xenovorans LB400, which is among the best aerobic polychlorinated biphenyl degraders known so far. We conducted differential gene expression profiling during exponential growth on succinate, benzoate, and biphenyl as sole carbon sources and investigated the transcriptome of early-stationary-phase cells grown on biphenyl. Based on these experiments, we outlined metabolic pathways and summarized other cellular functions in the organism relevant for biphenyl and benzoate degradation. All genes previously identified as being directly involved in biphenyl degradation were up-regulated when cells were grown on biphenyl compared to expression in succinate-grown cells. For benzoate degradation, however, genes for an aerobic coenzyme A activation pathway were up-regulated in biphenyl-grown cells, while the pathway for benzoate degradation via hydroxylation was up-regulated in benzoate-grown cells. The early-stationary-phase biphenyl-grown cells showed similar expression of biphenyl pathway genes, but a surprising up-regulation of C(1) metabolic pathway genes was observed. The microarray results were validated by quantitative reverse transcription PCR with a subset of genes of interest. The XeoChips showed a chip-to-chip variation of 13.9%, compared to the 21.6% variation for spotted oligonucleotide microarrays, which is less variation than that typically reported for PCR product microarrays.

Dennis, G., Jr., B. T. Sherman, et al. (2003). "DAVID: Database for Annotation, Visualization, and Integrated Discovery." Genome Biol **4**(5): P3.

BACKGROUND: Functional annotation of differentially expressed genes is a necessary and critical step in the analysis of microarray data. The distributed nature of biological knowledge frequently requires researchers to navigate through numerous web-accessible databases gathering information one gene at a time. A more judicious approach is to provide query-based access to an integrated database that disseminates biologically rich information across large datasets and displays graphic summaries of functional information. RESULTS: Database for Annotation, Visualization, and Integrated Discovery (DAVID; http://www.david.niaid.nih.gov) addresses this need via four web-based analysis modules: 1) Annotation Tool - rapidly appends descriptive data from several public databases to lists of genes; 2) GoCharts - assigns genes to Gene Ontology functional categories based on user selected classifications

and term specificity level; 3) KeggCharts - assigns genes to KEGG metabolic processes and enables users to view genes in the context of biochemical pathway maps; and 4) DomainCharts - groups genes according to PFAM conserved protein domains. CONCLUSIONS: Analysis results and graphical displays remain dynamically linked to primary data and external data repositories, thereby furnishing in-depth as well as broad-based data coverage. The functionality provided by DAVID accelerates the analysis of genome-scale datasets by facilitating the transition from data collection to biological meaning.

Deplancke, B. and H. R. Gaskins (2003). "Hydrogen sulfide induces serum-independent cell cycle entry in nontransformed rat intestinal epithelial cells." Faseb J **17**(10): 1310-2.

Hydrogen sulfide (H2S), produced by commensal sulfate-reducing bacteria, is an environmental insult that potentially contributes to chronic intestinal epithelial disorders. We tested the hypothesis that exposure of nontransformed intestinal epithelial cells (IEC-18) to the reducing agent sodium hydrogen sulfide (NaHS) activates molecular pathways that underlie epithelial hyperplasia, a phenotype common to both ulcerative colitis (UC) and colorectal cancer. Exposure of IEC-18 cells to NaHS rapidly increased the NADPH/NADP ratio, reduced the intracellular redox environment, and inhibited mitochondrial respiratory activity. The addition of 0.2-5 mM NaHS for 4 h increased the IEC-18 proliferative cell fraction ($P<0.05$), as evidenced by analysis of the cell cycle and proliferating cell nuclear antigen expression, while apoptosis occurred only at the highest concentration of NaHS. Thirty minutes of NaHS exposure increased ($P<0.05$) c-Jun mRNA concentrations, consistent with the observed activation of mitogen activated protein kinases (MAPK). Microarray analysis confirmed an increase ($P<0.05$) in MAPK-mediated proliferative activity, likely reflecting the reduced redox environment of NaHS-treated cells. These data identify functional pathways by which H2S may initiate epithelial dysregulation and thereby contribute to UC or colorectal cancer. Thus, it becomes crucial to understand how genetic background may affect epithelial responsiveness to this bacterial-derived environmental insult.

DeSantis, T. Z., I. Dubosarskiy, et al. (2003). "Comprehensive aligned sequence construction for automated design of effective probes (CASCADE-P) using 16S rDNA." Bioinformatics **19**(12): 1461-8.

MOTIVATION: Prokaryotic organisms have been identified utilizing the sequence variation of the 16S rRNA gene. Variations steer the design of DNA probes for the detection of taxonomic groups or specific organisms. The long-term goal of our project is to create probe arrays capable of identifying 16S rDNA sequences in unknown samples. This necessitated the authentication, categorization and alignment of the >75 000 publicly available '16S' sequences. Preferably, the entire process should be computationally administrated so the aligned collection could periodically absorb 16S rDNA sequences from the public records. A complete multiple sequence alignment would provide a foundation for computational probe selection and facilitates microbial taxonomy and phylogeny. RESULTS: Here we report the alignment and similarity clustering of 62 662 16S rDNA sequences and an approach for designing effective probes for each cluster. A novel alignment compression algorithm, NAST (Nearest Alignment Space Termination), was designed to produce the uniform multiple sequence alignment referred to as the prokMSA. From the prokMSA, 9020 Operational Taxonomic Units (OTUs) were found based on transitive sequence similarities. An automated approach to probe design was straightforward using the prokMSA clustered into OTUs. As a test case, multiple probes were computationally picked for each of the 27 OTUs that were identified within the Staphylococcus Group. The probes were incorporated into a customized microarray and were able to correctly categorize Staphylococcus aureus and Bacillus anthracis into their correct OTUs. Although a successful probe picking strategy is outlined, the main focus of creating the prokMSA was to provide a comprehensive, categorized, updateable 16S rDNA collection useful as a foundation for any probe selection algorithm.

Dessens, J. T., G. Margos, et al. (2000). "Identification of differentially regulated genes of Plasmodium by suppression subtractive hybridization." Parasitol Today **16**(8): 354-6.

Plasmodium, the causative agent of malaria, has many morphologically and functionally distinct

developmental stages. In the mosquito host alone, there are five transitions during the development of a gametocyte into a sporozoite. Determining which genes are expressed at the different developmental stages is vital to our understanding of the parasite. There are a growing number of techniques designed to study gene expression, including microarray. Here, Johannes Dessens, Gabrielle Margos, Maria del Carmen Rodriguez and Robert Sinden describe a novel method: suppression subtractive hybridization (SSH) and its successful application in obtaining mosquito midgut stage-specific genes of Plasmodium.

Dessus-Babus, S., S. T. Knight, et al. (2000). "Chlamydial infection of polarized HeLa cells induces PMN chemotaxis but the cytokine profile varies between disseminating and non-disseminating strains." Cell Microbiol **2**(4): 317-27.

While genital infections caused by Chlamydia trachomatis are generally asymptomatic, the density and pattern of inflammation varies considerably. The purpose of this study was to try to dissect the signalling in chlamydiae-infected epithelial cells that triggers innate responses and regulates polymorphonuclear neutrophil (PMN) chemotaxis. Polarized endocervical epithelial HeLa cells, grown in commercial inserts, were inoculated either with the non-disseminating (luminal) serovar E or the disseminating serovar L2. At 12-48 h after infection, the chambers were used in a quantitative chemotaxis assay, and cytokine production by infected cells was examined using cDNA microarray technology and confirmed by enzyme-linked immunosorbent assay (ELISA). Infection of HeLa cells with C. trachomatis E or L2 induced a strong and similar PMN chemotactic response, but larger amounts of interleukin (IL)-8 and IL-11 were released after infection with serovar L2. IL-6 was also produced in modest amounts after infection with either strain, but no IL-1alpha or tumour necrosis factor (TNF)-alpha was detected in any of the culture supernatants tested. IL-11 did not appear to influence the PMN response to chlamydial infection, but secretion of large amounts of this anti-inflammatory cytokine, mainly active on macrophages, in the very early stages of the infection may allow C. trachomatis to escape some innate defences to establish infection.

Detweiler, C. S., D. B. Cunanan, et al. (2001). "Host microarray analysis reveals a role for the Salmonella response regulator phoP in human macrophage cell death." Proc Natl Acad Sci U S A **98**(10): 5850-5.

Bacterial pathogens manipulate host cells to promote pathogen survival and dissemination. We used a 22,571 human cDNA microarray to identify host pathways that are affected by the Salmonella enterica subspecies typhimurium phoP gene, a transcription factor required for virulence, by comparing the expression profiles of human monocytic tissue culture cells infected with either the wild-type bacteria or a phoPTn10 mutant strain. Both wild-type and phoPTn10 bacteria induced a common set of genes, many of which are proinflammatory. Differentially expressed genes included those that affect host cell death, suggesting that the phoP regulatory system controls bacterial genes that alter macrophage survival. Subsequent experiments showed that the phoPTn10 mutant strain is defective for killing both cultured and primary human macrophages but is able to replicate intracellularly. These experiments indicate that phoP plays a role in Salmonella-induced human macrophage cell death.

Dhar, A. K., A. Dettori, et al. (2003). "Identification of differentially expressed genes in shrimp (Penaeus stylirostris) infected with White spot syndrome virus by cDNA microarrays." Arch Virol **148**(12): 2381-96.

White spot syndrome virus (WSSV) is currently the most important viral pathogen infecting penaeid shrimp worldwide. Although considerable progress has been made in characterizing the WSSV genome and developing detection methods, information pertaining to host genes involved in WSSV pathogenesis is limited. We examined the potential of cDNA microarray analysis to study gene expression in WSSV-infected shrimp. Shrimp cDNAs were printed as low-density arrays on glass slides and were hybridized with Cy3/Cy5 labeled probes derived from RNA isolated from healthy and WSSV-infected shrimp. Genes that code for proteins that are relevant to crustacean immunity, structural proteins, as well as proteins of unknown function were among those whose mRNA expression was altered upon

WSSV infection. To validate the microarray data, the temporal expression of three differentially expressed genes, an immune gene (C-type lectin-1), a structural gene (40S ribosomal protein), and a gene involved in lipid metabolism (fatty acid binding protein) was measured in healthy and WSSV-infected shrimp by real-time RT-PCR. The data suggest that WSSV infection alters the expression of a wide array of cellular genes, and provides a framework for further studies aimed at identifying genes whose function may provide insight into the mechanism of WSSV infection in shrimp.

Dharmadi, Y. and R. Gonzalez (2004). "DNA microarrays: experimental issues, data analysis, and application to bacterial systems." Biotechnol Prog **20**(5): 1309-24.

DNA microarrays are currently used to study the transcriptional response of many organisms to genetic and environmental perturbations. Although there is much room for improvement of this technology, its potential has been clearly demonstrated in the past 5 years. The general consensus is that the bottleneck is now located in the processing and analysis of transcriptome data and its use for purposes other than the quantification of changes in gene expression levels. In this article we discuss technological aspects of DNA microarrays, statistical and biological issues pertinent to the design of microarray experiments, and statistical tools for microarray data analysis. A review on applications of DNA microarrays in the study of bacterial systems is presented. Special attention is given to studies in the following areas: (1) bacterial response to environmental changes; (2) gene identification, genome organization, and transcriptional regulation; and (3) genetic and metabolic engineering. Soon, the use of DNA microarray technologies in conjunction with other genome/system-wide analyses (e.g., proteomics, metabolomics, fluxomics, phenomics, etc.) will provide a better assessment of genotype-phenotype relationships in bacteria, which serve as a basis for understanding similar processes in more complex organisms.

Di Pasquale, G., B. L. Davidson, et al. (2003). "Identification of PDGFR as a receptor for AAV-5 transduction." Nat Med **9**(10): 1306-12.

Understanding the process of vector transduction has important implications for the application and optimal use of a vector system for human gene therapy. Recent studies with vectors based on adeno-associated virus type 5 (AAV-5) have shown utility of this vector system in the lung, central nervous system, muscle and eye. To understand the natural tropism of this virus and to identify proteins necessary for AAV-5 transduction, we characterized 43 cell lines as permissive or nonpermissive for AAV-5 transduction and compared the gene expression profiles derived from cDNA microarray analyses of those cell lines. A statistically significant correlation was observed between expression of the platelet-derived growth factor receptor (PDGFR-alpha-polypeptide) and AAV-5 transduction. Subsequent experiments confirmed the role of PDGFR-alpha and PDGFR-beta as receptors for AAV-5. The tropism of AAV-5 in vivo also correlated with the expression pattern of PDGFR-alpha.

Diehl, S., F. Diehl, et al. (2002). "Analysis of stage-specific gene expression in the bloodstream and the procyclic form of Trypanosoma brucei using a genomic DNA-microarray." Mol Biochem Parasitol **123**(2): 115-23.

A microarray comprising 21,024 different PCR products spotted on glass slides was constructed for gene expression studies on Trypanosoma brucei. The arrayed fragments were generated from a T. brucei shotgun clone library, which had been prepared from randomly sheared and size-fractionated genomic DNA. For the identification of stage-specific gene activity, total RNA from in vitro cultures of the human, long slender form and the insect, procyclic form of the parasite was labelled and hybridised to the microarray. Approximately 75% of the genomic fragments produced a signal and about 2% exhibited significant differences between the transcript levels in the bloodstream and procyclic forms. A few results were confirmed by Northern blot analysis or reverse-transcription and PCR. Three hundred differentially regulated clones have been selected for sequencing. So far, of 33 clones that showed about 2-fold or more over-expression in bloodstream forms, 15 contained sequences similar to those of VSG expression sites

and at least six others appeared non-protein-coding. Of 29 procyclic-specific clones, at least eight appeared not to be protein-coding. A surprisingly large proportion of known regulated genes was already identified in this small sample, and some new ones were found, illustrating the utility of genomic arrays.

Diehn, M. and D. A. Relman (2001). "Comparing functional genomic datasets: lessons from DNA microarray analyses of host-pathogen interactions." Curr Opin Microbiol **4**(1): 95-101.

Functional genomic technologies such as high density DNA microarrays allow biologists to study the structure and behavior of thousands of genes in a single experiment. One of the fields in which microarrays have had an increasingly important impact is host-pathogen interactions. Early investigations in this area over the past two years not only emphasize the utility of this approach, but also highlight the stereotyped gene expression responses of different host cells to diverse infectious stimuli, and the potential value of broad dataset comparisons in revealing fundamental features of innate immunity. The comparative analysis of recently published datasets involving human gene expression responses to two bacterial respiratory pathogens illustrates many of these points. Comparisons between these large, highly parallel sets of experimental observations also emphasize important technical and experimental design issues as future challenges.

Dietz, A. B., P. A. Bulur, et al. (2000). "Maturation of human monocyte-derived dendritic cells studied by microarray hybridization." Biochem Biophys Res Commun **275**(3): 731-8.

We compared the transcript profiles of human myeloid immature dendritic (IDC) cells and mature dendritic cells (MDC) by hybridization of cell-derived cDNA to DNA probes immobilized on microarrays. The microarrays contained probes for 4110 known genes. We report maturation-dependent changes in transcription of clusters of differentiation, cytokines, cytokine receptors, chemokines, chemokine receptors, neuropeptides, adhesion molecules, and other genes. We identified 1124 transcripts expressed in IDC and 1556 transcripts expressed in MDC. Maturation increased the levels of 291 transcripts twofold or more and reduced the levels of 78 transcripts to one-half or less than in IDC. We identified a concerted maturation-stage-dependent transcription of the variable chains of the members of the gamma-chain-cytokine receptor family IL-4R, IL-7R, and IL-15R. Also, we found the reversal of the ratio of transcripts for galectin-3 and galectin-9 upon maturation. We identified maturation-dependent changes in the levels of transcripts for numerous genes encoding proteins previously undetected in dendritic cells such as indoleamine 2,3-deoxygenase, Epstein-Barr virus induced protein 3 and kinesin-2. Moreover, MDC transcribed and translated insulin like growth factor-1 receptor, transforming growth factor alpha, and neuropeptide Y.

Ding, H., G. G. Shi, et al. (2003). "Modulation of GdCl3 and Angelica sinensis polysaccharides on differentially expressed genes in liver of hepatic immunological injury mice by cDNA microarray." World J Gastroenterol **9**(5): 1072-6.

AIM: To study the modulating effect of GdCl(3) and Angelica Sinensis polysaccharides (ASP) on differentially expressed genes in liver of hepatic immunological mice by cDNA microarray. METHODS: Hepatic immunological injury was induced by lipopolysaccharide (LPS ip, 0.2 mg/kg(-1)) in bacillus calmetteguerin (BCG ip, 1 mg/kg(-1)) primed mice; A single dose of 20 mg/kg(-1) GdCl(3) was simultaneously pretreated and 30 mg/kg(-1) ASP (ig, qdX7 d) was administrated when the BCG+LPS was primed. The mice were sacrificed at the end of the 7(th) day after ip LPS for 6 h and the liver was removed quickly. The PCR products of 512 genes were spotted onto a chemical material-coated glass plate in array. The DNAs were fixed to the glass plate after series of treatments. The total RNAs were isolated from the liver tissue, and were purified to mRNAs by Oligotex. Both mRNAs from the normal liver tissue and the liver tissue from the mice with hepatic immunological injury or that pretreated with GdCl(3) or ASP were reversely transcribed to cDNAs with the incorporation of fluorescent dUTP to prepare the hybridization probes. The mixed probes were hybridized to the cDNA microarray. After high-stringent washing, the cDNA microarray was scanned for fluorescent signals and showed differences

between the two tissues. RESULTS: Among the 512 target genes, 18 differed in liver tissue of hepatic immunological injury mice, and 6 differed in those pretreated by ASP, 7 differed in those pretreated by GdCl(3). CONCLUSION: cDNA microarray technique is effective in screening the differentially expressed genes between two different kinds of tissue. Further analysis of those obtained genes will be helpful to understand the molecular mechanism of hepatic immunological injury and to study the intervention of drug. Both ASP and GdCl(3) can decrease the number of the differentially expressed genes in liver tissue of mice with hepatic immunological injury.

Dittmar, K. A., E. M. Mobley, et al. (2004). "Exploring the regulation of tRNA distribution on the genomic scale." J Mol Biol **337**(1): 31-47.

Though up to 20% of the total RNA in bacterial cells is tRNA, the regulation of tRNA distribution on the genomic level remains unclear. tRNA distribution is governed by four processes: transcription, processing of precursor tRNA, degradation of precursor tRNA and degradation of mature tRNA. To elucidate the relationship between these processes in the regulation of tRNA production, the relative tRNA distribution was measured using a microarray specifically designed for tRNA. We developed a procedure that selectively labels 3'-CCA-containing RNAs with the fluorophores Cy3 or Cy5. The labeled tRNAs were then hybridized to microarrays printed with complementary DNA probes. The regulation of tRNA distribution in Bacillus subtilis was explored for a wild-type strain and a mutant strain with significantly decreased levels of RNase P, the enzyme required for the 5' maturation of all tRNA. The strains were either grown under a variety of conditions at doubling times ranging from 0.1 to 2.2 doublings per hour to investigate growth-related changes in the tRNA abundance or treated with the transcriptional inhibitor rifampicin to analyze mature tRNA degradation. Our results confirm that transcription and processing contribute significantly to the distribution of the 35 tRNA species in B.subtilis, and suggest a role for the degradation of precursor tRNA. Mature tRNA degradation occurs with little specificity for individual tRNA species and on the hour time-scale, indicating that degradation of mature tRNA plays only a minor role in the regulation of tRNA distribution. Aside from transcription, the final tRNA distribution appears to be derived from a balance between processing and precursor degradation activities.

Dong, H., N. Toyoda, et al. (2002). "Gene expression profile analysis of the mouse liver during bacteria-induced fulminant hepatitis by a cDNA microarray system." Biochem Biophys Res Commun **298**(5): 675-86.

Fulminant hepatic failure (FHF) is a disease characterized by sudden and severe impairment of liver function. To elucidate the mechanism involved in FHF, we adopted a murine model of FHF by administrating mice with heat-killed Propionibacterium acnes (P. acnes), followed by a low dose of lipopolysaccharide (LPS), and analyzed the dynamic change of gene expression profile of the murine liver using an in-house cDNA microarray system which contained most of the cDNAs encoding chemokines/cytokines and their receptors (33 chemokines/21 chemokine receptors, 28 cytokines/35 cytokine receptors) as well as 230 liver related proteins mostly selected by serial analysis of gene expression (SAGE). Among them, 335 genes were found to differ by more than 2-fold in at least one time point comparing with normal liver. Hierarchical cluster analysis revealed that except for a few genes, such as heme oxygenase (HO)-1 and nicotinamide N-methyltransferase (NNMT) of which expression increased, the expression of most of the genes encoding drug metabolizing enzymes decreased with the progress of the disease. The expression of the genes encoding chemokines/cytokines was dramatically changed, such as Mig, IP-10, RANTES, TNF-alpha, and IFN-gamma. In addition, the expression of those that were not previously linked to this murine model was also identified to be changed. These include endogenous IL-18 binding protein (IL-18BP), CXCL16 (the ligand of Bonzo, CXCR6) as well as ESTs. Taken together this study has shown the systemic and comprehensive gene expression profile during FHF and may contribute to better understanding of the mechanism of FHF.

Dong, Y., J. D. Glasner, et al. (2001). "Genomic interspecies microarray hybridization: rapid discovery of three thousand genes in the maize endophyte, Klebsiella pneumoniae 342, by microarray hybridization with Escherichia coli K-12 open reading frames." Appl Environ Microbiol **67**(4): 1911-21.

In an effort to efficiently discover genes in the diazotrophic endophyte of maize, Klebsiella pneumoniae 342, DNA from strain 342 was hybridized to a microarray containing 96% (n = 4,098) of the annotated open reading frames from Escherichia coli K-12. Using a criterion of 55% identity or greater, 3,000 (70%) of the E. coli K-12 open reading frames were also found to be present in strain 342. Approximately 24% (n = 1,030) of the E. coli K-12 open reading frames are absent in strain 342. For 1.6% (n = 68) of the open reading frames, the signal was too low to make a determination regarding the presence or absence of the gene. Genes with high identity between the two organisms are those involved in energy metabolism, amino acid metabolism, fatty acid metabolism, cofactor synthesis, cell division, DNA replication, transcription, translation, transport, and regulatory proteins. Functions that were less highly conserved included carbon compound metabolism, membrane proteins, structural proteins, putative transport proteins, cell processes such as adaptation and protection, and central intermediary metabolism. Open reading frames of E. coli K-12 with little or no identity in strain 342 included putative regulatory proteins, putative chaperones, surface structure proteins, mobility proteins, putative enzymes, hypothetical proteins, and proteins of unknown function, as well as genes presumed to have been acquired by lateral transfer from sources such as phage, plasmids, or transposons. The results were in agreement with the physiological properties of the two strains. Whole genome comparisons by genomic interspecies microarray hybridization are shown to rapidly identify thousands of genes in a previously uncharacterized bacterial genome provided that the genome of a close relative has been fully sequenced. This approach will become increasingly more useful as more full genome sequences become available.

dos Reis, M., L. Wernisch, et al. (2003). "Unexpected correlations between gene expression and codon usage bias from microarray data for the whole Escherichia coli K-12 genome." Nucleic Acids Res **31**(23): 6976-85.

Escherichia coli has long been regarded as a model organism in the study of codon usage bias (CUB). However, most studies in this organism regarding this topic have been computational or, when experimental, restricted to small datasets; particularly poor attention has been given to genes with low CUB. In this work, correspondence analysis on codon usage is used to classify E.coli genes into three groups, and the relationship between them and expression levels from microarray experiments is studied. These groups are: group 1, highly biased genes; group 2, moderately biased genes; and group 3, AT-rich genes with low CUB. It is shown that, surprisingly, there is a negative correlation between codon bias and expression levels for group 3 genes, i.e. genes with extremely low codon adaptation index (CAI) values are highly expressed, while group 2 show the lowest average expression levels and group 1 show the usual expected positive correlation between CAI and expression. This trend is maintained over all functional gene groups, seeming to contradict the E.coli-yeast paradigm on CUB. It is argued that these findings are still compatible with the mutation-selection balance hypothesis of codon usage and that E.coli genes form a dynamic system shaped by these factors.

Dowd, C., I. W. Wilson, et al. (2004). "Gene expression profile changes in cotton root and hypocotyl tissues in response to infection with Fusarium oxysporum f. sp. vasinfectum." Mol Plant Microbe Interact **17**(6): 654-67.

Microarray analysis of large-scale temporal and tissue-specific plant gene expression changes occurring during a susceptible plant-pathogen interaction revealed different gene expression profile changes in cotton root and hypocotyl tissues. In hypocotyl tissues infected with Fusarium oxysporum f. sp. vasinfectum, increased expression of defense-related genes was observed, whereas few changes in the expression levels of defense-related genes were found in infected root tissues. In infected roots, more plant genes were repressed than were induced, especially at the earlier stages of infection. Although many known cotton defense responses were identified, including induction of pathogenesis-related genes and

gossypol biosynthesis genes, potential new defense responses also were identified, such as the biosynthesis of lignans. Many of the stress-related gene responses were common to both tissues. The repression of drought-responsive proteins such as aquaporins in both roots and hypocotyls represents a previously unreported response of a host to pathogen attack that may be specific to vascular wilt diseases. Gene expression results implicated the phytohormones ethylene and auxin in the disease process. Biochemical analysis of hormone level changes supported this observation.

Dubois, J. W., S. Hill, et al. (2004). "The development of a DNA microarray-based assay for the characterization of commercially formulated microbial products." J Microbiol Methods **58**(2): 251-62.

Commercially formulated bioproducts containing a complex consortia of bacteria as an active ingredient pose a significant challenge for regulatory agencies and companies seeking to assess the safety and efficacy of these bioproducts. The main challenge stems from how to characterize the bacterial composition of these products, for which there is presently a lack of suitable methods. A prototype DNA microarray composed of oligonucleotide probes for functional genes, virulence factors, and taxonomic genes for a number of bacterial species was developed to examine the utility of microarray technology as a molecular tool for characterizing consortia bioproducts. The genomic DNA from four different products was extracted by two methods and examined with the microarray prototype and by denaturing gradient gel electrophoresis (DGGE). Although the identity of the consortial species remains unknown, the microarray assay provided unique and reproducible hybridization patterns for all four products, and agreed with the fingerprints generated by DGGE. The ability to differentiate between a variety of consortia products demonstrates that DNA microarrays have the potential to be a powerful tool in monitoring complex microbial communities.

Dufva, M., J. Flodin, et al. (2002). "Epstein-Barr virus nuclear antigen 5 inhibits pre-mRNA cleavage and polyadenylation." Nucleic Acids Res **30**(10): 2131-43.

The long-standing suspicion that Epstein-Barr virus nuclear antigen 5 (EBNA5) is involved in transcription regulation was recently confirmed by the observation by several groups that EBNA5 cooperates with EBNA2 in activation of the LMP1 promoter. In attempts to elucidate the molecular basis for the EBNA5-mediated enhancement of EBNA2 transactivation, we obtained evidence of an additional function of EBNA5: at high but still biologically relevant levels, EBNA5 acted as a repressor of gene expression by interfering with the processing of pre-mRNA. Transient transfections with reporter plasmids revealed that EBNA5 repressed reporter mRNA and protein expression in the cytoplasm, but did not lower the steady-state level of reporter RNA in the total cellular RNA fraction. We have excluded that repression occurred as a consequence of cell death induced by EBNA5. Using the RNase protection assay with a probe comprising the pre-mRNA cleavage and polyadenylation site, EBNA5 was found to inhibit 3'-end cleavage and polyadenylation of pre-mRNAs from the reporter plasmids investigated. The effect of inhibitory levels of EBNA5 on chromosomal genes was examined in transient transfections by expression profiling using a cDNA microarray panel containing 588 genes. The results showed that EBNA5 could also inhibit the expression of chromosomal genes and did it in a discriminatory manner. This is consistent with the notion that a regulatory mechanism exists in the cell that confers specificity to the selection by EBNA5 of target genes for repression.

Ebrahimi, B., B. M. Dutia, et al. (2003). "Transcriptome profile of murine gammaherpesvirus-68 lytic infection." J Gen Virol **84**(Pt 1): 99-109.

The murine gammaherpesvirus-68 genome encodes 73 protein-coding open reading frames with extensive similarities to human gamma(2) herpesviruses, as well as unique genes and cellular homologues. We performed transcriptome analysis of stage-specific viral RNA during permissive infection using an oligonucleotide-based microarray. Using this approach, M4, K3, ORF38, ORF50, ORF57 and ORF73 were designated as immediate-early genes based on cycloheximide treatment. The microarray analysis also identified 10 transcripts with early expression kinetics, 32 transcripts with early-

late expression kinetics and 29 transcripts with late expression kinetics. The latter group consisted mainly of structural proteins, and showed high expression levels relative to other viral transcripts. Moreover, we detected all eight tRNA-like transcripts in the presence of cycloheximide and phosphonoacetic acid. Lytic infection with MHV-68 also resulted in a significant reduction in the expression of cellular transcripts included in the DNA chip. This global approach to viral transcript analysis offers a powerful system for examining molecular transitions between lytic and latent virus infections associated with disease pathogenesis.

Edwards, J. S. and J. R. Battista (2003). "Using DNA microarray data to understand the ionizing radiation resistance of Deinococcus radiodurans." Trends Biotechnol **21**(9): 381-2.

In a recent paper, Liu et al. documented the changes in gene expression as stationary phase Deinococcus radiodurans cultures recover from acute exposure to gamma radiation. Given that the biochemical details of the response of D. radiodurans to ionizing radiation are poorly understood, this work represents an important first step towards achieving an understanding of the ionizing radiation resistance in this species.

Edwards-Ingram, L. C., M. E. Gent, et al. (2004). "Comparative genomic hybridization provides new insights into the molecular taxonomy of the Saccharomyces sensu stricto complex." Genome Res **14**(6): 1043-51.

The science of taxonomy is constantly improving as new techniques are developed. Current practice is to construct phylogenetic trees based on the analysis of the DNA sequence of single genes, or parts of single genes. However, this approach has recently been brought into question as several tree topologies may be produced for the same clade when the sequences for various different genes are used. The availability of complete genome sequences for several organisms has seen the adoption of microarray technology to construct molecular phylogenies of bacteria, based on all of the genes. Similar techniques have been used to reveal the relationships between different strains of the yeast Saccharomyces cerevisiae. We have exploited microarray technology to construct a molecular phylogeny for the Saccharomyces sensu stricto complex of yeast species, which is based on all of the protein-encoding genes revealed by the complete genome sequence of the paradigmatic species, S. cerevisiae. We also analyze different strains of S. cerevisiae itself, as well as the putative species S. boulardii. We show that in addition to the phylogeny produced, we can identify and analyze individual ORF traits and interpret the results to give a detailed explanation of evolutionary events underlying the phylogeny.

Eguchi, Y., T. Oshima, et al. (2003). "Transcriptional regulation of drug efflux genes by EvgAS, a two-component system in Escherichia coli." Microbiology **149**(Pt 10): 2819-28.

A constitutively active mutant of histidine kinase sensor EvgS was found to confer multi-drug resistance (MDR) to an acrA-deficient Escherichia coli, indicating the relationship between the two-component system EvgAS and the expression of the MDR system. The observed MDR also depended on an outer-membrane channel, TolC. Microarray and S1 mapping assays indicated that, in the presence of this constitutive mutant EvgS, the level of transcription increased for some MDR genes, including the drug efflux genes emrKY, yhiUV, acrAB, mdfA and tolC. Transcription in vitro of emrK increased by the addition of phosphorylated EvgA. Transcription activation of tolC by the activated EvgS was, however, dependent on both EvgAS and PhoPQ (Mg(2+)-responsive two-component system), in agreement with the presence of the binding site (PhoP box) for the regulator PhoP in the tolC promoter region. Transcription in vitro of yhiUV also appears to require an as-yet-unidentified additional transcriptional factor besides EvgA. Taken together we propose that the expression of the MDR system is under a complex regulatory network, including the phosphorylated EvgA serving as the master regulator

Ehrt, S., D. Schnappinger, et al. (2001). "Reprogramming of the macrophage transcriptome in response to interferon-gamma and Mycobacterium tuberculosis: signaling roles of nitric oxide synthase-2 and

phagocyte oxidase." J Exp Med **194**(8): 1123-40.

Macrophage activation determines the outcome of infection by Mycobacterium tuberculosis (Mtb). Interferon-gamma (IFN-gamma) activates macrophages by driving Janus tyrosine kinase (JAK)/signal transducer and activator of transcription-dependent induction of transcription and PKR-dependent suppression of translation. Microarray-based experiments reported here enlarge this picture. Exposure to IFN-gamma and/or Mtb led to altered expression of 25% of the monitored genome in macrophages. The number of genes suppressed by IFN-gamma exceeded the number of genes induced, and much of the suppression was transcriptional. Five times as many genes related to immunity and inflammation were induced than suppressed. Mtb mimicked or synergized with IFN-gamma more than antagonized its actions. Phagocytosis of nonviable Mtb or polystyrene beads affected many genes, but the transcriptional signature of macrophages infected with viable Mtb was distinct. Studies involving macrophages deficient in inducible nitric oxide synthase and/or phagocyte oxidase revealed that these two antimicrobial enzymes help orchestrate the profound transcriptional remodeling that underlies macrophage activation.

El-Sayed, N. M., P. Hegde, et al. (2000). "The African trypanosome genome." Int J Parasitol **30**(4): 329-45.

The haploid nuclear genome of the African trypanosome, Trypanosoma brucei, is about 35 Mb and varies in size among different trypanosome isolates by as much as 25%. The nuclear DNA of this diploid organism is distributed among three size classes of chromosomes: the megabase chromosomes of which there are at least 11 pairs ranging from 1 Mb to more than 6 Mb (numbered I-XI from smallest to largest); several intermediate chromosomes of 200-900 kb and uncertain ploidy; and about 100 linear minichromosomes of 50-150 kb. Size differences of as much as four-fold can occur, both between the two homologues of a megabase chromosome pair in a specific trypanosome isolate and among chromosome pairs in different isolates. The genomic DNA sequences determined to date indicated that about 50% of the genome is coding sequence. The chromosomal telomeres possess TTAGGG repeats and many, if not all, of the telomeres of the megabase and intermediate chromosomes are linked to expression sites for genes encoding variant surface glycoproteins (VSGs). The minichromosomes serve as repositories for VSG genes since some but not all of their telomeres are linked to unexpressed VSG genes. A gene discovery program, based on sequencing the ends of cloned genomic DNA fragments, has generated more than 20 Mb of discontinuous single-pass genomic sequence data during the past year, and the complete sequences of chromosomes I and II (about 1 Mb each) in T. brucei GUTat 10.1 are currently being determined. It is anticipated that the entire genomic sequence of this organism will be known in a few years. Analysis of a test microarray of 400 cDNAs and small random genomic DNA fragments probed with RNAs from two developmental stages of T. brucei demonstrates that the microarray technology can be used to identify batteries of genes differentially expressed during the various life cycle stages of this parasite.

Eschbach, M., K. Schreiber, et al. (2004). "Long-term anaerobic survival of the opportunistic pathogen Pseudomonas aeruginosa via pyruvate fermentation." J Bacteriol **186**(14): 4596-604.

Denitrification and arginine fermentation are central metabolic processes performed by the opportunistic pathogen Pseudomonas aeruginosa during biofilm formation and infection of lungs of patients with cystic fibrosis. Genome-wide searches for additional components of the anaerobic metabolism identified potential genes for pyruvate-metabolizing NADH-dependent lactate dehydrogenase (ldhA), phosphotransacetylase (pta), and acetate kinase (ackA). While pyruvate fermentation alone does not sustain significant anaerobic growth of P. aeruginosa, it provides the bacterium with the metabolic capacity for long-term survival of up to 18 days. Detected conversion of pyruvate to lactate and acetate is dependent on the presence of intact ldhA and ackA-pta loci, respectively. DNA microarray studies in combination with reporter gene fusion analysis and enzyme activity measurements demonstrated the anr- and ihfA-dependent anaerobic induction of the ackA-pta promoter. Potential Anr and integration host

factor binding sites were localized. Pyruvate-dependent anaerobic long-term survival was found to be significantly reduced in anr and ihfA mutants. No obvious ldhA regulation by oxygen tension was observed. Pyruvate fermentation is pH dependent. Nitrate respiration abolished pyruvate fermentation, while arginine fermentation occurs independently of pyruvate utilization.

Eskra, L., A. Mathison, et al. (2003). "Microarray analysis of mRNA levels from RAW264.7 macrophages infected with Brucella abortus." Infect Immun **71**(3): 1125-33.

Identification of host responses at the gene transcription level provides a molecular profile of the events that occur following infection. Brucella abortus is a facultative intracellular pathogen of macrophages that induces chronic infection in humans and domestic animals. Using microarray technology, the response of macrophages 4 h following B. abortus infection was analyzed to identify early intracellular infection events that occur in macrophages. Of the >6,000 genes, we identified over 140 genes that were reproducibly differentially transcribed. First, an increase in the transcription of a number of proinflammatory cytokines and chemokines, such as tumor necrosis factor alpha, interleukin-1beta (IL-1beta), IL-1alpha, and members of the SCY family of proteins, that may constitute a general host recruitment of antibacterial defenses was evident. Alternatively, Brucella may subvert newly arriving macrophages for additional intracellular infection. Second, transcription of receptors and cytokines associated with antigen presentation, e.g., major histocompatibility complex class II and IL-12p40, were not evident at this 4-h period of infection. Third, Brucella inhibited transcription of various host genes involved in apoptosis, cell cycling, and intracellular vesicular trafficking. Identification of macrophage genes whose transcription was inhibited suggests that Brucella utilizes specific mechanisms to target certain cell pathways. In conclusion, these data suggest that B. abortus can alter macrophage pathways to recruit additional macrophages for future infection while simultaneously inhibiting apoptosis and innate immune mechanisms within the macrophage, permitting intracellular survival of the bacterium. These results provide insights into the pathogenic strategies used by Brucella for long-term survival within a hostile environment.

Feezor, R. J., C. Oberholzer, et al. (2003). "Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria." Infect Immun **71**(10): 5803-13.

Sepsis caused by gram-negative bacteria and that caused by gram-positive bacteria often manifest similar clinical features. We investigated plasma proinflammatory cytokine profiles in patients with sepsis due to gram-positive and gram-negative bacteria and studied the cytokine production and differential gene regulation of leukocytes stimulated ex vivo with Escherichia coli lipopolysaccharide or heat-killed Staphylococcus aureus. Concentrations of tumor necrosis factor alpha, interleukin 1 receptor antagonist (IL-1Ra), IL-8, IL-10, IL-18 binding protein, procalcitonin, and protein C in plasma did not differ between patients with sepsis due to gram-negative and gram-positive bacteria. However, plasma IL-1beta, IL-6, and IL-18 concentrations were significantly higher in patients with sepsis due to gram-positive bacteria. Ex vivo stimulation of whole blood with heat-killed S. aureus markedly increased IL-1beta and IL-18 levels more than E. coli lipopolysaccharide stimulation. Microarray analysis revealed at least 359 cross-validated probe sets (genes) significant at the $P < 0.001$ level whose expression discriminated among gram-negative-organism-stimulated, gram-positive-organism-stimulated, and unstimulated whole-blood leukocytes. The host inflammatory responses to gram-negative and gram-positive stimuli share some common response elements but also exhibit distinct patterns of cytokine appearance and leukocyte gene expression.

Feldgarden, M., N. Byrd, et al. (2003). "Gradual evolution in bacteria: evidence from Bacillus systematics." Microbiology **149**(Pt 12): 3565-73.

The bacterial genome projects have suggested a central role for horizontal transfer in bacterial adaptation, but it is difficult to rule out an adaptive role for ordinary genetic change in existing genes. The bacterial systematics literature can readily address the importance of gene acquisition in adaptive

evolution, since phenotypic characterization typically assesses presence versus absence of metabolic capabilities, and metabolic gains and losses are most likely due to horizontal transfer and/or gene loss. Bacterial systematists have not geared their studies toward quantitative differences in metabolic capabilities, which are more likely to involve adjustments of existing genes. Here, quantitative variation in metabolism within and between three closely related Bacillus taxa has been assayed. While these taxa show no qualitative (i.e. presence versus absence) differences in resource utilization, they are quantitatively different in utilization of 8 % of 95 resources tested. Moreover, 93 % of the resources tested showed significant quantitative variation among strains within a single taxon. These results suggest that ordinary genetic changes in existing genes may play an important role in adaptation. If these results are typical, future genomically based assays of quantitative variation in phenotype (e.g. microarray analysis of mRNA concentrations) may identify hundreds of genes whose expression has been modified. A protocol is presented for identifying those modifications of gene expression and those gene acquisitions that are most likely to have played a role in adaptive evolution.

Fiegler, H., P. Carr, et al. (2003). "DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones." Genes Chromosomes Cancer **36**(4): 361-74.

We have designed DOP-PCR primers specifically for the amplification of large insert clones for use in the construction of DNA microarrays. A bioinformatic approach was used to construct primers that were efficient in the general amplification of human DNA but were poor at amplifying E. coli DNA, a common contaminant of DNA preparations from large insert clones. We chose the three most selective primers for use in printing DNA microarrays. DNA combined from the amplification of large insert clones by use of these three primers and spotted onto glass slides showed more than a sixfold increase in the human to E. coli hybridization ratio when compared to the standard DOP-PCR primer, 6MW. The microarrays reproducibly delineated previously characterized gains and deletions in a cancer cell line and identified a small gain not detected by use of conventional CGH. We also describe a method for the bulk testing of the hybridization characteristics of chromosome-specific clones spotted on microarrays by use of DNA amplified from flow-sorted chromosomes. Finally, we describe a set of clones selected from the publicly available Golden Path of the human genome at 1-Mb intervals and a view in the Ensembl genome browser from which data required for the use of these clones in array CGH and other experiments can be downloaded across the Internet.

Firoved, A. M., S. R. Wood, et al. (2004). "Microarray analysis and functional characterization of the nitrosative stress response in nonmucoid and mucoid Pseudomonas aeruginosa." J Bacteriol **186**(12): 4046-50.

The type strain of Pseudomonas aeruginosa, PAO1, showed great upregulation of many nitrosative defense genes upon treatment with S-nitrosoglutathione, while the mucoid strain PAO578II showed no further upregulation above its constitutive upregulation of nor and fhp. NO* consumption however, showed that both strains mount functional, protein synthesis-dependent NO*-consumptive responses.

Fisher, M. A., B. B. Plikaytis, et al. (2002). "Microarray analysis of the Mycobacterium tuberculosis transcriptional response to the acidic conditions found in phagosomes." J Bacteriol **184**(14): 4025-32.

We used microarrays and real-time reverse transcription-PCR to analyze the global transcriptional response of Mycobacterium tuberculosis to low pH in vitro, which may mimic an environmental signal encountered by phagocytosed mycobacteria. Eighty-one genes were differentially expressed >1.5-fold, including many involved in fatty acid metabolism. The most highly induced genes showed homology with nonribosomal peptide synthetases/polyketide synthases.

Fitzgerald, J. R., S. D. Reid, et al. (2003). "Genome diversification in Staphylococcus aureus: Molecular evolution of a highly variable chromosomal region encoding the Staphylococcal exotoxin-like family of

proteins." Infect Immun **71**(5): 2827-38.

Recent genomic studies have revealed extensive variation in natural populations of many pathogenic bacteria. However, the evolutionary processes which contribute to much of this variation remain unclear. A previous whole-genome DNA microarray study identified variation at a large chromosomal region (RD13) of Staphylococcus aureus which encodes a family of proteins with homology to staphylococcal and streptococcal superantigens, designated staphylococcal exotoxin-like (SET) proteins. In the present study, RD13 was found in all 63 S. aureus isolates of divergent clonal, geographic, and disease origins but contained a high level of variation in gene content in different strains. A central variable region which contained from 6 to 10 different set genes, depending on the strain, was identified, and DNA sequence analysis suggests that horizontal gene transfer and recombination have contributed to the diversification of RD13. Phylogenetic analysis based on the RD13 DNA sequence of 18 strains suggested that loss of various set genes has occurred independently several times, in separate lineages of pathogenic S. aureus, providing a model to explain the molecular variation of RD13 in extant strains. In spite of multiple episodes of set deletion, analysis of the ratio of silent substitutions in set genes to amino acid replacements in their products suggests that purifying selection (selective constraint) is acting to maintain SET function. Further, concurrent transcription in vitro of six of the seven set genes in strain COL was detected, indicating that the expression of set genes has been maintained in contemporary strains, and Western immunoblot analysis indicated that multiple SET proteins are expressed during the course of human infections. Overall, we have shown that the chromosomal region RD13 has diversified extensively through episodes of gene deletion and recombination. The coexpression of many set genes and the production of multiple SET proteins during human infection suggests an important role in host-pathogen interactions.

Foldes-Papp, Z., R. Egerer, et al. (2004). "Detection of multiple human herpes viruses by DNA microarray technology." Mol Diagn **8**(1): 1-9.

BACKGROUND: The detailed characterization of virus DNA is a challenge, and the genotyping that has been achieved to date has only been possible because researchers have sent a great deal of time and effort to do so. Instead of the simultaneous detection of hundreds of viruses on a single high-density DNA-chip at very high costs per chip, we present here an alternative approach using a well-designed and tailored microarray which can establish whether or not a handful of viral genes are present in a clinical sample. METHODS: In this study we applied a new concept of microarray-based, optimized and robust biochemistry for molecular diagnostics of the herpesviruses. For comparison, all samples were genotyped using standard procedures. RESULTS: The biochemical procedure of a knowledge-based, low-density microarray was established based on the molecular diagnostics of human herpes viruses: herpes simplex virus (HSV) HSV-1, HSV-2, varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and HHV-6. The study attempted to optimize parameters of microarray design, surface chemistry, oligonucleotide probe spotting, sample labeling and DNA hybridization to the developed DNA microarray. The results of 12 900 hybridization reactions on about 150 configured herpes virus microarrays showed that the established microarray-based typing procedure was reproducible, virus-specific and sufficiently sensitive with a lower limit of 100 viral copies per mL sample. CONCLUSIONS: The developed method utilizes low-fluorescence background coverslips, epoxy surface chemistry, standardized oligonucleotide probe spotting, PCR-labeling with Cy3 of isolated DNA, array hybridization, and detecting of specific spot fluorescence by an automatic microarray reader. We expect the configured microarray approach to be the method for high-throughput associated studies on human herpes viruses.

Fortin, A., J. G. MacLaurin, et al. (2004). "The proapoptotic gene SIVA is a direct transcriptional target for the tumor suppressors p53 and E2F1." J Biol Chem **279**(27): 28706-14.

The p53 tumor suppressor gene is believed to play an important role in neuronal cell death in acute neurological disease and in neurodegeneration. The p53 signaling cascade is complex, and the

mechanism by which p53 induces apoptosis is cell type-dependent. Using DNA microarray analysis, we have found a striking induction of the proapoptotic gene, SIVA. SIVA is a proapoptotic protein containing a death domain and interacts with members of the tumor necrosis factor receptor family as well as anti-apoptotic Bcl-2 family proteins. SIVA is induced following direct p53 gene delivery, treatment with a DNA-damaging agent camptothecin, and stroke injury in vivo. SIVA up-regulation is sufficient to initiate the apoptotic cascade in neurons. Through isolation and analysis of the SIVA promoter, we have identified response elements for both p53 and E2F1. Like p53, E2F1 is another tumor suppressor gene involved in the regulation of apoptosis, including neuronal injury models. We have identified E2F consensus sites in the promoter region, whereas p53 recognition sequences were found in intron1. Sequence analysis has shown that these consensus sites are also conserved between mouse and human SIVA genes. Electrophoretic mobility shift assays reveal that both transcription factors are capable of binding to putative consensus sites, and luciferase reporter assays reveal that E2F1 and p53 can activate transcription from the SIVA promoter. Here, we report that the proapoptotic gene, SIVA, which functions in a broad spectrum of cell types, is a direct transcriptional target for both tumor suppressors, p53 and E2F1.

Fouts, D. E., R. B. Abramovitch, et al. (2002). "Genomewide identification of Pseudomonas syringae pv. tomato DC3000 promoters controlled by the HrpL alternative sigma factor." Proc Natl Acad Sci U S A **99**(4): 2275-80.

The ability of Pseudomonas syringae pv. tomato DC3000 to parasitize tomato and Arabidopsis thaliana depends on genes activated by the HrpL alternative sigma factor. To support various functional genomic analyses of DC3000, and specifically, to identify genes involved in pathogenesis, we developed a draft sequence of DC3000 and used an iterative process involving computational and gene expression techniques to identify virulence-implicated genes downstream of HrpL-responsive promoters. Hypersensitive response and pathogenicity (Hrp) promoters are known to control genes encoding the Hrp (type III protein secretion) machinery and a few type III effector proteins in DC3000. This process involved (i) identification of 9 new virulence-implicated genes in the Hrp regulon by miniTn5gus mutagenesis, (ii) development of a hidden Markov model (HMM) trained with known and transposon-identified Hrp promoter sequences, (iii) HMM identification of promoters upstream of 12 additional virulence-implicated genes, and (iv) microarray and RNA blot analyses of the HrpL-dependent expression of a representative subset of these DC3000 genes. We found that the Hrp regulon encodes candidates for 4 additional type III secretion machinery accessory factors, homologs of the effector proteins HopPsyA, AvrPpiB1 (2 copies), AvrPpiC2, AvrPphD (2 copies), AvrPphE, AvrPphF, and AvrXv3, and genes associated with the production or metabolism of virulence factors unrelated to the Hrp type III secretion system, including syringomycin synthetase (SyrE), N(epsilon)-(indole-3-acetyl)-l-lysine synthetase (IaaL), and a subsidiary regulon controlling coronatine production. Additional candidate effector genes, hopPtoA2, hopPtoB2, and an avrRps4 homolog, were preceded by Hrp promoter-like sequences, but these had HMM expectation values of relatively low significance and were not detectably activated by HrpL.

Francois, P., M. Bento, et al. (2003). "Comparison of fluorescence and resonance light scattering for highly sensitive microarray detection of bacterial pathogens." J Microbiol Methods **55**(3): 755-62.

Microarrays have emerged as potential tools for bacterial detection and identification. Given their high parallelism, they might represent a breakthrough in current diagnostic methods, provided they can be coupled to simplified labeling protocols and detected with adequate sensitivities. We describe here a technique to directly label total bacterial RNA, thus avoiding the multiple steps and possible biases associated with enzymatic amplification (e.g. PCR). We have then compared the performances of one white-light source and two laser-based fluorescence scanners for detection reliability and sensitivity. Our study reveals that nanoparticle-labeled bacterial RNA generates reproducible resonance light scattering signals that are at least 50 times more intense than state-of-the-art confocal-based fluorescence signals.

Frota, C. C., D. M. Hunt, et al. (2004). "Genome structure in the vole bacillus, Mycobacterium microti, a member of the Mycobacterium tuberculosis complex with a low virulence for humans." Microbiology **150**(Pt 5): 1519-27.

Mycobacterium microti, a member of the Mycobacterium tuberculosis complex, is phylogenetically closely related to M. tuberculosis, differing in a few biochemical properties. However, these species have different levels of virulence in different hosts; most notably M. microti shows lower virulence for humans than M. tuberculosis. This report presents genomic comparisons using DNA microarray analysis for an extensive study of the diversity of M. microti strains. Compared to M. tuberculosis H37Rv, 13 deletions were identified in 12 strains of M. microti, including the regions RD1 to RD10, which are also missing in Mycobacterium bovis BCG. In addition, four new deleted regions, named MiD1, RD1beta, MiD2 and MiD3, were identified. DNA sequencing was used to define the extent of most of the deletions in one strain. Although RD1 of M. bovis BCG and M. microti is thought to be crucial for attenuation, in this study, three of the four M. microti strains that were isolated from immunocompetent patients had the RD1 deletion. In fact, only the RD3 deletion was present in all of the strains examined, although deletions RD7, RD8 and MiD1 were found in almost all the M. microti strains. These deletions might therefore have some relation to the different host range of M. microti. It was also noticeable that of the 12 strains studied, only three were identical; these strains were all isolated from immunocompetent humans, suggesting that they could have arisen from a single source. Thus, this study shows that it is difficult to ascribe virulence to any particular pattern of deletion in M. microti.

Fu, M., X. Zhu, et al. (2003). "Egr-1 target genes in human endothelial cells identified by microarray analysis." Gene **315**: 33-41.

Early growth response factor 1 (Egr-1) is a key transcriptional factor to mediate gene expression after vascular injury. To better understand the role of Egr-1 in vasculature, we globally profiled Egr-1 target genes in human endothelial cells using adenoviral gene transfer and Affymetrix oligonucleotide-based microarray technology. More than 300 genes regulated by >/=3-fold with Egr-1 overexpression were identified and, partially, confirmed by Northern and Western blotting, including genes for transcriptional regulators, signaling proteins, cell cycle regulatory proteins, growth factors, and cytokines. Among them, thymus-expressed chemokine (TECK) and IP-30 were dramatically induced by Egr-1, but TNFalpha-related apoptosis inducing ligand (TRAIL) was significantly repressed by Egr-1, suggesting that Egr-1 is a key mediator of inflammation and apoptosis in vascular cells. These data provide novel Egr-1 target genes and contribute to the understanding of the role of Egr-1 in vasculature.

Fukiya, S., H. Mizoguchi, et al. (2004). "An improved method for deleting large regions of Escherichia coli K-12 chromosome using a combination of Cre/loxP and lambda Red." FEMS Microbiol Lett **234**(2): 325-31.

We have established an improved large deletion method in Escherichia coli genome using a combination of two different recombination systems, lambda Red and Cre/loxP. The loxP site could be rapidly and efficiently integrated in the genome by lambda Red and large deletions of both 117- and 165-kbp regions could be generated in 100% efficiency by Cre/loxP. Comparative genomic hybridization microarray experiments of deletion strains indicated that deletions were generated only in expected regions of the genome. These results have demonstrated that the method is useful for genome engineering in E. coli.

Fukiya, S., H. Mizoguchi, et al. (2004). "Extensive genomic diversity in pathogenic Escherichia coli and Shigella Strains revealed by comparative genomic hybridization microarray." J Bacteriol **186**(12): 3911-21.

Escherichia coli, including the closely related genus Shigella, is a highly diverse species in terms of genome structure. Comparative genomic hybridization (CGH) microarray analysis was used to compare the gene content of E. coli K-12 with the gene contents of pathogenic strains. Missing genes in a

pathogen were detected on a microarray slide spotted with 4,071 open reading frames (ORFs) of W3110, a commonly used wild-type K-12 strain. For 22 strains subjected to the CGH microarray analyses 1,424 ORFs were found to be absent in at least one strain. The common backbone of the E. coli genome was estimated to contain about 2,800 ORFs. The mosaic distribution of absent regions indicated that the genomes of pathogenic strains were highly diversified because of insertions and deletions. Prophages, cell envelope genes, transporter genes, and regulator genes in the K-12 genome often were not present in pathogens. The gene contents of the strains tested were recognized as a matrix for a neighbor-joining analysis. The phylogenic tree obtained was consistent with the results of previous studies. However, unique relationships between enteroinvasive strains and Shigella, uropathogenic, and some enteropathogenic strains were suggested by the results of this study. The data demonstrated that the CGH microarray technique is useful not only for genomic comparisons but also for phylogenic analysis of E. coli at the strain level.

Fukushima, K., H. Ogawa, et al. (2003). "Non-pathogenic bacteria modulate colonic epithelial gene expression in germ-free mice." Scand J Gastroenterol **38**(6): 626-34.

BACKGROUND: We established a bacterial reconstitution model to investigate epithelial cell-luminal bacteria interaction. The aim of the study was to identify the known genes directly or indirectly modulated by non-pathologic bacterial flora in the colonic epithelia of germ-free mice. METHODS: Germ-free mice were orally given a bacterial suspension prepared from specific pathogen-free counterparts (bacterial reconstitution). Colonic epithelial cells were isolated, then total and poly (A) RNA were extracted. We investigated differential gene expression in colonic epithelial cells among germ-free, bacteria-reconstituted, and specific pathogen-free mice by DNA microarray. Finally, differential expression was confirmed by Northern blot or quantitative RT-PCR. RESULTS: Thirty genes were initially selected as differentially expressed genes in DNA microarray analysis. We confirmed that genes associated with growth (Reg IIIbeta, Reg IIIgamma, guanylate nucleotide binding protein 2), apoptosis (Bcl-associated death promotor), cytoskeleton (tubulin alpha4, erythrocyte protein band 7.2), and immune response (lymphocyte antigen complex 6) were induced by bacterial reconstitution. In contrast, genes possibly participating in extracellular oxidant defence (selenoprotein P, metallothionein 1) and cellular metabolism (cytochrome P450, HMGCoA synthase 2, alcohol dehydrogenase 1 complex, aldehyde dehydrogenase family 1, carbonic anhydrase 1, glycoprotein galactosyltransferase alpha1,3) were down-regulated by bacterial challenge. CONCLUSION: Non-pathogenic bacteria modulated colonic gene expression in germ-free mice, suggesting that non-pathogenic bacteria possibly initiate epithelial change in genetically normal and/or abnormal hosts. The present study provides a basis for the functional study of each molecule in symbiosis with luminal bacteria in healthy and diseased colon.

Galindo, C. L., A. A. Fadl, et al. (2004). "Microarray analysis of Aeromonas hydrophila cytotoxic enterotoxin-treated murine primary macrophages." Infect Immun **72**(9): 5439-45.

We performed microarray analyses of murine peritoneal macrophages to examine cellular transcriptional responses to a cytotoxic enterotoxin of Aeromonas hydrophila. While 66% of altered genes were common to both primary macrophages and the murine macrophage cell line RAW 264.7, Act caused expression changes of 28 genes specifically in murine peritoneal macrophages.

Ganesan, K., L. Jiang, et al. (2002). "Stochastic versus stable transcriptional differences on Plasmodium falciparum DNA microarrays." Int J Parasitol **32**(13): 1543-50.

The recent availability of the Plasmodium falciparum genome sequence has opened up convenient, large-scale analysis of transcriptional products in malaria. Protocols for cDNA labelling, cDNA hybridisation, and fluorescent signal detection developed for other organisms can be applied directly to malaria. However, P. falciparum offers unique challenges in data analysis due to stochastic variability in expression of some gene products, such as variable erythrocyte surface proteins. Careful comparison of global transcriptional patterns in two well-studied clones of P. falciparum (Dd2 and HB3)

indicates that reliable, stable transcriptional alterations in malaria can be readily distinguished from stochastic processes. To do this, we utilised a complex experimental design which involves a combination of self-hybridisations and cross-hybridisations between two independently grown parasite populations for each clone being examined (for short, we call this a '2x2 CombiScan'). While even a simple 2x2 CombiScan required 12 microarray hybridisations, the effort generated output that was highly interpretable. Reliable RNA transcriptional differences between Dd2 and HB3 could be readily visualised using public algorithms for data normalisation and clustering.

Ganesh, R., D. A. Siegele, et al. (2003). "MOPAC: motif finding by preprocessing and agglomerative clustering from microarrays." Pac Symp Biocomput: 41-52.

We propose a novel strategy for discovering motifs from gene expression data. The gene expression data in our experiments comes from DNA Microarray analysis of the bacterium E. coli in response to recovery from nutrient starvation. We have annotated the data and identified the upregulated genes. Our interest is to find common regulatory motifs that are responsible for the upregulation of these specific genes. We assume that a common motif that a regulatory protein can bind to will be present in the upstream region of the upregulated genes and will not be present in the upstream regions of genes that showed a constant level of expression over time. Our objective is to find the common motifs that are present in at least some of the upstream sequences of upregulated genes and not present in the control set, which is the set of genes whose expression remained the same. Because it is possible that there could be several subsets of co-regulated genes under different control mechanisms among the co-expressed genes, we do not want to require motifs to be present in all upregulated sequences. Therefore, we propose a new algorithm for finding such motifs through stages of pre-processing, denoising, agglomerative clustering and consensus checking. Through this process, we have found some motifs that are good candidates for further validation.

Gao, J. J., V. Diesl, et al. (2003). "Bacterial LPS and CpG DNA differentially induce gene expression profiles in mouse macrophages." J Endotoxin Res **9**(4): 237-43.

Bacterial DNA containing unmethylated CpG dinucleotides (CpG DNA) is a potent immune stimulating agent that holds strong promise in the treatment of many disorders. Studies have established that CpG DNA triggers an immune response through activated expression of genes in immune cells including macrophages. To dissect further the molecular mechanism(s) by which CpG DNA activates the immune system, we studied macrophage gene expression profiles in response to CpG DNA using microarray technology. Since CpG DNA is reported to use the TLR9 receptor that shares homology with the TLR4 receptor used by bacterial lipopolysaccharide (LPS), we also evaluated gene expression profiles in macrophages stimulated by LPS versus CpG DNA. Both CpG DNA and LPS modulate expression of a large array of genes. However, LPS modulated the expression of a much greater number of genes than did CpG DNA and all genes induced or repressed by CpG DNA were also induced or repressed by LPS. These data indicate that the CpG DNA signaling pathway through TLR9 activates only a subset of genes induced by the LPS TLR4 signaling pathway.

Garcia-Cosio, M., A. Santon, et al. (2004). "Analysis of transcription factor OCT.1, OCT.2 and BOB.1 expression using tissue arrays in classical Hodgkin's lymphoma." Mod Pathol.

Hodgkin's lymphoma can be considered in most cases a B-cell lymphoma due to the presence of potentially functional immunoglobulin (Ig) gene rearrangements in the neoplastic cells. In contrast to lymphocyte-predominant Hodgkin's lymphoma, Hodgkin/Reed-Sternberg (HRS) cells from classical Hodgkin's lymphoma have low frequency of B-cell marker expression and lack Ig light and Ig heavy messenger RNA. Recent studies have shown transcription machinery deficiency in Hodgkin's lymphoma caused by an absence of the transcription factors OCT.1, OCT.2 and/or BOB.1. By using the tissue microarray technique, we have performed an immunohistochemical study of OCT.1, OCT.2 and BOB.1 in 325 classical Hodgkin's lymphoma cases. The results have been correlated with the expression of the

B-cell markers CD20, CD79a, B-cell-specific activator protein (BSAP) and MUM.1, the presence of Epstein-Barr virus and the histological subtype. The percentage of CD20 and CD79a positivity was low (18 and 18%, respectively), whereas MUM.1 and BSAP were positive in the majority of cases. Considering the positive cases with independence of the intensity of staining, 62% of them expressed OCT.2, 59% OCT.1 and 37% BOB.1. Nevertheless, when we considered only the strongly positive cases, the results were similar to those previously described by others. No statistical association was found between the transcription factor expression, histological subtype and Epstein-Barr virus presence. To our knowledge, this is the largest series of classical Hodgkin's lymphoma cases in which the expression of transcription factors has been studied. We have found a notorious percentage of cases displaying weak positivity for OCT.2 and BOB.1 factors in HRS cells. We propose that other mechanisms different from the absence of transcription factors OCT.2 and BOB.1 might be involved in the control of Ig transcription and B lineage in classical Hodgkin's lymphoma.Modern Pathology advance online publication, 16 July 2004; doi:10.1038/modpathol.3800227.

Gardner, S. N., T. A. Kuczmarski, et al. (2003). "Limitations of TaqMan PCR for detecting divergent viral pathogens illustrated by hepatitis A, B, C, and E viruses and human immunodeficiency virus." J Clin Microbiol **41**(6): 2417-27.

Recent events illustrate the imperative to rapidly and accurately detect and identify pathogens during disease outbreaks, whether they are natural or engineered. Particularly for our primary goal of detecting bioterrorist releases, detection techniques must be both species-wide (capable of detecting all known strains of a given species) and species specific. Due to classification restrictions on the publication of data for species that may pose a bioterror threat, we illustrate the challenges of finding such assays using five nonthreat organisms that are nevertheless of public health concern: human immunodeficiency virus (HIV) and four species of hepatitis viruses. Fluorogenic probe-based PCR assays (TaqMan; Perkin-Elmer Corp., Applied Biosystems, Foster City, Calif.) may be sensitive, fast methods for the identification of species in which the genome is conserved among strains, such as hepatitis A virus. For species such as HIV, however, the strains are highly divergent. We use computational methods to show that nine TaqMan primer and probe sequences, or signatures, are needed to ensure that all strains will be detected, but this is an unfeasible number, considering the cost of TaqMan probes. Strains of hepatitis B, C, and E viruses show intermediate divergence, so that two to three TaqMan signatures are required to detect all strains of each virus. We conclude that for species such as hepatitis A virus with high levels of sequence conservation among strains, signatures can be found computationally for detection by the TaqMan assay, which is a sensitive, rapid, and cost-effective method. However, for species such as HIV with substantial genetic divergence among strains, the TaqMan assay becomes unfeasible and alternative detection methods may be required. We compare the TaqMan assay with some of the alternative nucleic acid-based detection techniques of microarray, chip, and bead technologies in terms of sensitivity, speed, and cost.

Garg, N., V. L. Popov, et al. (2003). "Profiling gene transcription reveals a deficiency of mitochondrial oxidative phosphorylation in Trypanosoma cruzi-infected murine hearts: implications in chagasic myocarditis development." Biochim Biophys Acta **1638**(2): 106-20.

In this study, we report the host genetic responses that characterize Trypanosoma cruzi-induced myocarditis in a murine model of infection and disease development. The mRNA species from the myocardium of infected mice were assessed using cDNA microarray technology at immediate early, acute, and chronic stages of infection. The immediate early reaction of the host to T. cruzi infection was marked by up-regulation of transcripts indicative of proinflammatory and interferon-induced immune responses. Following acute infection, overexpression of transcripts for extracellular matrix (ECM) proteins, possibly initiated in response to myocardial injuries by invading and replicating parasites, was suggestive of active reparative and remodeling reactions. Surprisingly, progression to the cardiac disease phase was associated with coordinated down-regulation of a majority (>70%) of the differentially expressed genes. Among the most repressed genes were the troponins, essential for contractile function of

the myofibrils, and the genes encoding components of oxidative phosphorylation (OXPHOS) pathways. Reverse transcription-polymerase chain reaction (RT-PCR), Western blotting, and biochemical assays confirmed the microarray results and provided evidence for the deficiency of OXPHOS complex IV in the chagasic murine heart. We discuss the apparent role of OXPHOS dysfunction in the cardiac hypertrophic and remodeling processes with the development of chagasic cardiomyopathy (CCM).

Gauthier, B. R., T. Brun, et al. (2004). "Oligonucleotide microarray analysis reveals PDX1 as an essential regulator of mitochondrial metabolism in rat islets." J Biol Chem **279**(30): 31121-30.

Mutations in the transcription factor IPF1/PDX1 have been associated with type 2 diabetes. To elucidate beta-cell dysfunction, PDX1 was suppressed by transduction of rat islets with an adenoviral construct encoding a dominant negative form of PDX1. After 2 days, there was a marked inhibition of insulin secretion in response to glucose, leucine, and arginine. Increasing cAMP levels with forskolin and isobutylmethylxanthine restored glucose-stimulated insulin secretion, indicating normal capacity for exocytosis. To identify molecular targets implicated in the altered metabolism secretion coupling, DNA microarray analysis was performed on PDX1-deficient and control islets. Of the 2640 detected transcripts, 70 were up-regulated and 56 were down-regulated. Transcripts were subdivided into 12 clusters; the most prevalent were associated with metabolism. Quantitative reverse transcriptase-PCR confirmed increases in succinate dehydrogenase and ATP synthase mRNAs as well as pyruvate carboxylase and the transcript for the malate shuttle. In parallel there was a 50% reduction in mRNA levels for the mitochondrially encoded nd1 gene, a subunit of the NADH dehydrogenase comprising complex I of the mitochondrial respiratory chain. As a consequence, total cellular ATP concentration was drastically decreased by 75%, and glucose failed to augment cytosolic ATP, explaining the blunted glucose-stimulated insulin secretion. Rotenone, an inhibitor of complex I, mimicked this effect. Surprisingly, TFAM, a nuclear-encoded transcription factor important for sustaining expression of mitochondrial genes, was down-regulated in islets expressing DN79PDX1. In conclusion, loss of PDX1 function alters expression of mitochondrially encoded genes through regulation of TFAM leading to impaired insulin secretion.

Gaynor, E. C., S. Cawthraw, et al. (2004). "The genome-sequenced variant of Campylobacter jejuni NCTC 11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes." J Bacteriol **186**(2): 503-17.

The genome sequence of the enteric bacterial pathogen Campylobacter jejuni NCTC 11168 (11168-GS) was published in 2000, providing a valuable resource for the identification of C. jejuni-specific colonization and virulence factors. Surprisingly, the 11168-GS clone was subsequently found to colonize 1-day-old chicks following oral challenge very poorly compared to other strains. In contrast, we have found that the original clinical isolate from which 11168-GS was derived, 11168-O, is an excellent colonizer of chicks. Other marked phenotypic differences were also identified: 11168-O invaded and translocated through tissue culture cells far more efficiently and rapidly than 11168-GS, was significantly more motile, and displayed a different morphology. Serotyping, multiple high-resolution molecular genotyping procedures, and subtractive hybridization did not yield observable genetic differences between the variants, suggesting that they are clonal. However, microarray transcriptional profiling of these strains under microaerobic and severely oxygen-limited conditions revealed dramatic expression differences for several gene families. Many of the differences were in respiration and metabolism genes and operons, suggesting that adaptation to different oxygen tensions may influence colonization potential. This correlates biologically with our observation that anaerobically priming 11168-GS or aerobically passaging 11168-O caused an increase or decrease, respectively, in colonization compared to the parent strain. Expression differences were also observed for several flagellar genes and other less well-characterized genes that may participate in motility. Targeted sequencing of the sigma factors revealed specific DNA differences undetected by the other genomic methods. These observations highlight the capacity of C. jejuni to adapt to multiple environmental niches, the likelihood that this adaptation involves genetic evolution, and provides the first whole-genome molecular exploration of the effect of laboratory

culture and storage on colonization and virulence properties of this pathogen.

Geiman, D. E., D. Kaushal, et al. (2004). "Attenuation of late-stage disease in mice infected by the Mycobacterium tuberculosis mutant lacking the SigF alternate sigma factor and identification of SigF-dependent genes by microarray analysis." Infect Immun **72**(3): 1733-45.

The Mycobacterium tuberculosis alternate sigma factor, SigF, is expressed during stationary growth phase and under stress conditions in vitro. To better understand the function of SigF we studied the phenotype of the M. tuberculosis DeltasigF mutant in vivo during mouse infection, tested the mutant as a vaccine in rabbits, and evaluated the mutant's microarray expression profile in comparison with the wild type. In mice the growth rates of the DeltasigF mutant and wild-type strains were nearly identical during the first 8 weeks after infection. At 8 weeks, the DeltasigF mutant persisted in the lung, while the wild type continued growing through 20 weeks. Histopathological analysis showed that both wild-type and mutant strains had similar degrees of interstitial and granulomatous inflammation during the first 12 weeks of infection. However, from 12 to 20 weeks the mutant strain showed smaller and fewer lesions and less inflammation in the lungs and spleen. Intradermal vaccination of rabbits with the M. tuberculosis DeltasigF strain, followed by aerosol challenge, resulted in fewer tubercles than did intradermal M. bovis BCG vaccination. Complete genomic microarray analysis revealed that 187 genes were relatively underexpressed in the absence of SigF in early stationary phase, 277 in late stationary phase, and only 38 genes in exponential growth phase. Numerous regulatory genes and those involved in cell envelope synthesis were down-regulated in the absence of SigF; moreover, the DeltasigF mutant strain lacked neutral red staining, suggesting a reduction in the expression of envelope-associated sulfolipids. Examination of 5'-untranslated sequences among the downregulated genes revealed multiple instances of a putative SigF consensus recognition sequence: GGTTTCX(18)GGGTAT. These results indicate that in the mouse the M. tuberculosis DeltasigF mutant strain persists in the lung but at lower bacterial burdens than wild type and is attenuated by histopathologic assessment. Microarray analysis has identified SigF-dependent genes and a putative SigF consensus recognition site.

Geiss, G., G. Jin, et al. (2001). "A comprehensive view of regulation of gene expression by double-stranded RNA-mediated cell signaling." J Biol Chem **276**(32): 30178-82.

Double-stranded (ds) RNA, a common component of virus-infected cells, is a potent inducer of the type I interferon and other cellular genes. For identifying the full repertoire of human dsRNA-regulated genes, a cDNA microarray hybridization screening was conducted using mRNA from dsRNA-treated GRE cells. Because these cells lack all type I interferon genes, the possibility of gene induction by autocrine actions of interferon was eliminated. Our screen identified 175 dsRNA-stimulated genes (DSG) and 95 dsRNA-repressed genes. A subset of the DSGs was also induced by different inflammatory cytokines and viruses demonstrating interconnections among disparate signaling pathways. Functionally, the DSGs encode proteins involved in signaling, apoptosis, RNA synthesis, protein synthesis and processing, cell metabolism, transport, and structure. Induction of such a diverse family of genes by dsRNA has major implications in host-virus interactions and in the use of RNA(i) technology for functional ablation of specific genes.

Geiss, G. K., R. E. Bumgarner, et al. (2000). "Large-scale monitoring of host cell gene expression during HIV-1 infection using cDNA microarrays." Virology **266**(1): 8-16.

Human immunodeficiency virus type 1 (HIV-1) infection alters the expression of host cell genes at both the mRNA and protein levels. To obtain a more comprehensive view of the global effects of HIV infection of CD4-positive T-cells at the mRNA level, we performed cDNA microarray analysis on approximately 1500 cellular cDNAs at 2 and 3 days postinfection (p.i.) with HIV-1. Host cell gene expression changed little at 2 days p.i., but at 3 days p.i. 20 cellular genes were identified as differentially expressed. Genes involved in T-cell signaling, subcellular trafficking, and transcriptional regulation, as well as several uncharacterized genes, were among those whose mRNAs were differentially regulated.

These results support the hypothesis that HIV-1 infection alters expression of a broad array of cellular genes and provides a framework for future functional studies on the differentially expressed mRNA products.

Geiss, G. K., V. S. Carter, et al. (2003). "Gene expression profiling of the cellular transcriptional network regulated by alpha/beta interferon and its partial attenuation by the hepatitis C virus nonstructural 5A protein." J Virol **77**(11): 6367-75.

Alpha/beta interferons (IFN-alpha/beta) induce potent antiviral and antiproliferative responses and are used to treat a wide range of human diseases, including chronic hepatitis C virus (HCV) infection. However, for reasons that remain poorly understood, many HCV isolates are resistant to IFN therapy. To better understand the nature of the cellular IFN response, we examined the effects of IFN treatment on global gene expression by using several types of human cells, including HeLa cells, liver cell lines, and primary fetal hepatocytes. In response to IFN, 50 of the approximately 4,600 genes examined were consistently induced in each of these cell types and another 60 were induced in a cell type-specific manner. A search for IFN-stimulated response elements (ISREs) in genomic DNA located upstream of IFN-stimulated genes revealed both previously identified and novel putative ISREs. To determine whether HCV can alter IFN-regulated gene expression, we performed microarray analyses on IFN-treated HeLa cells expressing the HCV nonstructural 5A (NS5A) protein and on IFN-treated Huh7 cells containing an HCV subgenomic replicon. NS5A partially blocked the IFN-mediated induction of 14 IFN-stimulated genes, an effect that may play a role in HCV resistance to IFN. This block may occur through repression of ISRE-mediated transcription, since NS5A also inhibited the IFN-mediated induction of a reporter gene driven from an ISRE-containing promoter. In contrast, the HCV replicon had very little effect on IFN-regulated gene expression. These differences highlight the importance of comparing results from multiple model systems when investigating complex phenomena such as the cellular response to IFN and viral mechanisms of IFN resistance.

Gilbert, S. F. (2002). "The genome in its ecological context: philosophical perspectives on interspecies epigenesis." Ann N Y Acad Sci **981**: 202-18.

Epigenesis concerns the interactions through which the inherited potentials of the genome become actualized into an adult organism. In addition to epigenetic interactions occurring within the developing embryo, there are also critical epigenetic interactions occurring between the embryo and its environment. These interactions can determine the sex of the embryo, increase its fitness, or even be involved in the formation of particular organs. This essay will outline the history of environmental concerns in developmental biology and provide some reasons for the decline and resurgence of these ideas, and it will then focus on two areas that have recently gained much attention: predator-induced polyphenisms and developmental symbioses. Research in these two areas of interspecies cooperation in morphogenesis has profound implications for what we consider to be normal development and how we proceed to study it. Studies of predator-induced polyphenism have shown that soluble factors from predators can change the development of prey in specific ways. Prey has evolved mechanisms to sense compounds released from their predators and to use these chemical cues to change their development in ways that prevent predation. New techniques in molecular biology, especially polymerase chain reaction and microarray analysis, have shown that symbioses between embryos and bacteria are widespread and that animals may use bacterial cues to complete their development.

Gill, R. T., M. P. DeLisa, et al. (2001). "Genomic analysis of high-cell-density recombinant Escherichia coli fermentation and "cell conditioning" for improved recombinant protein yield." Biotechnol Bioeng **72**(1): 85-95.

The Escherichia coli stress gene transcription profile and response to recombinant protein overexpression were substantially altered at high cell density when compared with low cell density. Reverse trascription-polymerase chain reaction RT-PCR-amplified mRNA from low (4 g[DCW]/L) and

high-cell-density (43.5 g [DCW]/L) conditions were hybridized with a DNA microarray of Kohara clones encompassing 16% of the E. coli genome, and differentially displayed genes were identified. Transcript-specific RNA dot blots indicated that molecular chaperones (groEL, ibpA, degP), proteases (degP, ftsH), the lysis gene mltB, and DNA damage/bacteriophage-associated gene transcript levels (ftsH, recA, alpA, uvrB) increased 10- to 43-fold at high cell density. In addition, overexpression of recombinant green fluorescent protein (GFP(uv))/chloramphenicol acetyltransferase (CAT) fusion protein did not change the rates of cell growth or cell lysis. The stress gene transcription profile at high cell density was used to evaluate "cell conditioning" strategies to alter the levels of chaperones, proteases, and other intracellular proteins prior to recombinant protein overexpression. Interestingly, the addition of 1 g/L dithiothreitol (DTT) 20 min prior to induction of a GFP(uv)/CAT fusion protein resulted in a twofold increase in CAT activity when compared with the unconditioned controls. In addition, RNA dot blots of five stress genes confirmed that cell conditioning strategies significantly altered the dynamic stress gene response to foreign protein overexpression.

Gill, R. T., E. Katsoulakis, et al. (2002). "Genome-wide dynamic transcriptional profiling of the light-to-dark transition in Synechocystis sp. strain PCC 6803." J Bacteriol **184**(13): 3671-81.

We report the results of whole-genome transcriptional profiling of the light-to-dark transition with the model photosynthetic prokaryote Synechocystis sp. strain PCC 6803 (Synechocystis). Experiments were conducted by growing Synechocystis cultures to mid-exponential phase and then exposing them to two cycles of light/dark conditions, during which RNA samples were obtained. These samples were probed with a full-genome DNA microarray (3,169 genes, 20 samples) as well as a partial-genome microarray (88 genes, 29 samples). We concluded that (i) 30-min sampling intervals accurately captured transcriptional dynamics throughout the light/dark transition, (ii) 25% of the Synechocystis genes (783 genes) responded positively to the presence of light, and (iii) the response dynamics varied greatly for individual genes, with a delay of up to 120 to 150 min for some genes. Four classes of genes were identified on the basis of their dynamic gene expression profiles: class I (108 genes, 30-min response time), class II (279 genes, 60 to 90 min), class III (258 genes, 120 to 150 min), and class IV (138 genes, 180 min). The dynamics of several transcripts from genes involved in photosynthesis and primary energy generation are discussed. Finally, we applied Fisher discriminant analysis to better visualize the progression of the overall transcriptional program throughout the light/dark transition and to determine those genes most indicative of the lighting conditions during growth.

Gill, R. T., S. Wildt, et al. (2002). "Genome-wide screening for trait conferring genes using DNA microarrays." Proc Natl Acad Sci U S A **99**(10): 7033-8.

We report a DNA microarray-based method for genome-wide monitoring of competitively grown transformants to identify genes whose overexpression confers a specific cellular phenotype. Whereas transcriptional profiling identifies differentially expressed genes that are correlated with particular aspects of the cellular phenotype, this functional genomics approach determines genes that result in a specific physiology. This parallel gene-trait mapping method consists of transforming a strain with a genomic library, enriching the cell population in transformants containing the trait conferring gene(s), and finally using DNA microarrays to simultaneously isolate and identify the enriched gene inserts. Various methods of enrichment can be used; here, genes conferring low-level antibiotic resistance were identified by growth in selective media. We demonstrated the method by transforming Escherichia coli cells with a genomic E. coli library and selecting for transformants exhibiting a growth advantage in the presence of the anti-microbial agent Pine-Sol. Genes conferring Pine-Sol tolerance (19 genes) or sensitivity (27 genes) were identified by hybridizing, on DNA microarrays containing 1,160 E. coli gene probes, extra-chromosomal DNA isolated from transformed cells grown in the presence of various levels of Pine-Sol. Results were further validated by plating and sequencing of individual colonies, and also by assessing the Pine-Sol resistance of cells transformed with enriched plasmid library or individual resistance genes identified by the microarrays. Applications of this method beyond antibiotic resistance include

identification of genes resulting in resistance to chemotherapeutic agents, genes yielding resistance to toxic products (recombinant proteins, chemical feedstocks) in industrial fermentations, genes providing enhanced growth in cell culture or high cell density fermentations, genes facilitating growth on unconventional substrates, and others.

Girard, S., P. Shalhoub, et al. (2002). "An altered cellular response to interferon and up-regulation of interleukin-8 induced by the hepatitis C viral protein NS5A uncovered by microarray analysis." Virology **295**(2): 272-83.

There is evidence for an inhibition of interferon-alpha antiviral activity by the hepatitis C viral protein, NS5A. To identify the mechanisms through which NS5A blocks interferon activity, we compared the gene expression profile of interferon-treated Huh7 cells, stably expressing NS5A with control, using microarrays. Following interferon treatment, 50 genes were up-regulated by at least twofold in control clones, whereas induction of 9 of the 50 genes was significantly reduced in NS5A-expressing clones. The strongest effect of NS5A on interferon response was observed for the OAS-p69 gene. Remarkably, Huh7 cells expressing NS5A showed an up-regulation of interleukin-8. Up-regulation of interleukin-8 was also observed upon transient expression of NS5A mutants isolated from patients responsive or resistant to interferon therapy. Addition of interleukin-8 to Huh7 cells inhibited the antiviral activity of interferon and, similarly to NS5A, reduced the induction by interferon-alpha of selective genes including OAS-p69. Our findings provide a mechanism for NS5A-mediated interferon resistance.

Gjetting, T., T. L. Carver, et al. (2004). "Differential gene expression in individual papilla-resistant and powdery mildew-infected barley epidermal cells." Mol Plant Microbe Interact **17**(7): 729-38.

Resistance and susceptibility in barley to the powdery mildew fungus (Blumeria graminis f. sp. hordei) is determined at the single-cell level. Even in genetically compatible interactions, attacked plant epidermal cells defend themselves against attempted fungal penetration by localized responses leading to papilla deposition and reinforcement of their cell wall. This conveys a race-nonspecific form of resistance. However, this defense is not complete, and a proportion of penetration attempts succeed in infection. The resultant mixture of infected and uninfected leaf cells makes it impossible to relate powdery mildew-induced gene expression in whole leaves or even dissected epidermal tissues to resistance or susceptibility. A method for generating transcript profiles from individual barley epidermal cells was established and proven useful for analyzing resistant and successfully infected cells separately. Contents of single epidermal cells (resistant, infected, and unattacked controls) were collected, and after cDNA synthesis and PCR amplification, the resulting sample was hybridized to dot-blots spotted with genes, including some previously reported to be induced upon pathogen attack. Transcripts of several genes, (e.g., PR1a, encoding a pathogenesis related protein, and GLP4, encoding a germin-like protein) accumulated specifically in resistant cells, while GRP94, encoding a molecular chaperone, accumulated in infected cells. Thus, the single-cell method allows discrimination of transcript profiles from resistant and infected cells. The method will be useful for microarray expression profiling for simultaneous analysis of many genes.

Glanemann, C., A. Loos, et al. (2003). "Disparity between changes in mRNA abundance and enzyme activity in Corynebacterium glutamicum: implications for DNA microarray analysis." Appl Microbiol Biotechnol **61**(1): 61-8.

The relationship between changes in mRNA abundance and enzyme activity was determined for three genes over a span of nearly 3 h during amino acid production in Corynebacterium glutamicum. Gene expression changes during C. glutamicum fermentations were examined by complementary DNA (cDNA) microarrays and by a second method for quantitating RNA levels, competitive reverse transcriptase-PCR (RT-PCR). The results obtained independently by both methods were compared and found to be in agreement, thus validating the quantitative potential of DNA microarrays for gene expression profiling. Evidence of a disparity between mRNA abundance and enzyme activity is presented

and supports our belief that it is difficult to generally predict protein activity from quantitative transcriptome data. Homoserine dehydrogenase, threonine dehydratase, and homoserine kinase are enzymes involved in the biosynthesis of l-isoleucine and other aspartate-derived amino acids in C. glutamicum. Our data suggest that different underlying regulatory mechanisms may be connected with the expression of the genes encoding each of these three enzymes. Indeed, whereas in one case the increases in enzyme activity exceeded those in the corresponding mRNA abundance, in another case large increases in the levels of gene expression were not congruent with changes in enzyme activity.

Glasbey, C. A. and P. Ghazal (2003). "Combinatorial image analysis of DNA microarray features." Bioinformatics **19**(2): 194-203.

MOTIVATION: DNA and protein microarrays have become an established leading-edge technology for large-scale analysis of gene and protein content and activity. Contact-printed microarrays has emerged as a relatively simple and cost effective method of choice but its reliability is especially susceptible to quality of pixel information obtained from digital scans of spotted features in the microarray image. RESULTS: We address the statistical computation requirements for optimizing data acquisition and processing of digital scans. We consider the use of median filters to reduce noise levels in images and top-hat filters to correct for trends in background values. We also consider, as alternative estimators of spot intensity, discs of fixed radius, proportions of histograms and k-means clustering, either with or without a square-root intensity transformation and background subtraction. We identify, using combinatoric procedures, optimal filter and estimator parameters, in achieving consistency among the replicates of a gene on each microarray. Our results, using test data from microarrays of HCMV, indicate that a highly effective approach for improving reliability and quality of microarray data is to apply a 21 by 21 top-hat filter, then estimate spot intensity as the mean of the largest 20% of pixel values in the target region, after a square-root transformation, and corrected for background, by subtracting the mean of the smallest 70% of pixel values. AVAILABILITY: Fortran90 subroutines implementing these methods are available from the authors, or at http://www.bioss.ac.uk/~chris.

Glasner, J. D., P. Liss, et al. (2003). "ASAP, a systematic annotation package for community analysis of genomes." Nucleic Acids Res **31**(1): 147-51.

ASAP (a systematic annotation package for community analysis of genomes) is a relational database and web interface developed to store, update and distribute genome sequence data and functional characterization (https://asap.ahabs.wisc.edu/annotation/php/ASAP1.htm). ASAP facilitates ongoing community annotation of genomes and tracking of information as genome projects move from preliminary data collection through post-sequencing functional analysis. The ASAP database includes multiple genome sequences at various stages of analysis, corresponding experimental data and access to collections of related genome resources. ASAP supports three levels of users: public viewers, annotators and curators. Public viewers can currently browse updated annotation information for Escherichia coli K-12 strain MG1655, genome-wide transcript profiles from more than 50 microarray experiments and an extensive collection of mutant strains and associated phenotypic data. Annotators worldwide are currently using ASAP to participate in a community annotation project for the Erwinia chrysanthemi strain 3937 genome. Curation of the E. chrysanthemi genome annotation as well as those of additional published enterobacterial genomes is underway and will be publicly accessible in the near future.

Gottardo, R., J. A. Pannucci, et al. (2003). "Statistical analysis of microarray data: a Bayesian approach." Biostatistics **4**(4): 597-620.

The potential of microarray data is enormous. It allows us to monitor the expression of thousands of genes simultaneously. A common task with microarray is to determine which genes are differentially expressed between two samples obtained under two different conditions. Recently, several statistical methods have been proposed to perform such a task when there are replicate samples under each condition. Two major problems arise with microarray data. The first one is that the number of replicates is

very small (usually 2-10), leading to noisy point estimates. As a consequence, traditional statistics that are based on the means and standard deviations, e.g. t-statistic, are not suitable. The second problem is that the number of genes is usually very large (approximately 10,000), and one is faced with an extreme multiple testing problem. Most multiple testing adjustments are relatively conservative, especially when the number of replicates is small. In this paper we present an empirical Bayes analysis that handles both problems very well. Using different parametrizations, we develop four statistics that can be used to test hypotheses about the means and/or variances of the gene expression levels in both one- and two-sample problems. The methods are illustrated using experimental data with prior knowledge. In addition, we present the result of a simulation comparing our methods to well-known statistics and multiple testing adjustments.

Gutierrez-Rios, R. M., D. A. Rosenblueth, et al. (2003). "Regulatory network of Escherichia coli: consistency between literature knowledge and microarray profiles." Genome Res **13**(11): 2435-43.

The transcriptional network of Escherichia coli may well be the most complete experimentally characterized network of a single cell. A rule-based approach was built to assess the degree of consistency between whole-genome microarray experiments in different experimental conditions and the accumulated knowledge in the literature compiled in RegulonDB, a data base of transcriptional regulation and operon organization in E. coli. We observed a high and statistical significant level of consistency, ranging from 70%-87%. When effector metabolites of regulatory proteins are not considered in the prediction of the active or inactive state of the regulators, consistency falls by up to 40%. Similarly, consistency decreases when rules for multiple regulatory interactions are altered or when "on" and "off" entries were assigned randomly. We modified the initial state of regulators and evaluated the propagation of errors in the network that do not correlate linearly with the connectivity of regulators. We interpret this deviation mainly as a result of the existence of redundant regulatory interactions. Consistency evaluation opens a new space of dialogue between theory and experiment, as the consequences of different assumptions can be evaluated and compared.

Halgren, R. G., M. R. Fielden, et al. (2001). "Assessment of clone identity and sequence fidelity for 1189 IMAGE cDNA clones." Nucleic Acids Res **29**(2): 582-8.

This report documents the error rate in a commercially distributed subset of the IMAGE Consortium mouse cDNA clone collection. After isolation of plasmid DNA from 1189 bacterial stock cultures, only 62. 2% were uncontaminated and contained cDNA inserts that had significant sequence identity to published data for the ordered clones. An agarose gel electrophoresis pre-screening strategy identified 361 stock cultures that appeared to contain two or more plasmid species. Isolation of individual colonies from these stocks demonstrated that 7.1% of the original 1189 stocks contained both a correct and an incorrect plasmid. 5.9% of the original 1189 stocks contained multiple, distinct, incorrect plasmids, indicating the likelihood of multiple contaminating events. While only 739 of the stocks purchased contained the desired cDNA clone, agarose gel pre-screening, colony isolation and similarity searching of dbEST allowed for the identification of an additional 420 clones that would have otherwise been discarded. Considering the high error rate in this subset of the IMAGE cDNA clone set, the use of sequence verified clones for cDNA microarray construction is warranted. When this is not possible, pre-screening non-sequence verified clones with agarose gel electrophoresis provides an inexpensive and efficient method to eliminate contaminated clones from the probe set.

Halitschke, R., K. Gase, et al. (2003). "Molecular interactions between the specialist herbivore Manduca sexta (lepidoptera, sphingidae) and its natural host Nicotiana attenuata. VI. Microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates." Plant Physiol **131**(4): 1894-902.

Evidence is accumulating that insect-specific plant responses are mediated by constituents in the oral secretions and regurgitants (R) of herbivores, however the relative importance of the different

potentially active constituents remains unclear. Fatty acid-amino acid conjugates (FACs) are found in the R of many insect herbivores and have been shown to be necessary and sufficient to elicit a set of herbivore-specific responses when the native tobacco plant Nicotiana attenuata is attacked by the tobacco hornworm, Manduca sexta. Attack by this specialist herbivore results in a large transcriptional reorganization in N. attenuata, and 161 genes have been cloned from previous cDNA differential display-polymerase chain reaction and subtractive hybridization with magnetic beads analysis. cDNAs of these genes, in addition to those of 73 new R-responsive genes identified by cDNA-amplified fragment-length polymorphism display of R-elicited plants, were spotted on polyepoxide coated glass slides to create microarrays highly enriched in Manduca spp.- and R-induced genes. With these microarrays, we compare transcriptional responses in N. attenuata treated with R from the two most damaging lepidopteran herbivores of this plant in nature, M. sexta and Manduca quinquemaculata, which have very similar FAC compositions in their R, and with the two most abundant FACs in Manduca spp. R. More than 68% of the genes up- and down-regulated by M. sexta R were similarly regulated by M. quinquemaculata R. A majority of genes up-regulated (64%) and down-regulated (49%) by M. sexta R were similarly regulated by treatment with the two FACs. In contrast, few genes showed similar transcriptional changes after H(2)O(2)- and R-treatment. These results demonstrate that the two most abundant FACs in Manduca spp. R can account for the majority of Manduca spp.-induced alterations of the wound response of N. attenuata.

Hamels, S., J. L. Gala, et al. (2001). "Consensus PCR and microarray for diagnosis of the genus Staphylococcus, species, and methicillin resistance." Biotechniques **31**(6): 1364-6, 1368, 1370-2.

We propose the use of DNA microarray for the discrimination of homologous products after a single PCR amplification with consensus primers. The method was applied to Staphylococcus identification. The femA nucleotide sequences, which are phylogenetically conserved among the staphylococci, were first amplified using a consensus primer pair together with the mecA sequence, a molecular marker for methicillin resistance. Products were then identified on a glass array. The microarray contained five selective DNA capture probes for the simultaneous and differential identification of the five most clinically relevant staphylococcal species (S. aureus, S. epidermidis, S. haemolyticus, S. hominis, and S. saprophyticus), while a consensus capture probe could detect all femA sequences, allowing the identification of the genus Staphylococcus. The mecA sequence hybridized to a specific capture probe. The identification was univocal because only a single capture probe had to be present for each sequence to be identified. The hybridization and identification processes were completed in less than 2 h. Current results demonstrate that low-density microarrays are powerful multigenotypic post-PCR analyzers and could compete with conventional bacteria identification.

Hamon, M. A., N. R. Stanley, et al. (2004). "Identification of AbrB-regulated genes involved in biofilm formation by Bacillus subtilis." Mol Microbiol **52**(3): 847-60.

Bacillus subtilis is a ubiquitous soil bacterium that forms biofilms in a process that is negatively controlled by the transcription factor AbrB. To identify the AbrB-regulated genes required for biofilm formation by B. subtilis, genome-wide expression profiling studies of biofilms formed by spo0A abrB and sigH abrB mutant strains were performed. These data, in concert with previously published DNA microarray analysis of spo0A and sigH mutant strains, led to the identification of 39 operons that appear to be repressed by AbrB. Eight of these operons had previously been shown to be repressed by AbrB, and we confirmed AbrB repression for a further six operons by reverse transcription-PCR. The AbrB-repressed genes identified in this study are involved in processes known to be regulated by AbrB, such as extracellular degradative enzyme production and amino acid metabolism, and processes not previously known to be regulated by AbrB, such as membrane bioenergetics and cell wall functions. To determine whether any of these AbrB-regulated genes had a role in biofilm formation, we tested 23 mutants, each with a disruption in a different AbrB-regulated operon, for the ability to form biofilms. Two mutants had a greater than twofold defect in biofilm formation. A yoaW mutant exhibited a biofilm structure with

reduced depth, and a sipW mutant exhibited only surface-attached cells and did not form a mature biofilm. YoaW is a putative secreted protein, and SipW is a signal peptidase. This is the first evidence that secreted proteins have a role in biofilm formation by Bacillus subtilis.

Han, J., H. Y. Yoo, et al. (2000). "Selective transcriptional regulations in the human liver cell by hepatitis B viral X protein." Biochem Biophys Res Commun **272**(2): 525-30.

The hepatitis B viral X protein (HBx) is known as a transcription factor and potential oncogene. To gain a better view of the effect of HBx on the transcriptional regulation in the human liver cell, we constructed a HepG2 cell line stably expressing HBx (HepG2-HBx), and performed cDNA microarray analysis on 588 cellular cDNAs comparing with untransformed control cells. Two genes (IGFR-2, RhoA) of oncogenes, one gene (p55CDC) of cell cycle regulators, three genes (thrombin receptor, MLK-3, MacMARCKS) of intracellular transducers, one gene (HSP27) of stress response proteins, two genes (FAST kinase, Bak) of apoptosis response proteins, one gene (p21(WAF)) of transcription factors were highly up-regulated; one gene (transcription elongation factor SII) of transcription factors and two genes (monocyte chemotactic protein 1, T-lymphocyte-secreted protein I-309) of growth factors were highly down-regulated. These results showed selective transcriptional regulation by HBx in the human liver cell.

Hansen, E. H., M. A. Schembri, et al. (2004). "Elucidation of the antibacterial mechanism of the Curvularia haloperoxidase system by DNA microarray profiling." Appl Environ Microbiol **70**(3): 1749-57.

A novel antimicrobial enzyme system, the Curvularia haloperoxidase system, was examined with the aim of elucidating its mechanism of antibacterial action. Escherichia coli strain MG1655 was stressed with sublethal concentrations of the enzyme system, causing a temporary arrest of growth. The expression of genes altered upon exposure to the Curvularia haloperoxidase system was analyzed by using DNA microarrays. Only a limited number of genes were involved in the response to the Curvularia haloperoxidase system. Among the induced genes were the ibpA and ibpB genes encoding small heat shock proteins, a gene cluster of six genes (b0301-b0306) of unknown function, and finally, cpxP, a member of the Cpx pathway. Knockout mutants were constructed with deletions in b0301-b0306, cpxP, and cpxARP, respectively. Only the mutant lacking cpxARP was significantly more sensitive to the enzyme system than was the wild type. Our results demonstrate that DNA microarray technology cannot be used as the only technique to investigate the mechanisms of action of new antimicrobial compounds. However, by combining DNA microarray analysis with the subsequent creation of knockout mutants, we were able to pinpoint one of the specific responses of E. coli--namely, the Cpx pathway, which is important for managing the stress response from the Curvularia haloperoxidase system.

Hansen-Hagge, T. E., U. Trefzer, et al. (2001). "Identification of sample-specific sequences in mammalian cDNA and genomic DNA by the novel ligation-mediated subtraction (Limes)." Nucleic Acids Res **29**(4): E20.

The representational difference analysis (RDA) and other subtraction techniques are used to enrich sample-specific sequences by elimination of ubiquitous sequences existing in both the sample of interest (tester) and the subtraction partner (driver). While applying the RDA to genomic DNA of cutaneous lymphoma cells in order to identify tumor relevant alterations, we predominantly isolated repetitive sequences and artificial repeat-mediated fusion products of otherwise independent PCR fragments (PCR hybrids). Since these products severely interfered with the isolation of tester-specific fragments, we developed a considerably more robust and efficient approach, termed ligation-mediated subtraction (Limes). In first applications of Limes, genomic sequences and/or transcripts of genes involved in the regulation of transcription, such as transforming growth factor beta stimulated clone 22 related gene (TSC-22R), cell death and cytokine production (caspase-1) or antigen presentation (HLA class II sequences), were found to be completely absent in a cutaneous lymphoma line. On the assumption that mutations in tumor-relevant genes can affect their transcription pattern, a protocol was developed and

successfully applied that allows the identification of such sequences. Due to these results, Limes may substitute/supplement other subtraction/comparison techniques such as RDA or DNA microarray techniques in a variety of different research fields.

Hatfield, G. W., S. P. Hung, et al. (2003). "Differential analysis of DNA microarray gene expression data." Mol Microbiol **47**(4): 871-7.

Here, we review briefly the sources of experimental and biological variance that affect the interpretation of high-dimensional DNA microarray experiments. We discuss methods using a regularized t-test based on a Bayesian statistical framework that allow the identification of differentially regulated genes with a higher level of confidence than a simple t-test when only a few experimental replicates are available. We also describe a computational method for calculating the global false-positive and false-negative levels inherent in a DNA microarray data set. This method provides a probability of differential expression for each gene based on experiment-wide false-positive and -negative levels driven by experimental error and biological variance.

Hayward, R. E. (2000). "Plasmodium falciparum phosphoenolpyruvate carboxykinase is developmentally regulated in gametocytes." Mol Biochem Parasitol **107**(2): 227-40.

Plasmodium species have the capacity to fix carbon dioxide during intracellular development. This process contributes to the pool of free amino acids and metabolites, which are the end products of glucose metabolism in the malaria parasite. A gene encoding phosphoenolpyruvate carboxykinase (PEPCK), an enzyme known to catalyze CO(2) fixation was identified in the genome of the human parasite Plasmodium falciparum by DNA microarray analysis experiments and was cloned and characterized. PfPEPCK is a 66.2 kDa, ATP-dependent enzyme which is closely related to PEPCK from plants and yeast but markedly different from the host enzyme human PEPCK. PfPEPCK transcript and active enzyme levels are upregulated in the transmissible and zygote stages of parasite development relative to the asexual blood stages. Elevated expression of PfPEPCK during the extracellular zygote phase of P. falciparum development within the microenvironment of the mosquito midgut may reflect a glucose-rare medium and suggests a possible switch in carbohydrate metabolism to a gluconeogenesis pathway.

Hayward, R. E., J. L. Derisi, et al. (2000). "Shotgun DNA microarrays and stage-specific gene expression in Plasmodium falciparum malaria." Mol Microbiol **35**(1): 6-14.

Malaria infects over 200 million individuals and kills 2 million young children every year. Understanding the biology of malarial parasites will be facilitated by DNA microarray technology, which can track global changes in gene expression under different physiological conditions. However, genomes of Plasmodium sp. (and many other important pathogenic organisms) remain to be fully sequenced so, currently, it is not possible to construct gene-specific microarrays representing complete malarial genomes. In this study, 3648 random inserts from a Plasmodium falciparum mung bean nuclease genomic library were used to construct a shotgun DNA microarray. Through differential hybridization and sequencing of relevant clones, large differences in gene expression were identified between the blood stage trophozoite form of the malarial parasite and the sexual stage gametocyte form. The present study lengthens our list of stage-specific transcripts in malaria by at least an order of magnitude above all previous studies combined. The results offer an unprecedented number of leads for developing transmission blocking agents and for developing vaccines directed at blood stage antigens. A significant fraction of the stage-selective transcripts had no sequence homologues in the current genome data bases, thereby underscoring the importance of the shotgun approach. The malarial shotgun microarray will be useful for unravelling additional important aspects of malaria biology and the general approach may be applied to any organism, regardless of how much of its genome is sequenced.

Heffelfinger, G. S., A. Martino, et al. (2002). "Carbon sequestration in Synechococcus Sp.: from

molecular machines to hierarchical modeling." Omics **6**(4): 305-30.

The U.S. Department of Energy recently announced the first five grants for the Genomes to Life (GTL) Program. The goal of this program is to "achieve the most far-reaching of all biological goals: a fundamental, comprehensive, and systematic understanding of life." While more information about the program can be found at the GTL website (www.doegenomestolife.org), this paper provides an overview of one of the five GTL projects funded, "Carbon Sequestration in Synechococcus Sp.: From Molecular Machines to Hierarchical Modeling." This project is a combined experimental and computational effort emphasizing developing, prototyping, and applying new computational tools and methods to elucidate the biochemical mechanisms of the carbon sequestration of Synechococcus Sp., an abundant marine cyanobacteria known to play an important role in the global carbon cycle. Understanding, predicting, and perhaps manipulating carbon fixation in the oceans has long been a major focus of biological oceanography and has more recently been of interest to a broader audience of scientists and policy makers. It is clear that the oceanic sinks and sources of CO(2) are important terms in the global environmental response to anthropogenic atmospheric inputs of CO(2) and that oceanic microorganisms play a key role in this response. However, the relationship between this global phenomenon and the biochemical mechanisms of carbon fixation in these microorganisms is poorly understood. The project includes five subprojects: an experimental investigation, three computational biology efforts, and a fifth which deals with addressing computational infrastructure challenges of relevance to this project and the Genomes to Life program as a whole. Our experimental effort is designed to provide biology and data to drive the computational efforts and includes significant investment in developing new experimental methods for uncovering protein partners, characterizing protein complexes, identifying new binding domains. We will also develop and apply new data measurement and statistical methods for analyzing microarray experiments. Our computational efforts include coupling molecular simulation methods with knowledge discovery from diverse biological data sets for high-throughput discovery and characterization of protein-protein complexes and developing a set of novel capabilities for inference of regulatory pathways in microbial genomes across multiple sources of information through the integration of computational and experimental technologies. These capabilities will be applied to Synechococcus regulatory pathways to characterize their interaction map and identify component proteins in these pathways. We will also investigate methods for combining experimental and computational results with visualization and natural language tools to accelerate discovery of regulatory pathways. Furthermore, given that the ultimate goal of this effort is to develop a systems-level of understanding of how the Synechococcus genome affects carbon fixation at the global scale, we will develop and apply a set of tools for capturing the carbon fixation behavior of complex of Synechococcus at different levels of resolution. Finally, because the explosion of data being produced by high-throughput experiments requires data analysis and models which are more computationally complex, more heterogeneous, and require coupling to ever increasing amounts of experimentally obtained data in varying formats, we have also established a companion computational infrastructure to support this effort as well as the Genomes to Life program as a whole.

Helmann, J. D., M. F. Wu, et al. (2001). "Global transcriptional response of Bacillus subtilis to heat shock." J Bacteriol **183**(24): 7318-28.

In response to heat stress, Bacillus subtilis activates the transcription of well over 100 different genes. Many of these genes are members of a general stress response regulon controlled by the secondary sigma factor, sigma(B), while others are under control of the HrcA or CtsR heat shock regulators. We have used DNA microarrays to monitor the global transcriptional response to heat shock. We find strong induction of known sigma(B)-dependent genes with a characteristic rapid induction followed by a return to near prestimulus levels. The HrcA and CtsR regulons are also induced, but with somewhat slower kinetics. Analysis of DNA sequences proximal to newly identified heat-induced genes leads us to propose ~70 additional members of the sigma(B) regulon. We have also identified numerous heat-induced genes that are not members of known heat shock regulons. Notably, we observe very strong induction of

arginine biosynthesis and transport operons. Induction of several genes was confirmed by quantitative reverse transcriptase PCR. In addition, the transcriptional responses measured by microarray hybridization compare favorably with the numerous previous studies of heat shock in this organism. Since many different conditions elicit both specific and general stress responses, knowledge of the heat-induced general stress response reported here will be helpful for interpreting future microarray studies of other stress responses.

Hentzer, M., H. Wu, et al. (2003). "Attenuation of Pseudomonas aeruginosa virulence by quorum sensing inhibitors." Embo J **22**(15): 3803-15.

Traditional treatment of infectious diseases is based on compounds that kill or inhibit growth of bacteria. A major concern with this approach is the frequent development of resistance to antibiotics. The discovery of communication systems (quorum sensing systems) regulating bacterial virulence has afforded a novel opportunity to control infectious bacteria without interfering with growth. Compounds that can override communication signals have been found in the marine environment. Using Pseudomonas aeruginosa PAO1 as an example of an opportunistic human pathogen, we show that a synthetic derivate of natural furanone compounds can act as a potent antagonist of bacterial quorum sensing. We employed GeneChip microarray technology to identify furanone target genes and to map the quorum sensing regulon. The transcriptome analysis showed that the furanone drug specifically targeted quorum sensing systems and inhibited virulence factor expression. Application of the drug to P.aeruginosa biofilms increased bacterial susceptibility to tobramycin and SDS. In a mouse pulmonary infection model, the drug inhibited quorum sensing of the infecting bacteria and promoted their clearance by the mouse immune response.

Hernandez, A., N. Karrow, et al. (2003). "Evaluation of immune responses of cattle as a means to identify high or low responders and use of a human microarray to differentiate gene expression." Genet Sel Evol **35 Suppl 1**: S67-81.

An immune response (IR) index to identify cows with high (H) and low (L) antibody-mediated immune responses (AMIR) had been previously devised. High AMIR associated with decreased mastitis and improved response to vaccination. Measurement of cell-mediated immune response (CMIR) was not included in the index; therefore various antigen/adjuvant combinations were evaluated as inducers of DTH to be added to the IR-index. The Bacillus Calmette Guerin (BCG)-induced/purified protein derivative (PPD)-elicited tuberculin skin test is a reliable measure of DTH; however, its use to identify livestock with high CMIR may be confounded due to previous exposure to Mycobacteria tuberculosis. DTH to BCG/PPD was therefore compared with that induced by Mycobacteria phlei (saprophyte) and its derivative phlein as the test antigen. Antibody to OVA was also evaluated. The results indicated that BCG/PPD and M. phlei/phlein induced similar DTH, but cross reaction to PPD was evident following induction of DTH using M. phlei making it a less than ideal alternative for testing livestock. Nonetheless, cows could be ranked for both AMIR and CMIR. RNA from two cows with the highest and lowest IR ranks was then used to probe a human 1.7 kD microarray to determine the ability of a human array to provide information on bovine genes associated with H and L.

Higgins, M. A., B. R. Berridge, et al. (2003). "Gene expression analysis of the acute phase response using a canine microarray." Toxicol Sci **74**(2): 470-84.

The safety of pharmaceuticals is typically assessed in the dog and rat prior to investigation in humans. As a result, a greater understanding of adverse effects in these preclinical testing species would improve safety assessment. Despite this need, there is a lack of tools to examine mechanisms and identify biomarkers in the dog. To address this issue, we developed an Affymetrix-based oligonucleotide microarray capable of monitoring the expression of thousands of canine genes in parallel. The custom canine array contains 22,774 probe sets, consisting of 13,729 canine and 9045 human-derived probe sets. To improve cross-species hybridization with human-derived probes, the detection region was moved from

the variable 3' UTR to the more homologous coding region. Testing of this strategy was accomplished by comparing hybridization of naive dog liver RNA to the canine array (coding region design) and human U133A array (standard 3' design). Although raw signal intensity was greater with canine-specific probe sets, human-derived probes detected the expression of additional liver transcripts. To assess the ability of this tool to detect differential gene expression, the acute phase response was examined in beagle dogs given lipopolysaccharide (LPS). Hepatic gene expression 4 and 24 h post-LPS administration was compared to gene expression profiles of vehicle-treated dogs (n=3/group). Array data was consistent with an acute inflammatory response, with transcripts for multiple cytokines and acute phase proteins markedly induced 4 h after LPS challenge. Robust changes in the expression of transcripts involved with glucose homeostasis, biotransformation, and extracellular matrix remodeling were observed 24 h post-dose. In addition, the canine array identified several potential biomarkers of hepatic inflammation. Strong correlations were found between gene expression data and alterations in clinical chemistry parameters such as serum amyloid A (SAA), albumin, and alkaline phosphatase (ALP). In summary, this new genomic tool successfully detected basal canine gene expression and identified novel aspects of the acute phase response in dog that shed new light on mechanisms underlying inflammatory processes.

Hill, J. M., W. J. Lukiw, et al. (2001). "Gene expression analyzed by microarrays in HSV-1 latent mouse trigeminal ganglion following heat stress." Virus Genes **23**(3): 273-80.

An understanding of the cellular genes whose expression is altered during HSV reactivation will enable us to better understand host responses and biochemical pathways involved in the process. Furthermore, this knowledge could allow us to develop gene-targeted inhibitors to prevent viral reactivation. Mice latent with HSV-1 strain McKrae and uninfected control mice were subjected to hyperthermic stress (43 degrees C for 10 min) and their trigeminal ganglia (TG) collected 1 h later. Two additional groups included HSV-1 latently infected and uninfected mice not subjected to hyperthermic stress. Poly A+ mRNA was enriched from total mouse TG RNA and reverse transcribed using MMLV RT. Radioactively labeled cDNAs were analyzed by microarray analysis. A stress/toxicology array of 149 mouse genes on a nylon membrane was used. The labeled cDNAs prepared from latently infected, stressed mice demonstrated 3-fold or greater increases in certain mRNA-early response genes (ERGs) compared to cDNAs from uninfected, stressed control mice. The ERG mRNAs that showed increases included two heat shock proteins (HSP60 and HSP40), a basic transcription factor (BTF T62), a DNA repair enzyme, two kinases [MAP kinase and a stress-induced protein kinase (SADK)], an oxidative stress-induced protein, a manganese superoxide dismutase precursor-2 (SOD-2), and cyclooxygenase 2 (COX-2). The gene expression in unstressed, infected TGs was similar to the gene expression in unstressed, uninfected controls. These results suggest that there is a significant difference in the ERG expression profile in latently infected TGs undergoing stress-induced reactivation compared to uninfected Tgs.

Hiscott, J., N. Grandvaux, et al. (2003). "Convergence of the NF-kappaB and interferon signaling pathways in the regulation of antiviral defense and apoptosis." Ann N Y Acad Sci **1010**: 237-48.

The ubiquitously expressed interferon regulatory factor 3 (IRF-3) is directly activated following virus infection and functions as a key activator of the immediate-early Type 1 interferon (IFN) genes. Using DNA microarray analysis (8,556 genes) in Jurkat T cells inducibly expressing constitutively active IRF-3, several target genes directly regulated by IRF-3 were identified. Among the genes upregulated by IRF-3 were transcripts for a subset of known IFN-stimulated genes (ISGs), including ISG56, which functions as an inhibitor of translation initiation. Phosphorylation of C-terminal Ser/Thr residues--(382)GGASSLENTVDLHISNSHPLSLTSDQY(408)-is required for IRF-3 activation. Using C-terminal point mutations and a novel phosphospecific antibody, Ser396 was characterized as the minimal phosphoacceptor site required in vivo for IRF-3 activation following Sendai virus (SeV) infection, expression of viral nucleocapsid, or double-stranded RNA (dsRNA) treatment. The identity of the virus-activated kinase (VAK) activity that targets and activates IRF-3 and IRF-7 has remained a critical missing

link in the understanding of interferon signaling. We report that the IKK-related kinases-IKKepsilon/TBK-1-are components of VAK that mediate IRF-3 and IRF-7 phosphorylation and thus functionally link the NF-kappaB and IRF pathways in the development of the antiviral response.

Hoang, H. H., A. Becker, et al. (2004). "The LuxR homolog ExpR, in combination with the Sin quorum sensing system, plays a central role in Sinorhizobium meliloti gene expression." J Bacteriol **186**(16): 5460-72.

Quorum sensing, a population density-dependent mechanism for bacterial communication and gene regulation, plays a crucial role in the symbiosis between alfalfa and its symbiont Sinorhizobium meliloti. The Sin system, one of three quorum sensing systems present in S. meliloti, controls the production of the symbiotically active exopolysaccharide EPS II. Based on DNA microarray data, the Sin system also seems to regulate a multitude of S. meliloti genes, including genes that participate in low-molecular-weight succinoglycan production, motility, and chemotaxis, as well as other cellular processes. Most of the regulation by the Sin system is dependent on the presence of the ExpR regulator, a LuxR homolog. Gene expression profiling data indicate that ExpR participates in additional cellular processes that include nitrogen fixation, metabolism, and metal transport. Based on our microarray analysis we propose a model for the regulation of gene expression by the Sin/ExpR quorum sensing system and another possible quorum sensing system(s) in S. meliloti.

Hohlweg, U., M. Hosel, et al. (2003). "Intraperitoneal dissemination of Ad12-induced undifferentiated neuroectodermal hamster tumors: de novo methylation and transcription patterns of integrated viral and of cellular genes." Virus Res **98**(1): 45-56.

The intramuscular (i.m.) injection of human adenovirus type 12 (Ad12) into newborn Syrian hamsters caused widespread dissemination of up to 15 tumors over the entire peritoneal cavity in 70-90% of the animals within 30-50 days. Subcutaneous (s.c.) injections led to local tumor formation only. Independent of location, tumor histology revealed Homer-Wright rosette-like structures typical for primitive neuroectodermal tumors (PNET). All tumor cells showed markers indicative of neuroectodermal and mesenchymal derivations. Each Ad12-induced tumor cell carried multiple copies of integrated Ad12 genomes at one chromosomal site which was different for each tumor. For Ad12 tumor induction in hamsters, the patterns of Ad12 viral and cellular gene expression were important and were affected by changes in DNA methylation, both in the integrated Ad12 DNA and the cellular genome. By applying the bisulfite protocol, the de novo DNA methylation in the integrated Ad12 genomes was determined. These patterns were complex, characterized by regional initiation and by excluding genome segments in the E1A and E1B promoters. In all tumors, the Ad12 segments E1A, E1B, E2A, parts of E3 and E4 were similarly transcribed, as shown by the RT-PCR and DNA microarray methods. Changes in the transcription of a large number of cellular genes was assessed by using mouse gene microarrays encompassing about 1980 different mouse genes with 87-96% homology to hamster genes. Similarities and differences existed in the transcription of cellular genes of different functional classes among the different Ad12-induced tumors. These alterations in cellular gene transcription may be an important parameter in the oncogenic transformation by Ad12.

Hornbaek, T., A. K. Nielsen, et al. (2004). "The effect of inoculum age and solid versus liquid propagation on inoculum quality of an industrial Bacillus licheniformis strain." FEMS Microbiol Lett **236**(1): 145-51.

Shorter lag phases were obtained in cultivations of Bacillus licheniformis using early-compared to late-stationary growth phase inocula and using liquid versus solid propagation medium. Flow cytometry and fluorescence ratio imaging microscopy (FRIM) after staining with 5(6)-carboxyfluorescein diacetate succinimidyl ester (CFDA-SE), confirmed that liquid early-stationary growth phase inoculum had a higher vitality and was more homogeneous than solid late-stationary growth phase inoculum. DNA-microarray analyses indicated that liquid early-stationary growth phase inoculum was in a more active

state in terms of cell multiplication whereas solid late-stationary growth phase inoculum was induced to some spore formation potentially causing delayed growth initiation.

Hot, D., R. Antoine, et al. (2003). "Differential modulation of Bordetella pertussis virulence genes as evidenced by DNA microarray analysis." Mol Genet Genomics **269**(4): 475-86.

The production of most factors involved in Bordetella pertussis virulence is controlled by a two-component regulatory system termed BvgA/S. In the Bvg+ phase virulence-activated genes (vags) are expressed, and virulence-repressed genes (vrgs) are down-regulated. The expression of these genes can also be modulated by MgSO(4) or nicotinic acid. In this study we used microarrays to analyse the influence of BvgA/S or modulation on the expression of nearly 200 selected genes. With the exception of one vrg, all previously known vags and vrgs were correctly assigned as such, and the microarray analyses identified several new vags and vrgs, including genes coding for putative autotransporters, two-component systems, extracellular sigma factors, the adenylate cyclase accessory genes cyaBDE, and two genes coding for components of a type III secretion system. For most of the new vrgs and vags the results of the microarray analyses were confirmed by RT-PCR analysis and/or lacZfusions. The degree of regulation and modulation varied between genes, and showed a continuum from strongly BvgA/S-activated genes to strongly BvgA/S-repressed genes. The microarray analyses also led to the identification of a subset of vags and vrgs that are differentially regulated and modulated by MgSO(4) or nicotinic acid, indicating that these genes may be targets for multiple regulatory circuits. For example, the expression of bilA, a gene predicted to encode an intimin-like protein, was found to be activated by BvgA/S and up-modulated by nicotinic acid. Furthermore, surprisingly, in the strain analysed here, which produces only type 2 fimbriae, the fim3 gene was identified as a vrg, while fim2 was confirmed to be a vag.

Hottes, A. K., M. Meewan, et al. (2004). "Transcriptional profiling of Caulobacter crescentus during growth on complex and minimal media." J Bacteriol **186**(5): 1448-61.

Microarray analysis was used to examine gene expression in the freshwater oligotrophic bacterium Caulobacter crescentus during growth on three standard laboratory media, including peptone-yeast extract medium (PYE) and minimal salts medium with glucose or xylose as the carbon source. Nearly 400 genes (approximately 10% of the genome) varied significantly in expression between at least two of these media. The differentially expressed genes included many encoding transport systems, most notably diverse TonB-dependent outer membrane channels of unknown substrate specificity. Amino acid degradation pathways constituted the largest class of genes induced in PYE. In contrast, many of the genes upregulated in minimal media encoded enzymes for synthesis of amino acids, including incorporation of ammonia and sulfate into glutamate and cysteine. Glucose availability induced expression of genes encoding enzymes of the Entner-Doudoroff pathway, which was demonstrated here through mutational analysis to be essential in C. crescentus for growth on glucose. Xylose induced expression of genes encoding several hydrolytic exoenzymes as well as an operon that may encode a novel pathway for xylose catabolism. A conserved DNA motif upstream of many xylose-induced genes was identified and shown to confer xylose-specific expression. Xylose is an abundant component of xylan in plant cell walls, and the microarray data suggest that in addition to serving as a carbon source for growth of C. crescentus, this pentose may be interpreted as a signal to produce enzymes associated with plant polymer degradation.

Hu, Y., J. E. Ippolito, et al. (2002). "Molecular characterization of a metastatic neuroendocrine cell cancer arising in the prostates of transgenic mice." J Biol Chem **277**(46): 44462-74.

The features and functions of prostatic neuroendocrine (NE) cells remain ill-defined. Neuroendocrine differentiation (NED) in adenocarcinoma of the human prostate (CaP) is associated with more aggressive disease, but the underlying mediators are poorly understood. We examined these issues in transgenic mice that utilize regulatory elements from the cryptdin-2 gene (Defcr2) to express simian

virus 40 large T antigen (TAg) in prostatic NE cells. CR2-TAg mice develop prostatic intraepithelial neoplasia at 8 weeks of age, 1 week after the onset of TAg expression. An invasive phase follows 2-4 weeks later, with lymph node, liver, lung, brain, and bone metastases appearing within 16 weeks. DNA microarray studies revealed 122 mRNAs that were increased >/=2-fold in duplicate assays of 16-week-old CR2-TAg versus normal prostates. Thirty two transcripts encode proteins associated with neurons and endocrine cells (e.g. basic helix loop helix, SRY-related high mobility group box and sine-oculis homeobox transcription factors, Hu RNA-binding proteins, neuronatin, Racgap1, collapsin response mediator protein-1, synaptotagmin-1, proprotein convertase, and secretogranins). Follow-up studies of candidate mediators and biomarkers of differentiation/growth in the microarray data set involved real time quantitative reverse transcriptase-PCR assays of laser capture microdissected NE cells from CR2-TAg prostates plus liver metastases, and immunohistochemical comparisons of transgenic mouse prostates and 35 human CaP samples. Our findings include (a) expression of the bHLH mouse achaete-scute homolog (mASH1) in normal and CR2-TAg NE cells and foci of NED in human CaP, (b) glutamic acid decarboxylase and its product (gamma-aminobutyric acid) in neoplastic NE cells juxtaposed next to cohorts of normal gamma-aminobutyric acid receptor expressing secretory cells (a potential route for paracrine interactions between these two epithelial lineages), and (c) aromatic l-amino-acid decarboxylase, but not its dopamine/serotonin products, in CR2-TAg NE cells and NED. These results underscore the value of CR2-TAg mice for characterizing normal NE cell biology and tumorigenesis.

Hua, Q., C. Yang, et al. (2004). "Analysis of gene expression in Escherichia coli in response to changes of growth-limiting nutrient in chemostat cultures." Appl Environ Microbiol **70**(4): 2354-66.

Studies of steady-state metabolic fluxes in Escherichia coli grown in nutrient-limited chemostat cultures suggest remarkable flux alterations in response to changes of growth-limiting nutrient in the medium (Hua et al., J. Bacteriol. 185:7053-7067, 2003). To elucidate the physiological adaptation of cells to the nutrient condition through the flux change and understand the molecular mechanisms underlying the change in the flux, information on gene expression is of great importance. DNA microarray analysis was performed to investigate the global transcriptional responses of steady-state cells grown in chemostat cultures with limited glucose or ammonia while other environmental conditions and the growth rate were kept constant. In slow-growing cells (specific growth rate of 0.10 h(-1)), 9.8% of a total of 4,071 genes investigated, especially those involved in amino acid metabolism, central carbon and energy metabolism, transport system and cell envelope, were observed to be differentially expressed between the two nutrient-limited cultures. One important characteristic of E. coli grown under nutrient limitation was its capacity to scavenge carbon or nitrogen from the medium through elevating the expression of the corresponding transport and assimilation genes. The number of differentially expressed genes in faster-growing cells (specific growth rate of 0.55 h(-1)), however, decreased to below half of that in slow-growing cells, which could be explained by diverse transcriptional responses to the growth rate under different nutrient limitations. Independent of the growth rate, 92 genes were identified as being differentially expressed. Genes tightly related to the culture conditions were highlighted, some of which may be used to characterize nutrient-limited growth.

Huang, J., T. Fan, et al. (2004). "Lsh, an epigenetic guardian of repetitive elements." Nucleic Acids Res **32**(17): 5019-28.

The genome is burdened with repetitive sequences that are generally embedded in silenced chromatin. We have previously demonstrated that Lsh (lymphoid-specific helicase) is crucial for the control of heterochromatin at pericentromeric regions consisting of satellite repeats. In this study, we searched for additional genomic targets of Lsh by examining the effects of Lsh deletion on repeat regions and single copy gene sequences. We found that the absence of Lsh resulted in an increased association of acetylated histones with repeat sequences and transcriptional reactivation of their silenced state. In contrast, selected single copy genes displayed no change in histone acetylation levels, and their transcriptional rate was indistinguishable compared to Lsh-deficient cells and wild-type controls.

Microarray analysis of total RNA derived from brain and liver tissues revealed that <0.4% of the 15 247 examined loci were abnormally expressed in Lsh-/-embryos and almost two-thirds of these deregulated sequences contained repeats, mainly retroviral LTR (long terminal repeat) elements. Chromatin immunoprecipitation analysis demonstrated a direct interaction of Lsh with repetitive sites in the genome. These data suggest that the repetitive sites are direct targets of Lsh action and that Lsh plays an important role as 'epigenetic guardian' of the genome to protect against deregulation of parasitic retroviral elements.

Huang, X. and L. D. Hazlett (2003). "Analysis of Pseudomonas aeruginosa corneal infection using an oligonucleotide microarray." Invest Ophthalmol Vis Sci **44**(8): 3409-16.

PURPOSE: To compare the early gene expression pattern of normal versus Pseudomonas aeruginosa-infected corneas in resistant (cornea heals) versus susceptible (cornea perforates) mice. METHODS: A microarray analysis of normal versus postinfection (PI) day 1 BALB/c and B6 corneas was performed with a murine gene microarray. Real-time RT-PCR was used to confirm the microarray pattern selectively. RESULTS: The 1257 regulated transcripts detected were organized into nine clusters by a self-organizing map (SOM) algorithm according to their different behavior in each mouse group. At least three groups of genes associated with a CD4(+) T-cell type-1 (Th1) immune response and three clusters linked with a type-2 T-cell (Th2) response were identified. Biological categorization revealed that the cornea of B6 mice showed a dominant type-1-like immune response profile, whereas BALB/c mice showed a dominant type-2-like profile. In addition, expression of several genes that promote apoptosis (e.g., caspase-9) was upregulated in BALB/c mouse cornea, whereas genes with apoptosis-inhibiting activity (e.g., BCL2) were significantly upregulated in B6 mouse cornea. The infected cornea of BALB/c mice also showed increased gene expression of factors associated with matrix remodeling and tissue repair (e.g., tissue inhibitor of matrix metalloproteinase [TIMP-2] and epidermal growth factor [EGF]) and/or bacterial killing (e.g., inducible nitric oxide synthase [iNOS]). CONCLUSIONS: The data provide new insight into biological processes involved in Pseudomonas aeruginosa keratitis and confirm that B6 mice are Th1 and BALB/c mice are Th2 cytokine responsive to bacterial antigen early after challenge with P. aeruginosa.

Huang, Y., Y. Uchiyama, et al. (2001). "A human hepatoma cell line expressing hepatitis c virus nonstructural proteins tightly regulated by tetracycline." Biochem Biophys Res Commun **281**(3): 732-40.

Nonstructural (NS) proteins of hepatitis C virus (HCV) play major roles in viral replication and the pathogenesis of liver diseases. Current studies on antiviral strategies targeting these proteins have been hampered by the lack of efficient cell culture systems. Combining tetracycline-regulated gene expressing system and enhanced green fluorescent protein (EGFP), we generated a human hepatoma cell line inducibly expressing the HCV NS proteins. This cell line exhibited high induction of a full NS transcript (approximately 7 kb). In the absence of tetracycline, NS proteins 3, 4A, and 5A of mature sizes were detected by immunoblot analysis and the induction of NS proteins 3 to 5B are confirmed by immunofluorescent staining. Using DNA microarray analysis, we characterized the changes in mRNA expression profile of 6416 genes and identified several genes, whose mRNAs are specifically upregulated by the induction of NS proteins. This cell line provides a unique in vitro hepatoma cell system for the investigation of structural and functional properties of HCV NS proteins.

Huang, Z. X. and K. T. Yao (2004). "[Mining gene expression microarray data of nasopharyngeal carcinoma by literature profiling]." Di Yi Jun Yi Da Xue Xue Bao **24**(7): 798-801.

OBJECTIVE: To study abnormal signal pathway in nasopharyngeal carcinoma (NPC). METHOD: NPC gene expression microarray data was mined by analysis of literature profiles generated by extracting the frequencies of certain terms from the abstracts stored in the Medline literature database. The terms were then filtered on the basis of both repetitive occurrence and co-occurrence among multiple gene entries. Finally, clustering analysis was performed on the retained frequency values, shaping a coherent picture of the functional relationship among large and heterogeneous lists of genes. RESULT:

Sixteen function groups were found among 112 abnormally expressed genes, including 4 groups indicative of Epstein-Barr virus (EBV) infection, 6 groups indicative of normal nasopharyngeal tissues that acquired essential capabilities to develop into tumor, 2 groups involved in energy metabolism, 1 group suggesting abnormal phosphorylation of proteins, 2 groups related to other diseases, and 1 group associated with muscle activities. The pathways of p53 and Rb, which were frequently abnormal during tumor progression, were not found in these groups. CONCLUSION: Initiation and progression of NPC may be caused by special signal transduction pathways.

Hugot, K., M. P. Riviere, et al. (2004). "Coordinated regulation of genes for secretion in tobacco at late developmental stages: association with resistance against oomycetes." Plant Physiol **134**(2): 858-70.

Besides the systemic acquired resistance (SAR) induced in response to microbial stimulation, host plants may also acquire resistance to pathogens in response to endogenous stimuli associated with their own development. In tobacco (Nicotiana tabacum), the vegetative-to-flowering transition comes along with a susceptibility-to-resistance transition to the causal agent of black shank disease, the oomycete Phytophthora parasitica. This resistance affects infection effectiveness and hyphal expansion and is associated with extracellular accumulation of a cytotoxic activity that provokes in vitro cell death of P. parasitica zoospores. As a strategy to determine the extracellular events important for restriction of pathogen growth, we screened the tobacco genome for genes encoding secreted or membrane-bound proteins expressed in leaves of flowering plants. Using a signal sequence trap approach in yeast (Saccharomyces cerevisiae), 298 clones were selected that appear to encode for apoplastic, cell wall, or membrane-bound proteins involved in stress response, in plant defense, or in cell wall modifications. Microarray and northern-blot analyses revealed that, at late developmental stages, leaves were characterized by the coordinate up-regulation of genes involved in SAR and in peroxidative cross-linking of structural proteins to cell wall. This suggests the potential involvement of these genes in extracellular events that govern the expression of developmental resistance. The analysis of the influence of salicylic acid on mRNA accumulation also indicates a more complex network for regulation of gene expression at a later stage of tobacco development than during SAR. Further characterization of these genes will permit the formulation of hypotheses to explain resistance and to establish the connection with development.

Hui, D., J. Iqbal, et al. (2003). "Molecular interactions between the specialist herbivore Manduca sexta (lepidoptera, sphingidae) and its natural host Nicotiana attenuata: V. microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs." Plant Physiol **131**(4): 1877-93.

We extend our analysis of the transcriptional reorganization that occurs when the native tobacco, Nicotiana attenuata, is attacked by Manduca sexta larvae by cloning 115 transcripts by mRNA differential display reverse transcription-polymerase chain reaction and subtractive hybridization using magnetic beads (SHMB) from the M. sexta-responsive transcriptome. These transcripts were spotted as cDNA with eight others, previously confirmed to be differentially regulated by northern analysis on glass slide microarrays, and hybridized with Cy3- and Cy5-labeled probes derived from plants after 2, 6, 12, and 24 h of continuous attack. Microarray analysis proved to be a powerful means of verifying differential expression; 73 of the cloned genes (63%) were differentially regulated (in equal proportions from differential display reverse transcription-polymerase chain reaction and SHMB procedures), and of these, 24 (32%) had similarity to known genes or putative proteins (more from SHMB). The analysis provided insights into the signaling and transcriptional basis of direct and indirect defenses used against herbivores, suggesting simultaneous activation of salicylic acid-, ethylene-, cytokinin-, WRKY-, MYB-, and oxylipin-signaling pathways and implicating terpenoid-, pathogen-, and cell wall-related transcripts in defense responses. These defense responses require resources that could be made available by decreases in four photosynthetic-related transcripts, increases in transcripts associated with protein and nucleotide turnover, and increases in transcripts associated with carbohydrate metabolism. This putative up-regulation of defense-associated and down-regulation of growth-associated transcripts occur against a backdrop of altered transcripts for RNA-binding proteins, putative ATP/ADP translocators, chaperonins,

histones, and water channel proteins, responses consistent with a major metabolic reconfiguration that underscores the complexity of response to herbivore attack.

Hung, S. P., P. Baldi, et al. (2002). "Global gene expression profiling in Escherichia coli K12. The effects of leucine-responsive regulatory protein." J Biol Chem **277**(43): 40309-23.

Leucine-responsive regulatory protein (Lrp) is a global regulatory protein that affects the expression of multiple genes and operons in bacteria. Although the physiological purpose of Lrp-mediated gene regulation remains unclear, it has been suggested that it functions to coordinate cellular metabolism with the nutritional state of the environment. The results of gene expression profiles between otherwise isogenic lrp(+) and lrp(-) strains of Escherichia coli support this suggestion. The newly discovered Lrp-regulated genes reported here are involved either in small molecule or macromolecule synthesis or degradation, or in small molecule transport and environmental stress responses. Although many of these regulatory effects are direct, others are indirect consequences of Lrp-mediated changes in the expression levels of other global regulatory proteins. Because computational methods to analyze and interpret high dimensional DNA microarray data are still an early stage, much of the emphasis of this work is directed toward the development of methods to identify differentially expressed genes with a high level of confidence. In particular, we describe a Bayesian statistical framework for a posterior estimate of the standard deviation of gene measurements based on a limited number of replications. We also describe an algorithm to compute a posterior estimate of differential expression for each gene based on the experiment-wide global false positive and false negative level for a DNA microarray data set. This allows the experimenter to compute posterior probabilities of differential expression for each individual differential gene expression measurement.

Huser, A. T., A. Becker, et al. (2003). "Development of a Corynebacterium glutamicum DNA microarray and validation by genome-wide expression profiling during growth with propionate as carbon source." J Biotechnol **106**(2-3): 269-86.

A DNA microarray was developed to analyse global gene expression of the amino acid-producing bacterium Corynebacterium glutamicum. PCR products representing 93.4% of the predicted C. glutamicum genes were prepared and spotted in quadruplicate onto 3-aminopropyltrimethoxysilane-coated glass slides. The applicability of the C. glutamicum DNA microarray was demonstrated by co-hybridisation with fluorescently labelled cDNA probes. Analysis of the technical variance revealed that C. glutamicum genes detected with different intensities resulting in ratios greater than 1.52 or smaller than -1.52 can be regarded as differentially expressed with a confidence level of greater than 95%. In a validation example, we measured changes of the mRNA levels during growth of C. glutamicum with acetate and propionate as carbon sources. Acetate-grown C. glutamicum cultures were used as reference. At the 95% confidence interval, 117 genes revealed increased transcript levels in the presence of propionate, while 43 genes showed a decreased expression compared with the acetate-grown culture. Global expression profiling confirmed the induction of the prpD2B2C2 gene cluster already known to be essential for propionate degradation via the 2-methylcitrate cycle. Besides many genes of unknown function, the paralogous prpD1B1C1 gene cluster as well as fasI-B (encoding fatty-acid synthase IB), dtsR1 and dtsR2 (components of acyl-CoA carboxylases), gluABCD (glutamate transport system), putP (proline transport system), and pyc (pyruvate carboxylase) showed significantly increased expression levels. Differential expression of these genes was confirmed by real-time reverse transcription (RT) PCR assays.

Microarray Technology

I-P

Iizuka, N., M. Oka, et al. (2004). "Molecular signature in three types of hepatocellular carcinoma with

different viral origin by oligonucleotide microarray." Int J Oncol **24**(3): 565-74.

Chronic infection with hepatitis B or C virus (HBV or HCV) is the most clearly established risk factor for hepato-cellular carcinoma (HCC). One type of HCC (non-B, non-C HCC) also appears to develop in patients negative for both HBV and HCV. Using a supervised learning method, we investigated gene expression in 11 non-B, non-C HCCs with high-density oligonucleotide microarrays, and compared the patterns of gene expression with those of HBV-infected HCCs (B-type HCCs) and HCV-infected HCCs (C-type HCCs) in the previous dataset. Our gene selection identified 112 and 64 genes that were differentially expressed in non-B, non-C HCC in comparison with B- and C-type HCCs, respectively. In both gene selections, we found that the false discovery rate, the percentage of genes identified by chance, was less than 5%. Additionally, in combination with the previous data, our present data revealed a set of genes specific to each type of B- and C-type HCCs and non-B, non-C HCC. Among these, an interferon-induced gene, IFI27, was differentially expressed among all three types of HCCs, and this result was confirmed by RT-PCR. Thus, our present study provides a framework to characterize the molecular features in the three subtypes of HCC with different viral origin.

Iizuka, N., M. Oka, et al. (2003). "Differential gene expression in distinct virologic types of hepatocellular carcinoma: association with liver cirrhosis." Oncogene **22**(19): 3007-14.

Using oligonucleotide microarray data of 45 hepatocellular carcinoma (HCC) samples, we evaluated gene expression in hepatitis B virus-positive and hepatitis C virus-positive HCCs (HBV- and HCV-HCCs) for an association with liver cirrhosis (LC). In all, 89 genes were expressed differentially between HBV-HCCs associated with LC and those not associated with LC. Among them, tumors from LC patients showed significantly lower expression levels of 72 genes and significantly higher levels of 17 genes than the levels found in tumors from non-LC patients. The former included genes responsible for signal transduction, transcription, metabolism, and cell growth. The latter included a tumor suppressor gene and a cell-growth-related gene. Only eight genes were expressed differentially between HCV-HCCs associated with and without LC. Our findings provide as a framework for clarifying the role of LC in HBV- and HCV-related hepatocarcinogenesis.

Inaba, M., I. Suzuki, et al. (2003). "Gene-engineered rigidification of membrane lipids enhances the cold inducibility of gene expression in synechocystis." J Biol Chem **278**(14): 12191-8.

A sudden decrease in ambient temperature induces the expression of a number of genes in poikilothermic organisms. We report here that the cold inducibility of gene expression in Synechocystis sp. PCC 6803 was enhanced by the rigidification of membrane lipids that was engineered by disruption of genes for fatty acid desaturases. DNA microarray analysis revealed that cold-inducible genes could be divided into three groups according to the effects of the rigidification of membrane lipids. The first group included genes whose expression was not induced by cold in wild-type cells but became strongly cold-inducible upon rigidification of membrane lipids. This group included certain heat-shock genes, genes for subunits of the sulfate transport system, and the hik34 gene for a histidine kinase. The second group consisted of genes whose cold inducibility was moderately enhanced by the rigidification of membrane lipids. Most genes in this group encoded proteins of as yet unknown function. The third group consisted of genes whose cold inducibility was unaffected by the rigidification of membrane lipids. This group included genes for an RNA helicase and an RNA-binding protein. DNA microarray analysis also indicated that the rigidification of membrane lipids had no effect on the heat inducibility of gene expression. Hik33, a cold-sensing histidine kinase, regulated the expression of most genes in the second and third groups but of only a small number of genes in the first group, an observation that suggests that the cold-inducible expression of genes in the first group might be regulated by a cold sensor that remains to be identified.

Inoue, K., J. Chen, et al. (2002). "Specific growth inhibition by acetate of an Escherichia coli strain expressing Era-dE, a dominant negative Era mutant." J Mol Microbiol Biotechnol **4**(4): 379-88.

Escherichia coli Era is a GTP binding protein and essential for cell growth. We have previously reported that an Era mutant, designated Era-dE, causes a dominant negative effect on the growth and the loss of the ability to utilize TCA cycle metabolites as carbon source when overproduced. To investigate the role of Era, the gene expression in the cells overproducing Era-dE was examined by DNA microarray analysis. The expression of lipA and nadAB, which are involved in lipoic acid synthesis and NAD synthesis, respectively, was found to be reduced in the cells overproducing Era-dE. Lipoic acid and NAD are essential cofactors for the activities of pyruvate dehydrogenase complex, 2-oxoglutarate dehydrogenase complex and glycine cleavage enzyme complex. The expression of numerous genes involved in dissimilatory carbon metabolism and carbon source transport was increased. This set of genes partially overlaps with the set of genes controlled by cAMP-CAP in E coli. Moreover, the growth defect of Era-dE overproduction was specifically enhanced by acetate but not by TCA cycle metabolites both in rich and synthetic media. Intracellular serine pool in Era-dE overproducing cells was found to be increased significantly compared to that of the cells overproducing wild-type Era. It was further found that even the wild-type E. coli cells not overproducing Era-dE became sensitive to acetate in the presence of serine in a medium. We propose that when Era-dE is overproduced, carbon fluxes to the TCA cycle and to C1 units become impaired, resulting in a higher cellular serine concentration. We demonstrated that such cells with a high serine concentration became sensitive to acetate, however the reason for this acetate sensitivity is not known at the present.

Irving, P., L. Troxler, et al. (2001). "A genome-wide analysis of immune responses in Drosophila." Proc Natl Acad Sci U S A **98**(26): 15119-24.

Oligonucleotide DNA microarrays were used for a genome-wide analysis of immune-challenged Drosophila infected with Gram-positive or Gram-negative bacteria, or with fungi. Aside from the expression of an established set of immune defense genes, a significant number of previously unseen immune-induced genes were found. Genes of particular interest include corin- and Stubble-like genes, both of which have a type II transmembrane domain; easter- and snake-like genes, which may fulfil the roles of easter and snake in the Toll pathway; and a masquerade-like gene, potentially involved in enzyme regulation. The microarray data has also helped to greatly reduce the number of target genes in large gene groups, such as the proteases, helping to direct the choices for future mutant studies. Many of the up-regulated genes fit into the current conceptual framework of host defense, whereas others, including the substantial number of genes with unknown functions, offer new avenues for research.

Ishige, T., M. Krause, et al. (2003). "The phosphate starvation stimulon of Corynebacterium glutamicum determined by DNA microarray analyses." J Bacteriol **185**(15): 4519-29.

The phosphate (P(i)) starvation stimulon of Corynebacterium glutamicum was characterized by global gene expression analysis by using DNA microarrays. Hierarchical cluster analysis of the genes showing altered expression 10 to 180 min after a shift from P(i)-sufficient to P(i)-limiting conditions led to identification of five groups comprising 92 genes. Four of these groups included genes which are not directly involved in P metabolism and changed expression presumably due to the reduced growth rate observed after the shift or to the exchange of medium. One group, however, comprised 25 genes, most of which are obviously related to phosphorus (P) uptake and metabolism and exhibited 4- to >30-fold-greater expression after the shift to P(i) limitation. Among these genes, the RNA levels of the pstSCAB (ABC-type P(i) uptake system), glpQ (glycerophosphoryldiester phosphodiesterase), ugpAEBC (ABC-type sn-glycerol 3-phosphate uptake system), phoH (unknown function), nucH (extracellular nuclease), and Cgl0328 (5'-nucleotidase or related esterase) genes were increased, and pstSCAB exhibited a faster response than the other genes. Transcriptional fusion analyses revealed that elevated expression of pstSCAB and ugpAEBC was primarily due to transcriptional regulation. Several genes also involved in P uptake and metabolism were not affected by P(i) starvation; these included the genes encoding a PitA-like P(i) uptake system and a putative Na(+)-dependent P(i) transporter and the genes involved in the metabolism of pyrophosphate and polyphosphate. In summary, a global, time-resolved picture of the

response of C. glutamicum to P(i) starvation was obtained.

Islam, T. C., J. Lindvall, et al. (2002). "Expression profiling in transformed human B cells: influence of Btk mutations and comparison to B cell lymphomas using filter and oligonucleotide arrays." Eur J Immunol **32**(4): 982-93.

We have used both Clontech Atlas Human Hematology/Immunology cDNA microarrays, containing 588 genes, and Affymetrix oligonucleotide U95Av2 human array complementary to more than 12,500 genes to get a global view of genes expressed in Epstein-Barr virus (EBV)-transformed B cells and genes regulated by Bruton's tyrosine kinase (Btk). We compared EBV-transformed wild-type (WT) B cells from a healthy individual, WT1 and an X-linked agammaglobulinemia (XLA) patient cell line, XLA1, using the Clontech filters arrays. Eleven genes were > or =1.9-fold induced in absence of functional Btk. Furthermore, we analyzed a second patient cell line, XLA2, and compared this to two WT cell lines using oligonucleotide arrays. A total of 391 genes were found to be differentially expressed, including kinases and transcriptions factors. Furthermore, one expressed sequence tag and eight complementary DNA clones with unknown function were down-regulated in XLA2, indicating their biological role. Higher-fold inductions, Fyn (39.5), Hck (15.5) and Cyp1B1 (5.8), were observed using oligonucleotide array and were confirmed using real-time PCR for Fyn (20.8), Hck (6.7) and Cyp1B1 (10). Two genes, B cell translocation gene1 (BTG1) and B cell-specific OCT binding factor-1 (OBF-1) were induced > or =1.9-fold in both XLA1 and XLA2 analyzed by Atlas filter arrays andAffymetrix chips, respectively. Data from both filter and oligonucleotide arrays were compared to the gene clusters of a previously published lymphoma expression profile by linking to the UniGene transcript database. Our findings demonstrate for the first time the use of microarray to study the influence of Btk mutations and the use of functional annotation and validation of expression data by comparison of microarray analyses.

Isola, N. R., S. L. Allman, et al. (2001). "MALDI-TOF mass spectrometric method for detection of hybridized DNA oligomers." Anal Chem **73**(9): 2126-31.

Two new approaches for nucleic acid hybridizations by MALDI-TOF mass spectrometry are described. Hybridization using genomic DNA without polymerase chain reaction was demonstrated. Total genomic DNA of bacteriophages bound to charge-modified nylon membranes was identified by the hybridization of species-specific oligonucleotide probes. lambda-Phage DNA and M13 were used for the test with good success. Since MALDI-TOF mass spectrometry can be used to measure the molecular weights of different probes, mass spectrometry can be used for the detection of hybridizations with multiple probes. We demonstrate that multiple-probe hybridization can be resolved by mass spectrometry. Six probes with different mass tag were used for hybridization on a single spot. MALDI-TOF mass spectrometry was successfully used to measure these probes simultaneously. This provides a simple nonradioactive method for multiplex hybridization analysis. It has the potential to drastically increase the speed for microarray hybridization analysis in the future.

Israel, D. A., N. Salama, et al. (2001). "Helicobacter pylori genetic diversity within the gastric niche of a single human host." Proc Natl Acad Sci U S A **98**(25): 14625-30.

Isolates of the gastric pathogen Helicobacter pylori harvested from different individuals are highly polymorphic. Strain variation also has been observed within a single host. To more fully ascertain the extent of H. pylori genetic diversity within the ecological niche of its natural host, we harvested additional isolates of the sequenced H. pylori strain J99 from its human source patient after a 6-year interval. Randomly amplified polymorphic DNA PCR and DNA sequencing of four unlinked loci indicated that these isolates were closely related to the original strain. In contrast, microarray analysis revealed differences in genetic content among all of the isolates that were not detected by randomly amplified polymorphic DNA PCR or sequence analysis. Several ORFs from loci scattered throughout the chromosome in the archival strain did not hybridize with DNA from the recent strains, including multiple ORFs within the J99 plasticity zone. In addition, DNA from the recent isolates hybridized with probes for

ORFs specific for the other fully sequenced H. pylori strain 26695, including a putative traG homolog. Among the additional J99 isolates, patterns of genetic diversity were distinct both when compared with each other and to the original prototype isolate. These results indicate that within an apparently homogeneous population, as determined by macroscale comparison and nucleotide sequence analysis, remarkable genetic differences exist among single-colony isolates of H. pylori. Direct evidence that H. pylori has the capacity to lose and possibly acquire exogenous DNA is consistent with a model of continuous microevolution within its cognate host.

Izaguirre, M. M., A. L. Scopel, et al. (2003). "Convergent responses to stress. Solar ultraviolet-B radiation and Manduca sexta herbivory elicit overlapping transcriptional responses in field-grown plants of Nicotiana longiflora." Plant Physiol **132**(4): 1755-67.

The effects of solar ultraviolet (UV)-B (280-315 nm) on plants have been studied intensively over the last 2 decades in connection with research on the biological impacts of stratospheric ozone depletion. However, the molecular mechanisms that mediate plant responses to solar (ambient) UV-B and their interactions with response mechanisms activated by other stressors remain for the most part unclear. Using a microarray enriched in wound- and insect-responsive sequences, we examined expression responses of 241 genes to ambient UV-B in field-grown plants of Nicotiana longiflora Cav. Approximately 20% of the sequences represented on the array showed differential expression in response to solar UV-B. The expression responses to UV-B had parallels with those elicited by simulated Manduca sexta herbivory. The most obvious similarities were: (a) down-regulation of several photosynthesis-related genes, and (b) up-regulation of genes involved in fatty acid metabolism and oxylipin biosynthesis such as HPL (hydroperoxide lyase), alpha-DIOX (alpha-dioxygenase), LOX (13-lipoxygenase), and AOS (allene oxide synthase). Genes encoding a WRKY transcription factor, a ferredoxin-dependent glutamate-synthase, and several other insect-responsive genes of unknown function were also similarly regulated by UV-B and insect herbivory treatments. Our results suggest that UV-B and caterpillar herbivory activate common regulatory elements and provide a platform for understanding the mechanisms of UV-B impacts on insect herbivory that have been documented in recent field studies.

Jackson-Grusby, L., C. Beard, et al. (2001). "Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation." Nat Genet **27**(1): 31-9.

Cytosine methylation of mammalian DNA is essential for the proper epigenetic regulation of gene expression and maintenance of genomic integrity. To define the mechanism through which demethylated cells die, and to establish a paradigm for identifying genes regulated by DNA methylation, we have generated mice with a conditional allele for the maintenance DNA methyltransferase gene Dnmt1. Cre-mediated deletion of Dnmt1 causes demethylation of cultured fibroblasts and a uniform p53-dependent cell death. Mutational inactivation of Trp53 partially rescues the demethylated fibroblasts for up to five population doublings in culture. Oligonucleotide microarray analysis showed that up to 10% of genes are aberrantly expressed in demethylated fibroblasts. Our results demonstrate that loss of Dnmt1 causes cell-type-specific changes in gene expression that impinge on several pathways, including expression of imprinted genes, cell-cycle control, growth factor/receptor signal transduction and mobilization of retroelements.

Jakobsen, J. S., L. Jelsbak, et al. (2004). "Sigma54 enhancer binding proteins and Myxococcus xanthus fruiting body development." J Bacteriol **186**(13): 4361-8.

A search of the M1genome sequence, which includes 97% of the Myxococcus xanthus genes, identified 53 sequence homologs of sigma54-dependent enhancer binding proteins (EBPs). A DNA microarray was constructed from the M1genome that includes those homologs and 318 other M. xanthus genes for comparison. To screen the developmental program with this array, an RNA extract from growing cells was compared with one prepared from developing cells at 12 h. Previous reporter studies had shown that M. xanthus has initiated development and has begun to express many developmentally

regulated genes by 12 h. The comparison revealed substantial increases in the expression levels of 11 transcription factors that may respond to environmental stimuli. Six of the 53 EBP homologs were expressed at significantly higher levels at 12 h of development than during growth. Three were previously unknown genes, and they were inactivated to look for effects on fruiting body development. One knockout mutant produced fruiting bodies of abnormal shape that depended on the composition of the medium.

Janssen, P. J., B. Audit, et al. (2001). "Strain-specific genes of Helicobacter pylori: distribution, function and dynamics." Nucleic Acids Res **29**(21): 4395-404.

Whole-genome clustering of the two available genome sequences of Helicobacter pylori strains 26695 and J99 allows the detection of 110 and 52 strain-specific genes, respectively. This set of strain-specific genes was compared with the sets obtained with other computational approaches of direct genome comparison as well as experimental data from microarray analysis. A considerable number of novel function assignments is possible using database-driven sequence annotation, although the function of the majority of the identified genes remains unknown. Using whole-genome clustering, it is also possible to detect species-specific genes by comparing the two H.pylori strains against the genome sequence of Campylobacter jejuni. It is interesting that the majority of strain-specific genes appear to be species specific. Finally, we introduce a novel approach to gene position analysis by employing measures from directional statistics. We show that although the two strains exhibit differences with respect to strain-specific gene distributions, this is due to the extensive genome rearrangements. If these are taken into account, a common pattern for the genome dynamics of the two Helicobacter strains emerges, suggestive of certain spatial constraints that may act as control mechanisms of gene flux.

Jimenez, J. L., M. P. Mitchell, et al. (2003). "Microarray analysis of orthologous genes: conservation of the translational machinery across species at the sequence and expression level." Genome Biol **4**(1): R4.

BACKGROUND: Genome projects have provided a vast amount of sequence information. Sequence comparison between species helps to establish functional catalogues within organisms and to study how they are maintained and modified across phylogenetic groups during evolution. Microarray studies allow us to determine groups of genes with similar temporal regulation and perhaps also common regulatory upstream regions for binding of transcription factors. The integration of sequence and expression data is expected to refine our current annotations and provide some insight into the evolution of gene regulation across organisms. RESULTS: We have investigated how well the protein subcellular localization and functional categories established from clustering of orthologous genes agree with gene-expression data in Saccharomyces cerevisiae. An increase in the resolution of biologically meaningful classes is observed upon the combination of experiments under different conditions. The functional categories deduced by sequence comparison approaches are, in general, preserved at the level of expression and can sometimes interact into larger co-regulated networks, such as the protein translation process. Differences and similarities in the expression between cytoplasmic-mitochondrial and interspecies translation machineries complement evolutionary information from sequence similarity. CONCLUSIONS: Combination of several microarray experiments is a powerful tool for the identification of upstream regulatory motifs of yeast genes involved in protein synthesis. Comparison of these yeast co-regulated genes against the archaeal and bacterial operons indicates that the components of the protein translation process are conserved across organisms at the expression level with minor specific adaptations.

Johannes, G., M. S. Carter, et al. (1999). "Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray." Proc Natl Acad Sci U S A **96**(23): 13118-23.

Although most eukaryotic mRNAs need a functional cap binding complex eIF4F for efficient 5' end- dependent scanning to initiate translation, picornaviral, hepatitis C viral, and a few cellular RNAs

have been shown to be translated by internal ribosome entry, a mechanism that can operate in the presence of low levels of functional eIF4F. To identify cellular mRNAs that can be translated when eIF4F is depleted or in low abundance and that, therefore, may contain internal ribosome entry sites, mRNAs that remained associated with polysomes were isolated from human cells after infection with poliovirus and were identified by using a cDNA microarray. Approximately 200 of the 7000 mRNAs analyzed remained associated with polysomes under these conditions. Among the gene products encoded by these polysome-associated mRNAs were immediate-early transcription factors, kinases, and phosphatases of the mitogen-activated protein kinase pathways and several protooncogenes, including c-myc and Pim-1. In addition, the mRNA encoding Cyr61, a secreted factor that can promote angiogenesis and tumor growth, was selectively mobilized into polysomes when eIF4F concentrations were reduced, although its overall abundance changed only slightly. Subsequent tests confirmed the presence of internal ribosome entry sites in the 5' noncoding regions of both Cyr61 and Pim-1 mRNAs. Overall, this study suggests that diverse mRNAs whose gene products have been implicated in a variety of stress responses, including inflammation, angiogenesis, and the response to serum, can use translational initiation mechanisms that require little or no intact cap binding protein complex eIF4F.

Johnson, J., K. Jinneman, et al. (2004). "Natural atypical Listeria innocua strains with Listeria monocytogenes pathogenicity island 1 genes." Appl Environ Microbiol **70**(7): 4256-66.

Identification of bona fide Listeria isolates into the six species of the genus normally requires only a few tests. Aberrant isolates do occur, but even then only one or two extra confirmatory tests are generally needed for identification to species level. We have discovered a hemolytic-positive, rhamnose and xylose fermentation-negative Listeria strain with surprising recalcitrance to identification to the species level due to contradictory results in standard confirmatory tests. The issue had to be resolved by using total DNA-DNA hybridization testing and then confirmed by further specific PCR-based tests including a Listeria microarray assay. The results show that this isolate is indeed a novel one. Its discovery provides the first fully documented instance of a hemolytic Listeria innocua strain. This species, by definition, is typically nonhemolytic. The L. innocua isolate contains all the members of the PrfA-regulated virulence gene cluster (Listeria pathogenicity island 1) of L. monocytogenes. It is avirulent in the mouse pathogenicity test. Avirulence is likely at least partly due to the absence of the L. monocytogenes-specific allele of iap, as well as the absence of inlA, inlB, inlC, and daaA. At least two of the virulence cluster genes, hly and plcA, which encode the L. monocytogenes hemolysin (listeriolysin O) and inositol-specific phospholipase C, respectively, are phenotypically expressed in this L. innocua strain. The detection by PCR assays of specific L. innocua genes (lin0198, lin0372, lin0419, lin0558, lin1068, lin1073, lin1074, lin2454, and lin2693) and noncoding intergenic regions (lin0454-lin0455 and nadA-lin2134) in the strain is consistent with its L. innocua DNA-DNA hybridization identity. Additional distinctly different hemolytic L. innocua strains were also studied.

Jones, P. G., D. Allaway, et al. (2002). "Gene discovery and microarray analysis of cacao (Theobroma cacao L.) varieties." Planta **216**(2): 255-64.

The cacao bean harvest from the relatively under developed tropical tree cacao (Theobroma cacao L.) is subject to high losses in potential production due to pests and diseases. To discover and understand the stability of putative natural resistance mechanisms in this commodity crop, essential for chocolate production, we undertook a gene-discovery program and demonstrated its use in gene-expression arrays. Sequencing and assembling bean and leaf cDNA library inserts produced a unique contig set of 1,380 members. High-quality annotation of this gene set using Blast and MetaFam produced annotation for 75% of the contigs and allowed us to identify the types of gene expressed in cacao beans and leaves. Microarrays were constructed using amplified inserts of the uni-gene set and challenged with bean and leaf RNA from five cacao varieties. The microarray performed well across the five randomly chosen cacao genotypes and did not show a bias towards either leaf or bean tissues. This demonstrates that the gene sequences are useful for microarray analysis across cacao genotypes and tissue types. The array

results, when compared with real-time PCR results for selected genes, showed a correlation with differential gene-expression patterns.We intend that the resultant DNA sequences and molecular microarray platform will help the cacao community to understand the basis, likely stability and pathotype resistance range of candidate cacao plants.

Jouin, H., W. Daher, et al. (2004). "Double staining of Plasmodium falciparum nucleic acids with hydroethidine and thiazole orange for cell cycle stage analysis by flow cytometry." Cytometry **57A**(1): 34-8.

BACKGROUND: Microarray analyses of stage-specific gene expression of Plasmodium falciparum require purification of RNAs from highly synchronized cultures. To date, no reliable method to control the quality of synchronization of P. falciparum cultures is available. METHODS: A double-staining method using hydroethidine and thiazole orange for nucleic acid staining was carried out to compare by flow cytometric analysis the nucleic acid labeling of synchronized P. falciparum in cultures at different time points of the 48-h intraerythrocytic cycle. RESULTS: With this method, we determined the quality of culture synchronization in schizont and ring stages. Nucleic acid analysis, based on thiazole orange fluorescence, clearly showed that low levels of schizonts in ring cultures results in a high contamination of ring nucleic acids by schizonts. Conversely, nucleic acids from trophozoite or schizont cultures containing ring stages did not present a significant contamination by ring nucleic acids. CONCLUSION: The results demonstrated a very low nucleic acid content in the ring stage when compared with the high nucleic acid content of schizont-stage parasites. The rapid and reliable flow cytometric strategy using hydroethidine- and thiazole orange-stained parasite nucleic acids allows monitoring of the purity of the preparation, thus greatly improving the quality assessment of parasite cultures, a critical step to study gene expression patterns.

Joyce, E. A., K. Chan, et al. (2002). "Redefining bacterial populations: a post-genomic reformation." Nat Rev Genet **3**(6): 462-73.

Sexual reproduction and recombination are essential for the survival of most eukaryotic populations. Until recently, the impact of these processes on the structure of bacterial populations has been largely overlooked. The advent of large-scale whole-genome sequencing and the concomitant development of molecular tools, such as microarray technology, facilitate the sensitive detection of recombination events in bacteria. These techniques are revealing that bacterial populations are comprised of isolates that show a surprisingly wide spectrum of genetic diversity at the DNA level. Our new awareness of this genetic diversity is increasing our understanding of population structures and of how these affect host pathogen relationships.

Kabir, M. S., T. Sagara, et al. (2004). "Effects of mutations in the rpoS gene on cell viability and global gene expression under nitrogen starvation in Escherichia coli." Microbiology **150**(Pt 8): 2543-53.

Escherichia coli bearing an rpoS amber or disrupted mutation exhibited a significant decrease in the number of colony-forming units (c.f.u.) when exposed to nitrogen starvation, which was not observed in cells bearing a functional rpoS allele. The decrease in the number of c.f.u. that was observed about 25 h after initiation of nitrogen starvation was prevented by the addition of nitrogen within 3 h but not by the addition of nitrogen at more than 7 h after the initiation of nitrogen starvation, suggesting that a process leading to a decline in c.f.u. starts within this period. DNA microarray analysis of the rpoS mutant showed that a large number of genes including many functionally undefined genes were affected by nitrogen starvation. The expression levels of sigma(S) and sigma(H) regulon genes encoding acid-resistant proteins (hdeA, hdeB, gadA and gadB), DNA-binding protein (dps), chaperones (dnaK, ibpA, ibpB, dnaJ and htpG), chaperonins (mopB and mopA) and energy-metabolism-related proteins (hyaABCDF and gapA), and those of other genes encoding nucleotide-metabolism-related proteins (deoC and deoB), cell-division protein (ftsL), outer-membrane lipoprotein (slp) and DNA-binding protein (stpA) were significantly decreased by 10 h nitrogen starvation. The genes encoding transport/binding proteins

(nac, amtB, argT, artJ, potF and hisJ) and amino acid-metabolism-related proteins (glnA, trpB, argG, asnB, argC, gdhA, cstC, ntrB, asd and lysC) were significantly up-regulated under the same condition, some of which are known Ntr genes expressed under nitrogen limitation. On the basis of these results, possible causes of the decrease in the number of c.f.u. under nitrogen starvation are discussed.

Kahmann, R. and C. Basse (2001). "Fungal gene expression during pathogenesis-related development and host plant colonization." Curr Opin Microbiol **4**(4): 374-80.

To successfully infect plants, pathogenic fungi must recognize and communicate with their host during different stages of the disease cycle. In past years, techniques such as insertional mutagenesis, sensitive GFP-based reporter systems and microarray techniques have been developed to analyze these processes at the molecular level, and now novel insights into this fascinating aspect of pathogen-plant communication are beginning to emerge. This is exemplified by a number of pathogenicity genes functioning in distinct stages of pathogenic development in Magnaporthe grisea.

Kammanadiminti, S. J., B. J. Mann, et al. (2004). "Regulation of Toll-like receptor-2 expression by the Gal-lectin of Entamoeba histolytica." Faseb J **18**(1): 155-7.

The Gal/GalNAc lectin (Gal-lectin) of Entamoeba histolytica is a surface molecule involved in parasite adherence to host cells and is the most promising subunit vaccine candidate against amoebiasis. As macrophages are the major effector cells in host defense against amoebas, we studied the molecular mechanisms by which Gal-lectin activates macrophage. Microarray analysis showed that Gal-lectin up-regulated mRNAs of several cytokines and receptor genes involved in proinflammatory responses. The mechanism whereby the Gal-lectin regulates Toll-like receptor 2 (TLR-2) expression in macrophages was studied. Native Gal-lectin increased TLR-2 mRNA expression in a dose- and time-dependent fashion; peak response occurred with 1 microg/ml after 2 h stimulation. By immunoflourescence, enhanced surface expression of TLR-2 was observed after 12 h. With the use of nonoverlapping anti-Gal-lectin monoclonal antibodies that map to the carbohydrate recognition domain, amino acid 596-1082 was identified as the TLR-2 stimulating region. The Gal-lectin increased TLR-2 gene transcription, and the half-life of the mRNA transcripts was 1.4 h. Inhibition of nuclear factor (NF)-kappaB suppressed TLR-2 mRNA induction by the Gal-lectin. Moreover, cells pretreated with an inhibitor of p38 kinase (SB 208530) inhibited Gal-lectin induced TLR-2 mRNA expression by 40%. We conclude that the Gal-lectin activates NF-kappaB and MAP kinase-signaling pathways in macrophages culminating in the induction of several genes including TLR-2 and hypothesize that this could have a significant impact on macrophage activation and contribute to amoebic pathogenesis.

Kamme, F., R. Salunga, et al. (2003). "Single-cell microarray analysis in hippocampus CA1: demonstration and validation of cellular heterogeneity." J Neurosci **23**(9): 3607-15.

Laser capture microdissection in combination with microarrays allows for the expression analysis of thousands of genes in selected cells. Here we describe single-cell gene expression profiling of CA1 neurons in the rat hippocampus using a combination of laser capture, T7 RNA amplification, and cDNA microarray analysis. Subsequent cluster analysis of the microarray data identified two different cell types: pyramidal neurons and an interneuron. Cluster analysis also revealed differences among the pyramidal neurons, indicating that even a single cell type in vivo is not a homogeneous population of cells at the gene expression level. Microarray data were confirmed by quantitative RT-PCR and in situ hybridization. We also report on the reproducibility and sensitivity of this combination of methods. Single-cell gene expression profiling offers a powerful tool to tackle the complexity of the mammalian brain.

Kamme, F., J. Zhu, et al. (2004). "Single-cell laser-capture microdissection and RNA amplification." Methods Mol Med **99**: 215-23.

Generating gene-expression profiles from laser-captured cells requires the successful combination of laser-capture microdissection, RNA extraction, RNA amplification, and microarray analysis. To permit

single-cell gene-expression profiling, the RNA amplification method has to be sufficiently powerful to bridge the gap between the amount of RNA available from a single cell to what is required by the microarray, a gap that spans 5 to 6 orders of magnitude. This chapter focuses on the amplification of RNA using a two-round T7 RNA amplification method. The protocols described are adapted for laser-captured material and have been used to generate gene expression profiles from single laser-captured cells.

Kanamori, M., S. Watanabe, et al. (2004). "Epstein-Barr virus nuclear antigen leader protein induces expression of thymus- and activation-regulated chemokine in B cells." J Virol **78**(8): 3984-93.

Epstein-Barr virus (EBV) nuclear antigen leader protein (EBNA-LP) plays a critical role in transformation of primary B lymphocytes to continuously proliferating lymphoblastoid cell lines (LCLs). To identify cellular genes in B cells whose expression is regulated by EBNA-LP, we performed microarray expression profiling on an EBV-negative human B-cell line, BJAB cells, that were transduced by a retroviral vector expressing the EBV EBNA-LP (BJAB-LP cells) and on BJAB cells that were transduced with a control vector (BJAB-vec cells). Microarray analysis led to the identification of a cellular gene encoding the CC chemokine TARC as a novel target gene that was induced by EBNA-LP. The levels of TARC mRNA expression and TARC secretion were significantly up-regulated in BJAB-LP compared with BJAB-vec cells. Induction of TARC was also observed when a subline of BJAB cells was converted by a recombinant EBV. Among the EBV-infected B-cell lines with the latency III phenotype that were tested, the LCLs especially secreted significantly high levels of TARC. The level of TARC secretion appeared to correlate with the level of full-length EBNA-LP expression. These results indicate that EBV infection induces TARC expression in B cells and that EBNA-LP is one of the viral gene products responsible for the induction.

Kane, M. D., T. A. Jatkoe, et al. (2000). "Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays." Nucleic Acids Res **28**(22): 4552-7.

To examine the utility and performance of 50mer oligonucleotide (oligonucleotide probe) microarrays, gene-specific oligonucleotide probes were spotted along with PCR probes onto glass microarrays and the performance of each probe type was evaluated. The specificity of oligonucleotide probes was studied using target RNAs that shared various degrees of sequence similarity. Sensitivity was defined as the ability to detect a 3-fold change in mRNA. No significant difference in sensitivity between oligonucleotide probes and PCR probes was observed and both had a minimum reproducible detection limit of approximately 10 mRNA copies/cell. Specificity studies showed that for a given oligonucleotide probe any 'non-target' transcripts (cDNAs) >75% similar over the 50 base target may show cross-hybridization. Thus non-target sequences which have >75-80% sequence similarity with target sequences (within the oligonucleotide probe 50 base target region) will contribute to the overall signal intensity. In addition, if the 50 base target region is marginally similar, it must not include a stretch of complementary sequence >15 contiguous bases. Therefore, knowledge about the target sequence, as well as its similarity to other mRNAs in the target tissue or RNA sample, is required to design successful oligonucleotide probes for quality microarray results. Together these results validate the utility of oligonucleotide probe (50mer) glass microarrays.

Kanesaki, Y., I. Suzuki, et al. (2002). "Salt stress and hyperosmotic stress regulate the expression of different sets of genes in Synechocystis sp. PCC 6803." Biochem Biophys Res Commun **290**(1): 339-48.

Acclimation of microorganisms to environmental stress is closely related to the expression of various genes. We report here that salt stress and hyperosmotic stress have different effects on the cytoplasmic volume and gene expression in Synechocystis sp. PCC 6803. DNA microarray analysis indicated that salt stress strongly induced the genes for some ribosomal proteins. Hyperosmotic stress strongly induced the genes for 3-ketoacyl-acyl carrier protein reductase and rare lipoprotein A. Genes whose expression was induced both by salt stress and by hyperosmotic stress included those for heat-shock proteins and the enzymes for the synthesis of glucosylglycerol. We also found that each kind of

stress induced a number of genes for proteins of unknown function. Our findings suggest that Synechocystis recognizes salt stress and hyperosmotic stress as different stimuli, although mechanisms common to the responses to each form of stress might also contribute to gene expression.

Kapatral, V., J. W. Campbell, et al. (2004). "Gene array analysis of Yersinia enterocolitica FlhD and FlhC: regulation of enzymes affecting synthesis and degradation of carbamoylphosphate." Microbiology **150**(Pt 7): 2289-300.

This paper focuses on global gene regulation by FlhD/FlhC in enteric bacteria. Even though Yersinia enterocolitica FlhD/FlhC can complement an Escherichia coli flhDC mutant for motility, it is not known if the Y. enterocolitica FlhD/FlhC complex has an effect on metabolism similar to E. coli. To study metabolic gene regulation, a partial Yersinia enterocolitica 8081c microarray was constructed and the expression patterns of wild-type cells were compared to an flhDC mutant strain at 25 and 37 degrees C. The overlap between the E. coli and Y. enterocolitica FlhD/FlhC regulated genes was 25 %. Genes that were regulated at least fivefold by FlhD/FlhC in Y. enterocolitica are genes encoding urocanate hydratase (hutU), imidazolone propionase (hutI), carbamoylphosphate synthetase (carAB) and aspartate carbamoyltransferase (pyrBI). These enzymes are part of a pathway that is involved in the degradation of L-histidine to L-glutamate and eventually leads into purine/pyrimidine biosynthesis via carbamoylphosphate and carbamoylaspartate. A number of other genes were regulated at a lower rate. In two additional experiments, the expression of wild-type cells grown at 4 or 25 degrees C was compared to the same strain grown at 37 degrees C. The expression of the flagella master operon flhD was not affected by temperature, whereas the flagella-specific sigma factor fliA was highly expressed at 25 degrees C and reduced at 4 and 37 degrees C. Several other flagella genes, all of which are under the control of FliA, exhibited a similar temperature profile. These data are consistent with the hypothesis that temperature regulation of flagella genes might be mediated by the flagella-specific sigma factor FliA and not the flagella master regulator FlhD/FlhC.

Karaca, G., J. Anobile, et al. (2004). "Herpesvirus of turkeys: microarray analysis of host gene responses to infection." Virology **318**(1): 102-11.

Herpesvirus of turkeys (HVT) provides an economically important live vaccine for prevention of Marek's disease (MD) of chickens. MD, characterized by both immunosuppression and T-cell lymphoma, is caused by another herpesvirus termed Marek's disease virus (MDV). Microarrays were used to investigate the response of chicken embryonic fibroblasts (CEF) to infection with HVT. Genes responding to HVT infection include several induced by interferon along with others modulating signal transduction, transcription, scaffolding proteins, and the cytoskeleton. Results are compared with earlier studies examining the responses of CEF cells to infection with MDV.

Karaca, G., D. Hargett, et al. (2004). "Inhibition of the stress-activated kinase, p38, does not affect the virus transcriptional program of herpes simplex virus type 1." Virology **329**(1): 142-56.

To investigate the impact of stress kinase p38 activation on HSV-1 transcription, we performed a global transcript profile analysis of viral mRNA using an oligonucleotide-based DNA microarray. RNA was isolated from Vero cells infected with the KOS strain of HSV-1 in the presence or absence of SB203580, a pyridinyl imidazole inhibitor of p38. Under conditions that eliminated ATF2 activation but had no effect on c-Jun, and reduced virus yield by 85-90%, no effect on accumulation of viral IE, DE, or L transcripts was observed by array analysis or selected Northern blot analysis at 2, 4, and 6 h post infection. Results of array data from cells infected with the ICP27 mutant d27-1 in the presence or absence of SB203580 only reflected the known restricted transcription phenotype of the ICP27 mutant. This result is consistent with a role for p38 activation on virus replication lying downstream of the essential role of ICP27 in DE and perhaps late transcription regulation. No effect of SB203580 on transcription was detected after infection with the ICP0 mutant 7134, at 0.5 or 5.0 PFU/cell, though decreases in the rate of accumulation of all kinetic classes of mRNA could be detected, relative to wt

virus. These results indicate that inhibiting p38 activity in Vero cells, while significantly reducing wt virus yield, demonstrated no obvious impact on the program of viral transcription.

Kash, J. C., D. M. Cunningham, et al. (2002). "Selective translation of eukaryotic mRNAs: functional molecular analysis of GRSF-1, a positive regulator of influenza virus protein synthesis." J Virol **76**(20): 10417-26.

To understand the regulation of cap-dependent translation initiation mediated by specific 5' untranslated region (UTR) RNA-protein interactions in mammalian cells, we have studied the selective translation of influenza virus mRNAs. Previous work has shown that the host cell mRNA binding protein guanine-rich sequence factor 1 (GRSF-1) bound specifically to conserved viral 5' UTR sequences and stimulated translation of viral 5' UTR-driven mRNAs in vitro. In the present study, we have characterized the functional domains of GRSF-1 and mapped the RNA binding activity of GRSF-1 to RRM 2 (amino acids 194 to 275) with amino-terminal deletion glutathione S-transferase (GST)-GRSF-1 proteins. When these mutants were assayed for functional activity in vitro, deletion of an Ala-rich region (Delta[2-94]) appeared to diminish translational stimulation, while deletion of the Ala-rich region in addition to RRM 1 (Delta[2-194]) resulted in a 4-fold increase in translational activation over wild-type GRSF-1 (an overall 20-fold increase in activity). We have also mapped the GRSF-1 RNA binding site on influenza virus NP and NS1 5' UTRs, which was determined to be the sequence AGGGU. With polysome fractionation and cDNA microarray analysis, we have identified cellular and viral mRNAs containing putative GRSF-1 binding sites that were transcriptionally up-regulated and selectively recruited to polyribosomes following influenza virus infection. Taken together, these studies demonstrate that RRM 2 is critical for GRSF-1 RNA binding and translational activity. Further, our data suggest GRSF-1 functions by selectively recruiting cellular and viral mRNAs containing 5' UTR GRSF-1 binding sites to polyribosomes, which is mediated through interactions with cellular proteins.

Kasukabe, Y., L. He, et al. (2004). "Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic Arabidopsis thaliana." Plant Cell Physiol **45**(6): 712-22.

Polyamines play pivotal roles in plant defense to environmental stresses. However, stress tolerance of genetically engineered plants for polyamine biosynthesis has been little examined so far. We cloned spermidine synthase cDNA from Cucurbita ficifolia and the gene was introduced to Arabidopsis thaliana under the control of the cauliflower mosaic virus 35S promoter. The transgene was stably integrated and actively transcribed in the transgenic plants. As compared with the wild-type plants, the T2 and T3 transgenic plants exhibited a significant increase in spermidine synthase activity and spermidine content in leaves together with enhanced tolerance to various stresses including chilling, freezing, salinity, hyperosmosis, drought, and paraquat toxicity. During exposure to chilling stress (5 degrees C), the transgenics displayed a remarkable increase in arginine decarboxylase activity and conjugated spermidine contents in leaves compared to the wild type. A cDNA microarray analysis revealed that several genes were more abundantly transcribed in the transgenics than in the wild type under chilling stress. These genes included those for stress-responsive transcription factors such as DREB and stress-protective proteins like rd29A. These results strongly suggest an important role for spermidine as a signaling regulator in stress signaling pathways, leading to build-up of stress tolerance mechanisms in plants under stress conditions.

Katoh, H., R. K. Asthana, et al. (2004). "Gene expression in the cyanobacterium Anabaena sp. PCC7120 under desiccation." Microb Ecol **47**(2): 164-74.

The N2-fixing cyanobacterium Anabaena sp. PCC7120 showed an inherent capacity for desiccation tolerance. A DNA microarray covering almost the entire genome of Anabaena was used to determine the genome-wide gene expression under desiccation. RNA was extracted from cells at intervals starting from early to late desiccation. The pattern of gene expression in DNA fragments was categorized

into seven types, which include four types of up-regulated and three types of down-regulated fragments. Validation of the data was carried out by RT-PCR on selected up-regulated DNA fragments and was consistent with the changes in mRNA levels. Our conclusions regarding desiccation tolerance for Anabaena sp. PCC7120 are as follows: (i) Genes for osmoprotectant metabolisms and the K+ transporting system are up-regulated from early to mid-desiccation; (ii) genes induced by osmotic, salt, and low-temperature stress are up-regulated under desiccation; (iii) genes for heat shock proteins are up-regulated after mid-desiccation; (iv) genes for photosynthesis and the nitrogen-transporting system are down-regulated during early desiccation; and (v) genes for RNA polymerase and ribosomal protein are down-regulated between the early and the middle phase of desiccation. Profiles of gene expression are discussed in relation to desiccation acclimation.

Khadijah, S., S. Y. Neo, et al. (2003). "Identification of white spot syndrome virus latency-related genes in specific-pathogen-free shrimps by use of a microarray." J Virol **77**(18): 10162-7.

To investigate whether specific-pathogen-free (SPF) shrimps are asymptomatic carriers of white spot syndrome virus (WSSV), we used a WSSV-specific DNA microarray to measure WSSV gene expression in SPF and WSSV-infected shrimps. Three WSSV genes were found to be relatively highly expressed in SPF shrimps. Reverse transcription-PCR using nested primers as well as real-time detection confirmed that these genes have no detectable counterparts in GenBank; structural analysis of the putative proteins revealed helix-loop-helix and leucine zipper motifs. Viral sequences could be PCR amplified from genomic DNA of SPF shrimp, further supporting the suggestion that these shrimps are asymptomatic carriers.

Khodursky, A. B., B. J. Peter, et al. (2000). "DNA microarray analysis of gene expression in response to physiological and genetic changes that affect tryptophan metabolism in Escherichia coli." Proc Natl Acad Sci U S A **97**(22): 12170-5.

We investigated the global changes in mRNA abundance in Escherichia coli elicited by various perturbations of tryptophan metabolism. To do so we printed DNA microarrays containing 95% of all annotated E. coli ORFs. We determined the expression profile that is predominantly dictated by the activity of the tryptophan repressor. Only three operons, trp, mtr, and aroH, exhibited appreciable expression changes consistent with this profile. The quantitative changes we observed in mRNA levels for the five genes of the trp operon were consistent within a factor of 2, with expectations based on established Trp protein levels. Several operons known to be regulated by the TyrR protein, aroF-tyrA, aroL, aroP, and aroG, were down-regulated on addition of tryptophan. TyrR can be activated by any one of the three aromatic amino acids. Only one operon, tnaAB, was significantly activated by the presence of tryptophan in the medium. We uncovered a plethora of likely indirect effects of changes in tryptophan metabolism on intracellular mRNA pools, most prominent of which was the sensitivity of arginine biosynthetic operons to tryptophan starvation.

Kielian, T., E. D. Bearden, et al. (2004). "IL-1 and TNF-alpha play a pivotal role in the host immune response in a mouse model of Staphylococcus aureus-induced experimental brain abscess." J Neuropathol Exp Neurol **63**(4): 381-96.

Brain abscesses represent a significant medical problem despite recent advances made in detection and therapy. Using an established Staphylococcus aureus-induced brain abscess model, we have sought to define the functional importance of interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and IL-6 in the host anti-bacterial immune response using cytokine gene knockout (KO) mice. Previous studies from our laboratory revealed that these cytokines are among the main proinflammatory mediators produced during the acute stage of brain abscess development. The results presented here demonstrate that although they share many redundant activities, IL-1 and TNF-alpha are important for containing bacterial infection in evolving brain abscesses as evident by increased mortality and bacterial burdens in IL-1 and TNF-alpha KO mice compared to wild type (WT) animals. In contrast, IL-6 was not

found to be a major contributor to the host anti-bacterial immune response. Microarray analysis was used to evaluate the downstream consequences originating from the lack of IL-1 on subsequent proinflammatory mediator expression in brain abscesses from IL-1 KO and WT animals. Although numerous genes were significantly induced following S. aureus infection, only IL-1beta and 2 chemokines, CCL9 (macrophage inflammatory protein-1 gamma/MIP-1gamma) and CXCL13 (B lymphocyte chemoattractant/BLC), were differentially regulated in IL-1 KO versus WT animals. These results suggest that IL-1 and TNF-alpha play a pivotal role during the acute stage of brain abscess development through regulating the ensuing anti-bacterial inflammatory response.

Kielian, T., M. McMahon, et al. (2004). "S. aureus-dependent microglial activation is selectively attenuated by the cyclopentenone prostaglandin 15-deoxy-Delta12,14- prostaglandin J2 (15d-PGJ2)." J Neurochem **90**(5): 1163-72.

Microglial activation is a hallmark of brain abscess. The continual release of proinflammatory mediators by microglia following bacterial challenge may contribute, in part, to the destruction of surrounding normal tissue characteristic of brain abscess. Therefore, attenuating chronic microglial activation during the course of CNS bacterial infections may have therapeutic benefits. The purpose of this study was to evaluate the ability of the natural peroxisome proliferator-activated receptor (PPAR)-gamma agonist 15-deoxy-Delta12,14- prostaglandin J2 (15d-PGJ2) to modulate microglial activation in response to Staphylococcus aureus, one of the main etiologic agents of brain abscess in humans. 15d-PGJ2 was a potent inhibitor of proinflammatory cytokine (IL-1beta, TNF-alpha, IL-12 p40) and CC chemokine (MIP-1beta, MCP-1) production in primary microglia, but had no effect upon the expression of select CXC chemokines (MIP-2, KC). 15d-PGJ2 also selectively inhibited the S. aureus-dependent increase in microglial TLR2, CD14, MHC class II, and CD40 expression, whereas it had no effect on the co-stimulatory molecules CD80 and CD86. Microarray analysis revealed additional inflammatory mediators modulated by 15d-PGJ2 in primary microglia following S. aureus exposure, the majority of which were chemokines. These results suggest that suppressing microglial activation through the use of 15d-PGJ2 may lead to the sparing of damage to normal brain parenchyma that often results from brain abscess.

Kim, C. C. and S. Falkow (2003). "Significance analysis of lexical bias in microarray data." BMC Bioinformatics **4**(1): 12.

BACKGROUND: Genes that are determined to be significantly differentially regulated in microarray analyses often appear to have functional commonalities, such as being components of the same biochemical pathway. This results in certain words being under- or overrepresented in the list of genes. Distinguishing between biologically meaningful trends and artifacts of annotation and analysis procedures is of the utmost importance, as only true biological trends are of interest for further experimentation. A number of sophisticated methods for identification of significant lexical trends are currently available, but these methods are generally too cumbersome for practical use by most microarray users. RESULTS: We have developed a tool, LACK, for calculating the statistical significance of apparent lexical bias in microarray datasets. The frequency of a user-specified list of search terms in a list of genes which are differentially regulated is assessed for statistical significance by comparison to randomly generated datasets. The simplicity of the input files and user interface targets the average microarray user who wishes to have a statistical measure of apparent lexical trends in analyzed datasets without the need for bioinformatics skills. The software is available as Perl source or a Windows executable. CONCLUSION: We have used LACK in our laboratory to generate biological hypotheses based on our microarray data. We demonstrate the program's utility using an example in which we confirm significant upregulation of SPI-2 pathogenicity island of Salmonella enterica serovar Typhimurium by the cation chelator dipyridyl.

Kim, C. C., E. A. Joyce, et al. (2002). "Improved analytical methods for microarray-based genome-

composition analysis." Genome Biol **3**(11): RESEARCH0065.

BACKGROUND: Whereas genome sequencing has given us high-resolution pictures of many different species of bacteria, microarrays provide a means of obtaining information on genome composition for many strains of a given species. Genome-composition analysis using microarrays, or 'genomotyping', can be used to categorize genes into 'present' and 'divergent' categories based on the level of hybridization signal. This typically involves selecting a signal value that is used as a cutoff to discriminate present (high signal) and divergent (low signal) genes. Current methodology uses empirical determination of cutoffs for classification into these categories, but this methodology is subject to several problems that can result in the misclassification of many genes. RESULTS: We describe a method that depends on the shape of the signal-ratio distribution and does not require empirical determination of a cutoff. Moreover, the cutoff is determined on an array-to-array basis, accounting for variation in strain composition and hybridization quality. The algorithm also provides an estimate of the probability that any given gene is present, which provides a measure of confidence in the categorical assignments. CONCLUSIONS: Many genes previously classified as present using static methods are in fact divergent on the basis of microarray signal; this is corrected by our algorithm. We have reassigned hundreds of genes from previous genomotyping studies of Helicobacter pylori and Campylobacter jejuni strains, and expect that the algorithm should be widely applicable to genomotyping data.

Kim, H., E. C. Snesrud, et al. (2003). "Gene expression analyses of Arabidopsis chromosome 2 using a genomic DNA amplicon microarray." Genome Res **13**(3): 327-40.

The gene predictions and accompanying functional assignments resulting from the sequencing and annotation of a genome represent hypotheses that can be tested and used to develop a more complete understanding of the organism and its biology. In the model plant Arabidopsis thaliana, we developed a novel approach to constructing whole-genome microarrays based on PCR amplification of the 3' ends of each predicted gene from genomic DNA, and constructed an array representing more than 94% of the predicted genes and pseudogenes on chromosome 2. With this array, we examined various tissues and physiological conditions, providing expression-based validation for 84% of the gene predictions and providing clues as to the functions of many predicted genes. Further, by examining the distribution of expression along the physical chromosome, we were able to identify a region of repressed transcription that may represent a previously undescribed heterochromatic region.

Kimura, N., R. Oda, et al. (2004). "Attachment of oligonucleotide probes to poly carbodiimide-coated glass for microarray applications." Nucleic Acids Res **32**(7): e68.

Oligonucleotide-based DNA microarrays are becoming increasingly useful tools for the analysis of gene expression and single nucleotide polymorphisms (SNPs). Here, we present a method that permits the manufacture of microarrays from non-modified oligonucleotides on a poly carbodiimide-coated glass surface by UV-irradiation. The use of UV-irradiation facilitates an increase in the level of signal intensity, but it does not affect signal discrimination by the oligonucleotides immobilized on the surface. The signal intensity obtained for an array fabricated using non-modified oligonucleotides with UV-irradiation is approximately 7-fold greater than that without UV-irradiation. The detection of SNPs was tested to ascertain whether this technique could discriminate specific hybridization signals without causing significant UV-irradiation-induced damage to the immobilized oligonucleotides. We found that this immobilization method provides greater hybridization signals and a better match/mismatch ratio of SNPs than do the established aminosilane techniques. Application of this technology to manufacturing DNA microarrays for sequence analysis is discussed.

Kimura, T., M. Gotoh, et al. (2003). "hCDC4b, a regulator of cyclin E, as a direct transcriptional target of p53." Cancer Sci **94**(5): 431-6.

To identify p53-target genes we have been using a cDNA-microarray system to assess gene expression in a p53-mutated glioblastoma cell line (U373MG) after adenovirus-mediated transfer of wild-

type p53 into the p53-deficient cells. In the work reported here, expression of hCDC4b, which encodes one of the four subunits of the SCF (ubiquitin ligase) complex responsible for degradation of cyclin E, was dramatically up-regulated by infection with Ad-p53. An electrophoretic mobility-shift assay and a chromatin immunoprecipitation assay indicated that a potential p53-binding site (p53BS) present in exon 1b of the hCDC4 gene was able to bind to p53, and a reporter assay confirmed that this p53BS had p53-dependent transcriptional activity. Expression of endogenous hCDC4b, but not the alternative transcript of this gene, hCDC4a, was induced in a p53-dependent manner in response to genotoxic stresses caused by UV irradiation and adriamycin treatment, suggesting that each transcript has a different functional role. These results suggest that hCDC4b is a previously unrecognized transcriptional target of the p53 protein, and that by negatively regulating cyclin E through induction of hCDC4b, p53 might stop cell-cycle progression at G0-G1. This would represent a novel mechanism for p53-dependent control of the cell cycle, in addition to the well-known p21(WAF1) machinery.

Kissinger, J. C., B. Gajria, et al. (2003). "ToxoDB: accessing the Toxoplasma gondii genome." Nucleic Acids Res **31**(1): 234-6.

ToxoDB (http://ToxoDB.org) provides a genome resource for the protozoan parasite Toxoplasma gondii. Several sequencing projects devoted to T. gondii have been completed or are in progress: an EST project (http://genome.wustl.edu/est/index.php?toxoplasma=1), a BAC clone end-sequencing project (http://www.sanger.ac.uk/Projects/T_gondii/) and an 8X random shotgun genomic sequencing project (http://www.tigr.org/tdb/e2k1/tga1/). ToxoDB was designed to provide a central point of access for all available T. gondii data, and a variety of data mining tools useful for the analysis of unfinished, un-annotated draft sequence during the early phases of the genome project. In later stages, as more and different types of data become available (microarray, proteomic, SNP, QTL, etc.) the database will provide an integrated data analysis platform facilitating user-defined queries across the different data types.

Kivi, M., X. Liu, et al. (2002). "Determining the genomic locations of repetitive DNA sequences with a whole-genome microarray: IS6110 in Mycobacterium tuberculosis." J Clin Microbiol **40**(6): 2192-8.

The mycobacterial insertion sequence IS6110 has been exploited extensively as a clonal marker in molecular epidemiologic studies of tuberculosis. In addition, it has been hypothesized that this element is an important driving force behind genotypic variability that may have phenotypic consequences. We present here a novel, DNA microarray-based methodology, designated SiteMapping, that simultaneously maps the locations and orientations of multiple copies of IS6110 within the genome. To investigate the sensitivity, accuracy, and limitations of the technique, it was applied to eight Mycobacterium tuberculosis strains for which complete or partial IS6110 insertion site information had been determined previously. SiteMapping correctly located 64% (38 of 59) of the IS6110 copies predicted by restriction fragment length polymorphism analysis. The technique is highly specific; 97% of the predicted insertion sites were true insertions. Eight previously unknown insertions were identified and confirmed by PCR or sequencing. The performance could be improved by modifications in the experimental protocol and in the approach to data analysis. SiteMapping has general applicability and demonstrates an expansion in the applications of microarrays that complements conventional approaches in the study of genome architecture.

Klucky, B., B. Koch, et al. (2004). "Polyomavirus tumorantigens have a profound effect on gene expression in mouse fibroblasts." Oncogene **23**(27): 4707-21.

Polyomavirus (Py) large and small tumorantigens together are competent to induce S phase in growth-arrested mouse fibroblasts. The capacity of the large tumorantigen to bind the pocket proteins, pRB, p130 and p107, is important for the transactivation of DNA synthesis enzymes and the cyclins E and A, while the interference of small tumorantigen with protein phosphatase PP2A causes a destabilization of the cdk2 inhibitor p27, and thus leads to strong cyclin E- and cyclin A-dependent cdk2

activity. Py small tumorantigen, in addition, is able to transactivate cyclin A. Hence, this protein might have a much wider effect on gene expression in arrested mouse fibroblasts than hitherto suspected. This may have a profound part in the known capacity of Py to form tumors in mice. Therefore, it was interesting to gain an insight into the spectrum of transcriptional deregulation by Py tumorantigens. Accordingly, we performed microarray analysis of quiescent mouse fibroblasts in the absence and presence of small or large tumorantigen. We found that the viral proteins can induce or repress a great variety of genes beyond those involved in the S phase induction and DNA synthesis. The results of the microarray analysis were confirmed for selected genes by several methods, including real-time PCR. Interestingly, a mutation of the binding site for pocket proteins in case of LT and for PP2A in case of ST has a variable effect on the deregulation of genes by the viral proteins depending on the gene in question. In fact, some genes are transactivated by LT as well as ST completely independent of an interaction with their major cellular targets, pocket proteins and PP2A, respectively.

Kluger, Y., H. Yu, et al. (2003). "Relationship between gene co-expression and probe localization on microarray slides." BMC Genomics **4**(1): 49.

BACKGROUND: Microarray technology allows simultaneous measurement of thousands of genes in a single experiment. This is a potentially useful tool for evaluating co-expression of genes and extraction of useful functional and chromosomal structural information about genes. RESULTS: In this work we studied the association between the co-expression of genes, their location on the chromosome and their location on the microarray slides by analyzing a number of eukaryotic expression datasets, derived from the S. cerevisiae, C. elegans, and D. melanogaster. We find that in several different yeast microarray experiments the distribution of the number of gene pairs with correlated expression profiles as a function of chromosomal spacing is peaked at short separations and has two superimposed periodicities. The longer periodicity has a spacing of 22 genes (approximately 42 Kb), and the shorter periodicity is 2 genes (approximately 4 Kb). CONCLUSION: The relative positioning of DNA probes on microarray slides and source plates introduces subtle but significant correlations between pairs of genes. Careful consideration of this spatial artifact is important for analysis of microarray expression data. It is particularly relevant to recent microarray analyses that suggest that co-expressed genes cluster along chromosomes or are spaced by multiples of a fixed number of genes along the chromosome.

Kniazeva, M., Q. T. Crawford, et al. (2004). "Monomethyl Branched-Chain Fatty Acids Play an Essential Role in Caenorhabditis elegans Development." PLoS Biol **2**(9): E257.

Monomethyl branched-chain fatty acids (mmBCFAs) are commonly found in many organisms from bacteria to mammals. In humans, they have been detected in skin, brain, blood, and cancer cells. Despite a broad distribution, mmBCFAs remain exotic in eukaryotes, where their origin and physiological roles are not understood. Here we report our study of the function and regulation of mmBCFAs in Caenorhabditis elegans, combining genetics, gas chromatography, and DNA microarray analysis. We show that C. elegans synthesizes mmBCFAs de novo and utilizes the long-chain fatty acid elongation enzymes ELO-5 and ELO-6 to produce two mmBCFAs, C15ISO and C17ISO. These mmBCFAs are essential for C. elegans growth and development, as suppression of their biosynthesis results in a growth arrest at the first larval stage. The arrest is reversible and can be overcome by feeding the arrested animals with mmBCFA supplements. We show not only that the levels of C15ISO and C17ISO affect the expression of several genes, but also that the activities of some of these genes affect biosynthesis of mmBCFAs, suggesting a potential feedback regulation. One of the genes, lpd-1, encodes a homolog of a mammalian sterol regulatory element-binding protein (SREBP 1c). We present results suggesting that elo-5 and elo-6 may be transcriptional targets of LPD-1. This study exposes unexpected and crucial physiological functions of C15ISO and C17ISO in C. elegans and suggests a potentially important role for mmBCFAs in other eukaryotes.

Kobayashi, K., M. Ogura, et al. (2001). "Comprehensive DNA microarray analysis of Bacillus subtilis

two-component regulatory systems." J Bacteriol **183**(24): 7365-70.

It has recently been shown through DNA microarray analysis of Bacillus subtilis two-component regulatory systems (DegS-DegU, ComP-ComA, and PhoR-PhoP) that overproduction of a response regulator of the two-component systems in the background of a deficiency of its cognate sensor kinase affects the regulation of genes, including its target ones. The genome-wide effect on gene expression caused by the overproduction was revealed by DNA microarray analysis. In the present work, we newly analyzed 24 two-component systems by means of this strategy, leaving out 8 systems to which it was unlikely to be applicable. This analysis revealed various target gene candidates for these two-component systems. It is especially notable that interesting interactions appeared to take place between several two-component systems. Moreover, the probable functions of some unknown two-component systems were deduced from the list of their target gene candidates. This work is heuristic but provides valuable information for further study toward a comprehensive understanding of the B. subtilis two-component regulatory systems. The DNA microarray data obtained in this work are available at the KEGG Expression Database website (http://www.genome.ad.jp/kegg/expression).

Kobayashi, M., T. Ishizuka, et al. (2004). "Response to oxidative stress involves a novel peroxiredoxin gene in the unicellular cyanobacterium Synechocystis sp. PCC 6803." Plant Cell Physiol **45**(3): 290-9.

Exposure to methyl viologen in the presence of light facilitates the production of superoxide that gives severe damage on photosynthetic apparatus as well as many cellular processes in cyanobacteria and plants. The effects of methyl viologen on global gene expression of a unicellular cyanobacterium Synechocystis sp. strain PCC 6803 were determined by DNA microarray. The ORFs sll1621, slr1738, slr0074, slr0075, and slr0589 were significantly induced by treatment of methyl viologen for 15 min commonly under conditions of normal and high light. One of these genes, slr1738, which encodes a ferric uptake repressor (Fur)-type transcriptional regulator, is located divergently next to another induced gene, sll1621, in the genome. We deleted slr1738, and compared the global gene expression patterns of this mutant to that of wild type under non-stressed conditions. It was found that sll1621 was derepressed to the greatest extent, while many other genes including slr0589 but not slr0074 or slr0075 were derepressed to lesser extent in the mutant. Genetic disruption of sll1621, which encodes a putative type 2 peroxiredoxin, indicates that it is essential for aerobic phototrophic growth in both liquid and solid media in high light and on solid medium even in low light. Slr1738 was prepared as a His-tagged recombinant protein and shown to specifically bind to the intergenic region between sll1621 and slr1738. The binding was enhanced by dithiothreitol and abolished by hydrogen peroxide. We concluded that the Fur homolog, Slr1738, plays a regulatory role in the induction of a potent antioxidant gene, sll1621, in response to oxidative stress.

Kodama, K., T. Kobayashi, et al. (2002). "Amplification of Hot DNA segments in Escherichia coli." Mol Microbiol **45**(6): 1575-88.

In Escherichia coli, a replication fork blocking event at a DNA replication terminus (Ter) enhances homologous recombination at the nearby sister chromosomal region, converting the region into a recombination hotspot, Hot, site. Using a RNaseH negative (rnhA-) mutant, we identified eight kinds of Hot DNAs (HotA-H). Among these, enhanced recombination of three kinds of Hot DNAs (HotA-C) was dependent on fork blocking events at Ter sites. In the present study, we examined whether HotA DNAs are amplified when circular DNA (HotA plus a drug-resistance DNA) is inserted into the homologous region on the chromosome of a rnhA- mutant. The resulting HotA DNA transformants were analysed using pulsed-field gel electrophoresis, fluorescence in situ hybridization and DNA microarray technique. The following results were obtained: (i) HotA DNA is amplified by about 40-fold on average; (ii) whereas 90% of the cells contain about 6-10 copies of HotA DNA, the remaining 10% of cells have as many as several hundred HotA copies; and (iii) amplification is detected in all other Hot DNAs, among which HotB and HotG DNAs are amplified to the same level as HotA. Furthermore, HotL DNA, which is activated by blocking the clockwise oriC-starting replication fork at the artificially inserted TerL site in

the fork-blocked strain with a rnhA+ background, is also amplified, but is not amplified in the non-blocked strain. From these data, we propose a model that can explain production of three distinct forms of Hot DNA molecules by the following three recombination pathways: (i) unequal intersister recombination; (ii) intrasister recombination, followed by rolling-circle replication; and (iii) intrasister recombination, producing circular DNA molecules.

Koizumi, Y., J. J. Kelly, et al. (2002). "Parallel characterization of anaerobic toluene- and ethylbenzene-degrading microbial consortia by PCR-denaturing gradient gel electrophoresis, RNA-DNA membrane hybridization, and DNA microarray technology." Appl Environ Microbiol **68**(7): 3215-25.

A mesophilic toluene-degrading consortium (TDC) and an ethylbenzene-degrading consortium (EDC) were established under sulfate-reducing conditions. These consortia were first characterized by denaturing gradient gel electrophoresis (DGGE) fingerprinting of PCR-amplified 16S rRNA gene fragments, followed by sequencing. The sequences of the major bands (T-1 and E-2) belonging to TDC and EDC, respectively, were affiliated with the family Desulfobacteriaceae. Another major band from EDC (E-1) was related to an uncultured non-sulfate-reducing soil bacterium. Oligonucleotide probes specific for the 16S rRNAs of target organisms corresponding to T-1, E-1, and E-2 were designed, and hybridization conditions were optimized for two analytical formats, membrane and DNA microarray hybridization. Both formats were used to characterize the TDC and EDC, and the results of both were consistent with DGGE analysis. In order to assess the utility of the microarray format for analysis of environmental samples, oil-contaminated sediments from the coast of Kuwait were analyzed. The DNA microarray successfully detected bacterial nucleic acids from these samples, but probes targeting specific groups of sulfate-reducing bacteria did not give positive signals. The results of this study demonstrate the limitations and the potential utility of DNA microarrays for microbial community analysis.

Kraft, A. D., D. A. Johnson, et al. (2004). "Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult." J Neurosci **24**(5): 1101-12.

Binding of the transcription factor nuclear factor E2-related factor 2 (Nrf2) to the antioxidant response element (ARE) in neural cells results in the induction of a battery of genes that can coordinate a protective response against a variety of oxidative stressors. In this study, tert-butylhydroquinone (tBHQ) and sulforaphane were used as activators of this pathway. Consistent with previous studies, treatment of primary cortical cultures from ARE reporter mice revealed selective promoter activity in astrocytes. This activation protected neurons from hydrogen peroxide and nonexcitotoxic glutamate toxicity. tBHQ treatment of cultures from Nrf2 knock-out animals resulted in neither ARE activation nor neuroprotection. By reintroducing Nrf2 via infection with a replication-deficient adenovirus (ad), both the genetic response and neuroprotection were rescued. Conversely, infection with adenovirus encoding dominant-negative (DN) Nrf2 (ad-DN-Nrf2) or pretreatment with the selective phosphatidylinositol-3 kinase inhibitor LY294002 inhibited the tBHQ-mediated promoter response and corresponding neuroprotection. Interestingly, the adenoviral infection showed a high selectivity for astrocytes over neurons. In an attempt to reveal some of the cell type-specific changes resulting from ARE activation, cultures were infected with adenovirus encoding green fluorescent protein (GFP) (ad-GFP) or ad-DN-Nrf2 (containing GFP) before tBHQ treatment. A glia-enriched population of GFP-infected cells was then isolated from a population of uninfected neurons using cell-sorting technology. Microarray analysis was used to evaluate potential glial versus neuron-specific contributions to the neuroprotective effects of ARE activation and Nrf2 dependence. Strikingly, the change in neuronal gene expression after tBHQ treatment was dependent on Nrf2 activity in the astrocytes. This suggests that Nrf2-dependent genetic changes alter neuron-glia interactions resulting in neuroprotection.

Kravets, A., Z. Hu, et al. (2004). "Biliverdin reductase, a novel regulator for induction of activating transcription factor-2 and heme oxygenase-1." J Biol Chem **279**(19): 19916-23.

Biliverdin IXalpha reductase (BVR) catalyzes reduction of the HO activity product, biliverdin, to bilirubin. hBVR is a serine/threonine kinase that contains a bZip domain. Presently, regulation of gene expression by hBVR was examined. 293A cells were infected with adenovirus-doxycycline (Ad-Dox)-inducible hBVR cDNA. High level expression of hBVR was determined at mRNA, protein, and activity levels 8 h after induction. Cell signal transduction microarray analysis of cells infected with expression or with the control Ad-inverted (INV)-hBVR vector identified ATF-2 among several up-regulated genes. ATF-2 is a bZip transcription factor for activation of cAMP response element (CRE) and a dimeric partner to c-jun in MAPK pathway that regulates the stress protein, HO-1, expression. Northern and Western blot analyses showed increases of approximately 10-fold in ATF-2 mRNA and protein at 16 and 24 h after Dox addition. Ad-INV-hBVR did not effect ATF-2 expression. In hBVR-infected cells, levels of HO-1 mRNA and protein were increased. In vitro translated hBVR and nuclear extract containing hBVR in gel mobility-shift assay bound to AP-1 sites in the ATF-2 promoter region and to an oligonucleotide containing the CRE site. Both bindings could be competed out by excess unlabeled probe; in the presence of hBVR antibody, they displayed shifted bands. Co-transfection of hBVR with ATF-2 or c-jun promoters caused a severalfold increase in luciferase activity. hBVR modulation of ATF-2 and HO-1 expression suggests it has a potential role in regulation of AP-1 and cAMP-regulated genes and a role in cell signaling. We propose that increased expression of the protein can be used to alter the gene expression profile in the cell.

Kuipers, O. P., A. de Jong, et al. (2002). "Transcriptome analysis and related databases of Lactococcus lactis." Antonie Van Leeuwenhoek **82**(1-4): 113-22.

Several complete genome sequences of Lactococcus lactis and their annotations will become available in the near future, next to the already published genome sequence of L. lactis ssp. lactis IL 1403. This will allow intraspecies comparative genomics studies as well as functional genomics studies aimed at a better understanding of physiological processes and regulatory networks operating in lactococci. This paper describes the initial set-up of a DNA-microarray facility in our group, to enable transcriptome analysis of various Gram-positive bacteria, including a ssp. lactis and a ssp. cremoris strain of Lactococcus lactis. Moreover a global description will be given of the hardware and software requirements for such a set-up, highlighting the crucial integration of relevant bioinformatics tools and methods. This includes the development of MolGenIS, an information system for transcriptome data storage and retrieval, and LactococCye, a metabolic pathway/genome database of Lactococcus lactis.

Kumar, S., G. K. Christophides, et al. (2003). "The role of reactive oxygen species on Plasmodium melanotic encapsulation in Anopheles gambiae." Proc Natl Acad Sci U S A **100**(24): 14139-44.

Malaria transmission depends on the competence of some Anopheles mosquitoes to sustain Plasmodium development (susceptibility). A genetically selected refractory strain of Anopheles gambiae blocks Plasmodium development, melanizing, and encapsulating the parasite in a reaction that begins with tyrosine oxidation, and involves three quantitative trait loci. Morphological and microarray mRNA expression analysis suggest that the refractory and susceptible strains have broad physiological differences, which are related to the production and detoxification of reactive oxygen species. Physiological studies corroborate that the refractory strain is in a chronic state of oxidative stress, which is exacerbated by blood feeding, resulting in increased steady-state levels of reactive oxygen species, which favor melanization of parasites as well as Sephadex beads.

Kuroda, M., H. Kuroda, et al. (2003). "Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in Staphylococcus aureus." Mol Microbiol **49**(3): 807-21.

DNA microarray covering the whole genome of Staphylococcus aureus strain N315 was prepared to investigate transcription profiles. The microarray analyses revealed that vancomycin induces transcription of 139 genes. Forty-six genes among them failed to be induced in the vraSR null mutant KVR. Part of the genes regulated by VraSR system is associated with cell-wall biosynthesis, such as

PBP2, SgtB and MurZ. Other cell-wall synthesis inhibitors also induced VraSR, suggesting that the sensor kinase VraS responds to the damage of cell-wall structure or inhibition of cell-wall biosynthesis. Additionally, the vraSR null mutants derived from hetero- and homo-methicillin-resistant S. aureus showed significant decrease of resistance against teicoplanin, beta-lactam, bacitracin and fosfomycin but not of D-cycloserine and levofloxacin. The observation strongly indicates that VraSR constitutes a positive regulator of cell-wall peptidoglycan synthesis, and that is deeply involved in the expression of beta-lactam and glycopeptide resistance in S. aureus.

Kurokawa, Y., R. Matoba, et al. (2003). "Molecular features of non-B, non-C hepatocellular carcinoma: a PCR-array gene expression profiling study." J Hepatol **39**(6): 1004-12.

BACKGROUND/AIMS: Hepatocellular carcinoma (HCC) usually develops following chronic liver inflammation caused by hepatitis C or B virus. Through expression profiling in a rare type of HCC, for which the causes are unknown, we sought to find key genes responsible for each step of hepatocarcinogenesis in the absence of viral influence. METHODS: We used 68 non-B, non-C liver tissues (20 HCC, 17 non-tumor, 31 normal liver) for expression profiling with PCR-array carrying 3072 genes known to be expressed in liver tissues. To select the differentially expressed genes, we performed random permutation testing. A weighted voting classification algorithm was used to confirm the reliability of gene selection. We then compared these genes with the results of previous expression profiling studies. RESULTS: A total of 220 differentially expressed genes were selected by random permutation tests. The classification accuracies using these genes were 91.8, 92.0 and 100.0% by a leave-one-out cross-validation, an additional PCR-array dataset and a Stanford DNA microarray dataset, respectively. By comparing our results with previous reports on virus-infected HCC, four genes (ALB, A2M, ECHS1 and IGFBP3) were commonly selected in some studies. CONCLUSIONS: The 220 differentially expressed genes selected by PCR-array are potentially responsible for hepatocarcinogenesis in the absence of viral influence.

Kuster, H., N. Hohnjec, et al. (2004). "Construction and validation of cDNA-based Mt6k-RIT macro- and microarrays to explore root endosymbioses in the model legume Medicago truncatula." J Biotechnol **108**(2): 95-113.

To construct macro- and microarray tools suitable for expression profiling in root endosymbioses of the model legume Medicago truncatula, we PCR-amplified a total of 6048 cDNA probes representing genes expressed in uninfected roots, mycorrhizal roots and young root nodules [Nucleic Acids Res. 30 (2002) 5579]. Including additional probes for either tissue-specific or constitutively expressed control genes, 5651 successfully amplified gene-specific probes were used to grid macro- and to spot microarrays designated Mt6k-RIT (M. truncatula 6k root interaction transcriptome). Subsequent to a technical validation of microarray printing, we performed two pilot expression profiling experiments using Cy-labeled targets from Sinorhizobium meliloti-induced root nodules and Glomus intraradices-colonized arbuscular mycorrhizal roots. These targets detected marker genes for nodule and arbuscular mycorrhiza development, amongst them different nodule-specific leghemoglobin and nodulin genes as well as a mycorrhiza-specific phosphate transporter gene. In addition, we identified several dozens of genes that have so far not been reported to be differentially expressed in nodules or arbuscular mycorrhiza thus demonstrating that Mt6k-RIT arrays serve as useful tools for an identification of genes relevant for legume root endosymbioses. A comprehensive profiling of such candidate genes will be very helpful to the development of breeding strategies and for the improvement of cultivation management targeted at increasing legume use in sustainable agricultural systems.

Kutalik, Z., J. Inwald, et al. (2004). "Advanced significance analysis of microarray data based on weighted resampling: a comparative study and application to gene deletions in Mycobacterium bovis." Bioinformatics **20**(3): 357-63.

MOTIVATION: When analyzing microarray data, non-biological variation introduces

uncertainty in the analysis and interpretation. In this paper we focus on the validation of significant differences in gene expression levels, or normalized channel intensity levels with respect to different experimental conditions and with replicated measurements. A myriad of methods have been proposed to study differences in gene expression levels and to assign significance values as a measure of confidence. In this paper we compare several methods, including SAM, regularized t-test, mixture modeling, Wilk's lambda score and variance stabilization. From this comparison we developed a weighted resampling approach and applied it to gene deletions in Mycobacterium bovis. RESULTS: We discuss the assumptions, model structure, computational complexity and applicability to microarray data. The results of our study justified the theoretical basis of the weighted resampling approach, which clearly outperforms the others.

Lanford, R. E., C. Bigger, et al. (2001). "The chimpanzee model of hepatitis C virus infections." Ilar J **42**(2): 117-26.

The chimpanzee (Pan troglodytes) is the only experimental animal susceptible to infection with hepatitis C virus (HCV). The chimpanzee model of HCV infection was instrumental in the initial studies on non-A, non-B hepatitis, including observations on the clinical course of infection, determination of the physical properties of the virus, and eventual cloning of the HCV nucleic acid. This review focuses on more recent aspects of the use of the chimpanzee in HCV research. The chimpanzee model has been critical for the analysis of early events in HCV infection because it represents a population for which samples are available from the time of exposure and all exposed animals are examined. For this reason, the chimpanzee represents a truly nonselected population. In contrast, human cohorts are often selected for disease status or antibody reactivity and typically include individuals that have been infected for decades. The chimpanzee model is essential to an improved understanding of the factors involved in viral clearance, analysis of the immune response to infection, and the development of vaccines. The development of infectious cDNA clones of HCV was dependent on the use of chimpanzees, and they will continue to be needed in the use of reverse genetics to evaluate critical sequences for viral replication. In addition, chimpanzees have been used in conjunction with DNA microarray technology to probe the entire spectrum of changes in liver gene expression during the course of HCV infection. The chimpanzee will continue to provide a critical aspect to the understanding of HCV disease and the development of therapeutic modalities.

Lawhon, S. D., J. G. Frye, et al. (2003). "Global regulation by CsrA in Salmonella typhimurium." Mol Microbiol **48**(6): 1633-45.

CsrA is a regulator of invasion genes in Salmonella enterica serovar Typhimurium. To investigate the wider role of CsrA in gene regulation, we compared the expression of Salmonella genes in a csrA mutant with those in the wild type using a DNA microarray. As expected, we found that expression of Salmonella pathogenicity island 1 (SPI-1) invasion genes was greatly reduced in the csrA mutant, as were genes outside the island that encode proteins translocated into eukaryotic cells by the SPI-1 type III secretion apparatus. The flagellar synthesis operons, flg and fli, were also poorly expressed, and the csrA mutant was aflagellate and non-motile. The genes of two metabolic pathways likely to be used by Salmonella in the intestinal milieu also showed reduced expression: the pdu operon for utilization of 1,2-propanediol and the eut operon for ethanolamine catabolism. Reduced expression of reporter fusions in these two operons confirmed the microarray data. Moreover, csrA was found to regulate co-ordinately the cob operon for synthesis of vitamin B12, required for the metabolism of either 1,2-propanediol or ethanolamine. Additionally, the csrA mutant poorly expressed the genes of the mal operon, required for transport and use of maltose and maltodextrins, and had reduced amounts of maltoporin, normally a dominant protein of the outer membrane. These results show that csrA controls a number of gene classes in addition to those required for invasion, some of them unique to Salmonella, and suggests a co-ordinated bacterial response to conditions that exist at the site of bacterial invasion, the intestinal tract of a host animal.

Laytragoon-Lewin, N., F. Chen, et al. (2003). "Cytokines and Epstein Barr virus (EBV) genes expression in blood chronic lymphocytic leukaemia (CLL) cells and their immortalised CLL cell lines." Anticancer Res **23**(5A): 4017-22.

We have encountered two unique chronic lymphocytic leukaemia (CLL) patients, PG and NN. Some blood CLL cells of these patients have been infected and carry Epstein Barr virus (EBV) in vivo. In spite of their early-activated G0/G1 stage of post germinal center (GC) memory cells, ex vivo EBV-carrying blood CLL cells of PG clone expressed LMPs and used specific QUK splice for their EBNA1 expression, similar to the EBV-carrying cells of non-B origin. Interestingly, EBV-carrying CLL cells of NN clone expressed LMP2a and used UK-splice for their EBNA1 expression, similar to the in vivo EBV-carrying high density normal B cells in the blood of healthy individuals. The CLL-derived lines but not normal lymphoblastoid cell line (LCL) used QUK- and YUK-splice for their EBNA1 expression. As expected, LCL and their permanent CLL-derived lines used Cp promoter and up-regulated their EBNA2 expression. Blood CLL cells and the CLL-derived cell lines of these patients spontaneously produced cytokines as shown by microarray assay. The types and quantities of cytokines might relate to their CLL origin and viral strain in the given CLL cells. Neither blood CLL nor their CLL-derived cell lines express any detectable apoptosis-inducer ligands, CD95L or Apo 3L. As a consequence of cell cycle progression, CLL-derived cell lines up-regulated their co-stimulator molecules CD80 and apoptosis-related receptor CD95. Since only the rare EBV-carrying CLL cells grew in vitro, the combination of viral genome and cytokines seems to be critical for the outgrowth of EBV-carrying CLL cells over their EBV-negative counterpart in vitro but not in vivo.

Le Roch, K. G., Y. Zhou, et al. (2002). "Monitoring the chromosome 2 intraerythrocytic transcriptome of Plasmodium falciparum using oligonucleotide arrays." Am J Trop Med Hyg **67**(3): 233-43.

To test the feasibility of using short oligonucleotide probes to monitor transcript levels in Plasmodium falciparum, a microarray was manufactured containing 4,167 (25 base single-stranded) probes derived from the predicted coding region of P. falciparum chromosome 2. RNA samples from three asexual stages (rings, trophozoites, and schizonts) were labeled and hybridized to the arrays. These results were reproducible, and transcripts were detected for 69% of the 210 genes on chromosome 2. In addition, of the 145 expressed genes, 1/3 appeared to be differentially transcribed during the asexual cycle. Some regions of the chromosome appeared to be transcriptionally silent. Results were confirmed by Northern blot analysis and by quantitative reverse transcriptase-polymerase chain reaction. These data validate the use of relatively short 25-mers for monitoring the expression of a genome that is 82% AT rich.

Lee, B. S., M. Paulose-Murphy, et al. (2002). "Suppression of tetradecanoyl phorbol acetate-induced lytic reactivation of Kaposi's sarcoma-associated herpesvirus by K1 signal transduction." J Virol **76**(23): 12185-99.

The K1 protein of Kaposi's sarcoma-associated herpesvirus (KSHV) contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic region and elicits cellular signal transduction through this motif. To investigate the role of K1 signal transduction in KSHV replication, we expressed full-length K1 and CD8-K1 chimeras in BCBL1 cells. Unlike its strong signaling activity in uninfected B lymphocytes, K1 did not induce intracellular calcium mobilization or NF-AT activation at detectable levels in KSHV-infected BCBL1 cells. Instead, K1 signaling dramatically suppressed KSHV lytic reactivation induced by tetradecanoyl phorbol acetate (TPA) stimulation, but not by ORF50 ectopic expression. Mutational analysis showed that the cytoplasmic ITAM sequence of K1 was required for this suppression. Viral microarray and immunoblot analyses demonstrated that K1 signaling suppressed the TPA-mediated increase in the expression of a large subset of viral lytic genes in KSHV-infected BCBL1 cells. Furthermore, electrophoretic mobility shift assays demonstrated that TPA-induced activation of AP-1, NF-kappaB, and Oct-1 activities was severely diminished in BCBL1 cells expressing the K1 cytoplasmic domain. The reduced activities of these transcription factors may confer the observed

reduction in viral lytic gene expression. These results demonstrate that K1-mediated signal transduction in KSHV-infected cells is profoundly different from that in KSHV-negative cells. Furthermore, K1 signal transduction efficiently suppresses TPA-mediated viral reactivation in an ITAM-dependent manner, and this suppression may contribute to the establishment and/or maintenance of KSHV latency in vivo.

Lehnen, D., C. Blumer, et al. (2002). "LrhA as a new transcriptional key regulator of flagella, motility and chemotaxis genes in Escherichia coli." Mol Microbiol **45**(2): 521-32.

The function of the LysR-type regulator LrhA of Escherichia coli was defined by comparing whole-genome mRNA profiles from wild-type E. coli and an isogenic lrhA mutant on a DNA microarray. In the lrhA mutant, a large number (48) of genes involved in flagellation, motility and chemotaxis showed relative mRNA abundances increased by factors between 3 and 80. When a representative set of five flagellar, motility and chemotaxis genes was tested in lacZ reporter gene fusions, similar factors for derepression were found in the lrhA mutant. In gel retardation experiments, the LrhA protein bound specifically to flhD and lrhA promoter DNA (apparent K(D) approximately 20 nM), whereas the promoters of fliC, fliA and trg were not bound by LrhA. The expression of flhDC (encoding FlhD(2)C(2)) was derepressed by a factor of 3.5 in the lrhA mutant. FlhD(2)C(2) is known as the master regulator for the expression of flagellar and chemotaxis genes. By DNase I footprinting, LrhA binding sites at the flhDC and lrhA promoters were identified. The lrhA gene was under positive autoregulation by LrhA as shown by gel retardation and lrhA expression studies. It is suggested that LrhA is a key regulator controlling the transcription of flagellar, motility and chemotaxis genes by regulating the synthesis and concentration of FlhD(2)C(2).

Li, H., A. K. Singh, et al. (2004). "Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of Synechocystis sp. strain PCC 6803." J Bacteriol **186**(11): 3331-45.

We utilized a full genome cDNA microarray to identify the genes that comprise the peroxide stimulon in the cyanobacterium Synechocystis sp. strain PCC 6803. We determined that a gene (slr1738) encoding a protein similar to PerR in Bacillus subtilis was induced by peroxide. We constructed a PerR knockout strain and used it to help identify components of the PerR regulon, and we found that the regulatory properties were consistent with the hypothesis that PerR functions as a repressor. This effort was guided by finding putative PerR boxes in positions upstream of specific genes and by careful statistical analysis. PerR and sll1621 (ahpC), which codes for a peroxiredoxin, share a divergent promoter that is regulated by PerR. We found that isiA, encoding a Chl protein that is induced under low-iron conditions, was strongly induced by a short-term peroxide stress. Other genes that were strongly induced by peroxide included sigD, sigB, and genes encoding peroxiredoxins and Dsb-like proteins that have not been studied yet in this strain. A gene (slr1894) that encoded a protein similar to MrgA in B. subtilis was upregulated by peroxide, and a strain containing an mrgA knockout mutation was highly sensitive to peroxide. A number of genes were downregulated, including key genes in the chlorophyll biosynthesis pathway and numerous regulatory genes, including those encoding histidine kinases. We used PerR mutants and a thioredoxin mutant (TrxA1) to study differential expression in response to peroxide and determined that neither PerR nor TrxA1 is essential for the peroxide protective response.

Li, L., W. L. Ma, et al. (2003). "A modified restriction display PCR method in sample-labelling of DNA microarray." J Virol Methods **114**(1): 71-5.

The restriction display PCR is a useful technique for studying the diversity of gene expression. This method involves ligating the digested genes with adapters and amplifying the gene fragments by PCR using universal and selective primers. In this study, we improved this restriction display PCR method by using Cy3-UP, a fluorescently labelled universal primer, in place of Cy3-dCTP in sample-labelling for DNA microarray. The results show that this new method increases significantly the sensitivity of the assay, and will have a wide application in the DNA microarray field.

Li, X., W. L. Ma, et al. (2003). "Cloning and sequence analysis of Bacillus thuringiensis gene fragments isolated by restriction digest PCR." Di Yi Jun Yi Da Xue Xue Bao **23**(4): 323-5.

OBJECTIVE: To clone and analyze Bacillus thuringiensis gene fragments isolated by restriction digest PCR (RD-PCR). METHOD: Specific primers were designed to amplify the genes of Bacillus thuringiensis israelensis (Bti), and the PCR products were classified and re-amplified by RD-PCR to obtain the fragments for subsequent purification and cloning into the pMD18-T vectors, followed by rapid identification. The recombinant plasmids were extracted from positive clones and the target gene fragments were sequenced. RESULTS: Sequence analysis showed that all the fragments amplified were Bti genes. CONCLUSION: RD-PCR is reliable in breaking down large gene fragments into confined and shorter gene fragments for preparing microarray probes.

Li, Y. and S. Altman (2003). "A specific endoribonuclease, RNase P, affects gene expression of polycistronic operon mRNAs." Proc Natl Acad Sci U S A **100**(23): 13213-8.

The rnpA mutation, A49, in Escherichia coli reduces the level of RNase P at 43 degrees C because of a temperature-sensitive mutation in C5 protein, the protein subunit of the enzyme. Microarray analysis reveals the expression of several noncoding intergenic regions that are increased at 43 degrees C compared with 30 degrees C. These regions are substrates for RNase P, and they are cleaved less efficiently than, for example, tRNA precursors. An analysis of the tna, secG, rbs, and his operons, all of which contain RNase P cleavage sites, indicates that RNase P affects gene expression for regions downstream of its cleavage sites.

Li, Y. and M. A. Lazar (2002). "Differential gene regulation by PPARgamma agonist and constitutively active PPARgamma2." Mol Endocrinol **16**(5): 1040-8.

The PPARgamma is a key adipogenic determination factor. Ligands for PPARgamma such as antidiabetic thiazolidinedione (TZD) compounds are adipogenic, and many adipocyte genes that are activated by TZDs contain binding sites for PPARgamma. Like ligands for other nuclear receptors, TZDs can regulate genes positively or negatively. Here, we sought to understand the importance of positive regulation of gene expression by PPARgamma in adipogenesis. Fusion of the potent viral transcriptional activator VP16 to PPARgamma2 (VP16-PPARgamma) created a transcription factor that constitutively and dramatically activated transcription of PPARgamma-responsive genes in the absence of ligand. Forced expression of VP16-PPARgamma in 3T3-L1 preadipocytes using retroviral vectors led to adipogenesis in the absence of standard differentiating medium or any exogenous PPARgamma ligand. Gene microarray analysis revealed that VP16-PPARgamma induced many of the genes associated with adipogenesis and adipocyte function. Thus, direct up-regulation of gene expression by PPARgamma is sufficient for adipogenesis. TZD-induced adipogenesis up-regulated many of the same genes, although some were divergently regulated, including resistin, whose gene expression was reduced inVP16-PPARgamma adipocytes treated with TZDs. These results show that, although activation of PPARgamma by a heterologous activation domain is sufficient for adipogenesis, it is not equivalent to TZD treatment. This conclusion has important implications for understanding biological effects of the TZDs on adipogenesis and insulin sensitization.

Lee, G. P., B. E. Min, et al. (2003). "Plant virus cDNA chip hybridization for detection and differentiation of four cucurbit-infecting Tobamoviruses." J Virol Methods **110**(1): 19-24.

A plant virus cDNA chip was developed by using viral cDNA clones and microarray technology. The cDNA chip was designed for detection and differentiation of the four species of selected cucurbit-infecting tobamoviruses [target viruses: Cucumber green mottle mosaic virus (CGMMV); Cucumber fruit mottle mosaic virus (CFMMV); Kyuri green mottle mosaic virus (KGMMV); and Zucchini green mottle mosaic virus (ZGMMV)]. The chip consisted of cDNA clones of the four cucurbit-infecting tobamoviruses, two target-related tobamoviruses, and another three unrelated plant viruses. Polymerase chain reaction products were amplified from the selected cDNA clones and arrayed onto slide glass. The

cDNA chip, which was called cucurbit-virus chip, detected successfully specific target viruses. When applied to probes made from ZGMMV-infected samples, ZGMMV reacted strongly with its homologous cDNA and moderately reacted with KGMMV and CFMMV, while it did not react with CGMMV on the same chip. CGMMV probe gave strong signal intensity to its homologous cDNA spot and weakly reacted with ZGMMV, KGMMV, and CFMMV. The signal intensity of all combinations of probe and target was correlated significantly with nucleotide sequence identities between the probes and target viruses based on scatter diagrams. The signals could be made as image files for specific virus detection, and this could be useful for virus identification and differentiation. This is the first report of plant virus detection by using cDNA chip technology.

Lee, J. M., S. Zhang, et al. (2001). "RNA expression analysis using an antisense Bacillus subtilis genome array." J Bacteriol **183**(24): 7371-80.

We have developed an antisense oligonucleotide microarray for the study of gene expression and regulation in Bacillus subtilis by using Affymetrix technology. Quality control tests of the B. subtilis GeneChip were performed to ascertain the quality of the array. These tests included optimization of the labeling and hybridization conditions, determination of the linear dynamic range of gene expression levels, and assessment of differential gene expression patterns of known vitamin biosynthetic genes. In minimal medium, we detected transcripts for approximately 70% of the known open reading frames (ORFs). In addition, we were able to monitor the transcript level of known biosynthetic genes regulated by riboflavin, biotin, or thiamine. Moreover, novel transcripts were also detected within intergenic regions and on the opposite coding strand of known ORFs. Several of these novel transcripts were subsequently correlated to new coding regions.

Lee, P. S., L. B. Shaw, et al. (2003). "Insights into the relation between mrna and protein expression patterns: II. Experimental observations in Escherichia coli." Biotechnol Bioeng **84**(7): 834-41.

There is a need for improved appreciation of the importance of genome-wide mRNA and protein expression measurements and their role in understanding translation and in relation to genome-wide mathematical frameworks for gene expression regulation. We investigated the use of a high-density microarray technique for mRNA expression analysis and a two-dimensional protein electrophoresis-tandem mass spectrometry method for protein analysis to monitor changes in gene expression. We applied these analytical tools in the context of an environmental perturbation of Escherichia coli cells-the addition of varying amounts of IPTG. We also tested the application of these tools to the study of a genetic perturbation of Escherichia coli cells-the ability of certain strains to hypersecrete the hemolysin protein. We observed a lack of correspondence between mRNA and protein expression profiles. Although our data do not include measurements on all expressed genes (because the ability to measure protein expression profiles is limiting), we observed that the qualitative and quantitative behavior of the measurements of a subset of expressed genes is similar to the behavior of the entire system. The change in observed average mRNA and protein amplification factors for 77 and 52 genes coincided with the observed change in mRNA amplification factor for the entire system. Furthermore, we found that the use of relative changes in expression could be used to elucidate mechanisms of gene expression regulation for the system studied, even when measurements were made on a small subset of the system.

Li, Y. Q., P. L. Chen, et al. (2004). "A pair of two-component regulatory genes ecrA1/A2 in S. coelicolor." J Zhejiang Univ Sci **5**(2): 173-9.

Two-component genes are kinds of genetic elements involved in regulation of antibiotic production in Streptomyces coelicolor. DNA microarray analysis revealed that ecrA1/A2, which mapped at distant sites from red locus and encode respectively the kinase and regulator, expressed coordinately with genes of Red specific biosynthetic pathway. ecrA1 and ecrA2 gene-disruptive mutants were constructed using homogenotisation by reciprocal double crossover. Fermentation data showed that the undecylprodigiosin (Red) level of production was lower than that of wild-type strain. However, the

change of the actinorhodin (Act) production level was not significant compared with wild type. Thus, these experiment results confirmed that the two-component system ecrA1/A2 was positive regulatory element for red gene cluster.

Liang, F. T., F. K. Nelson, et al. (2002). "DNA microarray assessment of putative Borrelia burgdorferi lipoprotein genes." Infect Immun **70**(6): 3300-3.

A DNA microarray containing fragments of 137 Borrelia burgdorferi B31 putative lipoprotein genes was used to examine Lyme disease spirochetes. DNA from B. burgdorferi sensu stricto B31, 297, and N40; Borrelia garinii IP90; and Borrelia afzelii P/Gau was fluorescently labeled and hybridized to the microarray, demonstrating the degree to which the individual putative lipoprotein genes were conserved among the genospecies. These data show that a DNA microarray can globally examine the genes encoding B. burgdorferi lipoproteins.

Lievens, S., S. Goormachtig, et al. (2001). "A critical evaluation of differential display as a tool to identify genes involved in legume nodulation: looking back and looking forward." Nucleic Acids Res **29**(17): 3459-68.

Screening for differentially expressed genes is a straightforward approach to study the molecular basis of a biological system. In the last 10 years, differential screening technology has evolved rapidly and currently high-throughput tools for genome-wide transcript profiling, such as expressed sequence tags and microarray analysis, are becoming widely available. Here, an overview of this (r)evolution is given with emphasis on the differential display method, which for many years has been the preferred technique of scientists in diverse fields of research. Differential display has also been the method of choice for the identification of genes involved in the symbiotic interaction between Azorhizobium caulinodans and Sesbania rostrata. The advantages with respect to tissue specificity of this particular model system for legume nodulation and the results of a screening for early nodulation-related genes have been considered in the context of transcriptome analyses in other rhizobium-legume interactions.

Lilly, J. W., J. E. Maul, et al. (2002). "The Chlamydomonas reinhardtii organellar genomes respond transcriptionally and post-transcriptionally to abiotic stimuli." Plant Cell **14**(11): 2681-706.

The Chlamydomonas reinhardtii plastid and mitochondrial transcriptomes were surveyed for changes in RNA profiles resulting from growth in 12 culture conditions representing 8 abiotic stimuli. Organellar RNA abundance exhibited marked changes during nutrient stress and exposure to UV light, as revealed by both RNA gel blot and DNA microarray analyses. Of particular note were large increases in tufA and clpP transcript abundance during nutrient limitation. Phosphate and sulfur limitation resulted in the most global, yet opposite, effects on organellar RNA abundance, changes that were dissected further using run-on transcription assays. Removal of sulfate from the culture medium, which is known to reduce photosynthesis, resulted in 2-fold to 10-fold decreases in transcription rates, which were reflected in lower RNA abundance. The decrease in transcriptional activity was completely reversible and recovered to twice the control level after sulfate replenishment. Conversely, phosphate limitation resulted in a twofold to threefold increase in RNA abundance that was found to be a post-transcriptional effect, because it could be accounted for by increased RNA stability. This finding is consistent with the known metabolic slowdown under phosphate stress. Additionally, inhibitor studies suggested that unlike those in higher plants, Chlamydomonas chloroplasts lack a nucleus-encoded plastid RNA polymerase. The apparently single type of polymerase could contribute to the rapid and genome-wide transcriptional responses observed within the chloroplast.

Liu, C. L., S. L. Schreiber, et al. (2003). "Development and validation of a T7 based linear amplification for genomic DNA." BMC Genomics **4**(1): 19.

BACKGROUND: Genomic maps of transcription factor binding sites and histone modification patterns provide unique insight into the nature of gene regulatory networks and chromatin structure.

These systematic studies use microarrays to analyze the composition of DNA isolated by chromatin immunoprecipitation. To obtain quantities sufficient for microarray analysis, the isolated DNA must be amplified. Current protocols use PCR-based approaches to amplify in exponential fashion. However, exponential amplification protocols are highly susceptible to bias. Linear amplification strategies minimize amplification bias and have had a profound impact on mRNA expression analysis. These protocols have yet to be applied to the analysis of genomic DNA due to the lack of a suitable tag such as the polyA tail. RESULTS: We have developed a novel linear amplification protocol for genomic DNA. Terminal transferase is used to add polyT tails to the ends of DNA fragments. Tail length uniformity is ensured by including a limiting concentration of the terminating nucleotide ddCTP. Second strand synthesis using a T7-polyA primer adapter yields double stranded templates suitable for in vitro transcription (IVT). Using this approach, we are able to amplify as little as 2.5 ng of genomic DNA, while retaining the size distribution of the starting material. In contrast, we find that PCR amplification is biased towards species of greater size. Furthermore, extensive microarray-based analyses reveal that our linear amplification protocol preserves dynamic range and species representation more effectively than a commonly used PCR-based approach. CONCLUSION: We present a T7-based linear amplification protocol for genomic DNA. Validation studies and comparisons with existing methods suggest that incorporation of this protocol will reduce amplification bias in genome mapping experiments.

Liu, H. C., M. Niikura, et al. (2003). "Identification of chicken lymphocyte antigen 6 complex, locus E (LY6E, alias SCA2) as a putative Marek's disease resistance gene via a virus-host protein interaction screen." Cytogenet Genome Res **102**(1-4): 304-8.

Marek's disease virus (MDV) is a naturally occurring oncogenic avian herpesvirus that causes neurological disorders and T cell lymphoma disease in domestic chickens. Identification and functional characterization of the individual factors involved in Marek's disease (MD) resistance or pathogenesis will enhance our understanding of MDV pathogenesis and further genetic improvement of chickens. To study the genetic basis for resistance to MD, a strategy that combined protein-protein interaction screens followed by linkage analysis was performed. The MDV protein US10 was used as the bait in an E. COLI two-hybrid screening of a cDNA library derived from activated splenic T cells. The chicken LY6E, also known as SCA2 and TSA1, was found to specifically interact with US10. This interaction was confirmed by an in vitro protein-binding assay. Furthermore, LY6E was found to be significantly associated with MD traits in an MD resource population comprised of commercial chickens. Previously, LY6E was implicated in two independent DNA microarray experiments evaluating differential gene expression following MDV infection. Given that LY6E is involved in T cell differentiation and activation, we suggest that LY6E is a candidate gene for MD resistance and deserves further investigation on its role in MDV pathogenesis, especially with respect to the binding of US10.

Liu, J., K. Tan, et al. (2003). "Computational identification of the Spo0A-phosphate regulon that is essential for the cellular differentiation and development in Gram-positive spore-forming bacteria." Nucleic Acids Res **31**(23): 6891-903.

Spo0A-phosphate is essential for the initiation of cellular differentiation and developmental processes in Gram-positive spore-forming bacteria. Here we combined comparative genomics with analyses of microarray expression profiles to identify the Spo0A-phosphate regulon in Bacillus subtilis. The consensus Spo0A-phosphate DNA-binding motif identified from the training set based on different computational algorithms is an 8 bp sequence, TTGTCGAA. The same motif was identified by aligning the upstream regulatory sequences of spo0A-dependent genes obtained from the expression profile of Sad67 (a constitutively active form of Spo0A) and their orthologs. After the transcription units (TUs) having putative Spo0A-phosphate binding sites were obtained, conservation of regulons among the genomes of B.subtilis, Bacillus halodurans and Bacillus anthracis, and expression profiles were employed to identify the most confident predictions. Besides genes already known to be directly under the control of Spo0A-phosphate, 276 novel members (organized in 109 TUs) of the Spo0A-phosphate regulon in

B.subtilis are predicted in this study. The sensitivity and specificity of our predictions are estimated based on known sites and combinations of different types of evidence. Further characterization of the novel candidates will provide information towards understanding the role of Spo0A-phosphate in the sporulation process, as well as the entire genetic network governing cellular differentiation and developmental processes in B.subtilis.

Liu, L., A. A. Roberts, et al. (2003). "By IL-1 signaling, monocyte-derived cells dramatically enhance the epidermal antimicrobial response to lipopolysaccharide." J Immunol **170**(1): 575-80.

Epithelia react to microbial pathogens by mounting a defensive response that includes the production of antimicrobial peptides. In this study, we show that, in human epidermal cultures, Escherichia coli LPS was a very weak direct inducer of human beta-defensin (HBD)-2 mRNA and peptide, but the induction was greatly amplified when monocyte-derived cells (MoDeC) acted as intermediaries between LPS and the epidermis. IL-1R antagonist largely reversed the effect of MoDeC on epidermal HBD-2, indicating that, from among the many products of MoDeC, IL-1 was the dominant inducer of HBD-2 synthesis. In normal fresh human skin, which contains Langerhans cells and other myeloid cell types, in addition to keratinocytes, LPS also induced HBD-2 in an IL-1-dependent manner. In DNA microarray expression studies, HBD-2 was one of the most abundant mRNAs induced in epidermis by LPS-treated MoDeC, and its induction was reversed by IL-1Ra. Thus, epidermal response to LPS is potently amplified by MoDeC through IL-1-mediated signaling, leading to a selective increase in the synthesis of the antimicrobial peptide HBD-2. This pattern of responses establishes a key role for both IL-1 and HBD-2 in the host defense reaction of the epidermis.

Liu, S., D. J. Gallo, et al. (2002). "Role of toll-like receptors in changes in gene expression and NF-kappa B activation in mouse hepatocytes stimulated with lipopolysaccharide." Infect Immun **70**(7): 3433-42.

The liver is an important site of host-microbe interaction. Although hepatocytes have been reported to be responsive to lipopolysaccharide (LPS), the global gene expression changes by LPS and mechanism(s) by which LPS stimulates cultured hepatocytes remain uncertain. Cultures of primary mouse hepatocytes were incubated with LPS to assess its effects on the global gene expression, hepatic transcription factors, and mitogen-activated protein (MAP) kinase activation. DNA microarray analysis indicated that LPS modulates the selective expression of more than 80 genes and expressed sequence tags. We have shown previously that hepatocytes express CD14, which is required both for uptake and responsiveness to LPS. In other cells, responsiveness to microbial products requires expression of Toll-like receptors (TLR) and their associated accessory molecules. Hepatocytes expressed TLR1 through TLR9 as well as MyD88 and MD-2 transcripts, as shown by reverse transcriptase PCR analysis, indicating that hepatocytes express all known microbe recognition molecules. The MAP kinase extracellular signal-regulated kinase 1/2 was phosphorylated in response to LPS in mouse hepatocytes, and the levels of phosphorylation were lower in hepatocytes from TLR4-null mice. NF-kappa B activation was reduced in TLR4-mutant or -null hepatocytes compared to control hepatocytes, and this defect was partially restored by adenoviral transduction of mouse TLR4. Thus, hepatocytes respond to nanogram concentrations of LPS through a TLR4 response pathway.

Liu, W. T., A. D. Mirzabekov, et al. (2001). "Optimization of an oligonucleotide microchip for microbial identification studies: a non-equilibrium dissociation approach." Environ Microbiol **3**(10): 619-29.

The utility of a high-density oligonucleotide microarray (microchip) for identifying strains of five closely related bacilli (Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus medusa and Bacillus subtilis) was demonstrated using an approach that compares the non-equilibrium dissociation rates ('melting curves') of all probe-target duplexes simultaneously. For this study, a hierarchical set of 30 oligonucleotide probes targeting the 16S ribosomal RNA of these bacilli at multiple levels of specificity (approximate taxonomic ranks of domain, kingdom, order, genus and species) was designed and immobilized in a high-density matrix of gel pads on a glass slide. Reproducible melting curves for probes

with different levels of specificity were obtained using an optimized salt concentration. Clear discrimination between perfect match (PM) and mismatch (MM) duplexes was achieved. By normalizing the signals to an internal standard (a universal probe), a more than twofold discrimination (> 2.4x) was achieved between PM and 1-MM duplexes at the dissociation temperature at which 50% of the probe-target duplexes remained intact. This provided excellent differentiation among representatives of different Bacillus species, both individually and in mixtures of two or three. The overall pattern of hybridization derived from this hierarchical probe set also provided a clear 'chip fingerprint' for each of these closely related Bacillus species.

Liu, X., D. L. Brutlag, et al. (2001). "BioProspector: discovering conserved DNA motifs in upstream regulatory regions of co-expressed genes." Pac Symp Biocomput: 127-38.

The development of genome sequencing and DNA microarray analysis of gene expression gives rise to the demand for data-mining tools. BioProspector, a C program using a Gibbs sampling strategy, examines the upstream region of genes in the same gene expression pattern group and looks for regulatory sequence motifs. BioProspector uses zero to third-order Markov background models whose parameters are either given by the user or estimated from a specified sequence file. The significance of each motif found is judged based on a motif score distribution estimated by a Monte Carlo method. In addition, BioProspector modifies the motif model used in the earlier Gibbs samplers to allow for the modeling of gapped motifs and motifs with palindromic patterns. All these modifications greatly improve the performance of the program. Although testing and development are still in progress, the program has shown preliminary success in finding the binding motifs for Saccharomyces cerevisiae RAP1, Bacillus subtilis RNA polymerase, and Escherichia coli CRP. We are currently working on combining BioProspector with a clustering program to explore gene expression networks and regulatory mechanisms.

Liu, X. and P. De Wulf (2004). "Probing the ArcA-P modulon of Escherichia coli by whole genome transcriptional analysis and sequence recognition profiling." J Biol Chem **279**(13): 12588-97.

The ArcB/ArcA two-component signal transduction system of Escherichia coli regulates gene expression in response to the redox conditions of growth. Over the years, genetic screens have lead to the identification of about 30 ArcA-P-controlled operons that are involved in redox metabolism. However, the discovery of 3 targets that are not implicated in respiratory metabolism (the tra operon for plasmid conjugation, psi site for Xer-based recombination, and oriC site for chromosome replication) suggests that the Arc modulon may comprise additional operons that are involved in a myriad of functions. To identify these operons, we derived the ArcA-P-dependent transcription profile of E. coli using oligonucleotide-based microarray analysis. The findings indicated that 9% of all open reading frames in E. coli are affected either directly or indirectly by ArcA-P. To identify which operons are under the direct control of ArcA-P, we developed the ArcA-P recognition weight matrix from footprinting data and used it to scan the genome, yielding an ArcA-P sequence affinity map. By overlaying both methods, we identified 55 new Arc-regulated operons that are implicated in energy metabolism, transport, survival, catabolism, and transcriptional regulation. The data also suggest that the Arc response pathway, which translates into a net global downscaling of gene expression, overlaps partly with the FNR regulatory network. A conservative but reasonable assessment is that the Arc pathway recruits 100-150 operons to mediate a role in cellular adaptation that is more extensive than hitherto anticipated.

Liu, Y., J. Zhou, et al. (2003). "Transcriptome dynamics of Deinococcus radiodurans recovering from ionizing radiation." Proc Natl Acad Sci U S A **100**(7): 4191-6.

Deinococcus radiodurans R1 (DEIRA) is a bacterium best known for its extreme resistance to the lethal effects of ionizing radiation, but the molecular mechanisms underlying this phenotype remain poorly understood. To define the repertoire of DEIRA genes responding to acute irradiation (15 kGy), transcriptome dynamics were examined in cells representing early, middle, and late phases of recovery by

using DNA microarrays covering approximately 94% of its predicted genes. At least at one time point during DEIRA recovery, 832 genes (28% of the genome) were induced and 451 genes (15%) were repressed 2-fold or more. The expression patterns of the majority of the induced genes resemble the previously characterized expression profile of recA after irradiation. DEIRA recA, which is central to genomic restoration after irradiation, is substantially up-regulated on DNA damage (early phase) and down-regulated before the onset of exponential growth (late phase). Many other genes were expressed later in recovery, displaying a growth-related pattern of induction. Genes induced in the early phase of recovery included those involved in DNA replication, repair, and recombination, cell wall metabolism, cellular transport, and many encoding uncharacterized proteins. Collectively, the microarray data suggest that DEIRA cells efficiently coordinate their recovery by a complex network, within which both DNA repair and metabolic functions play critical roles. Components of this network include a predicted distinct ATP-dependent DNA ligase and metabolic pathway switching that could prevent additional genomic damage elicited by metabolism-induced free radicals.

Lizewski, S. E., J. R. Schurr, et al. (2004). "Identification of AlgR-regulated genes in Pseudomonas aeruginosa by use of microarray analysis." J Bacteriol **186**(17): 5672-84.

The Pseudomonas aeruginosa transcriptional regulator AlgR controls a variety of different processes, including alginate production, type IV pilus function, and virulence, indicating that AlgR plays a pivotal role in the regulation of gene expression. In order to characterize the AlgR regulon, Pseudomonas Affymetrix GeneChips were used to generate the transcriptional profiles of (i) P. aeruginosa PAO1 versus its algR mutant in mid-logarithmic phase, (ii) P. aeruginosa PAO1 versus its algR mutant in stationary growth phase, and (iii) PAO1 versus PAO1 harboring an algR overexpression plasmid. Expression analysis revealed that, during mid-logarithmic growth, AlgR activated the expression of 58 genes while it repressed the expression of 37 others, while during stationary phase, it activated expression of 45 genes and repression of 14 genes. Confirmatory experiments were performed on two genes found to be AlgR repressed (hcnA and PA1557) and one AlgR-activated operon (fimU-pilVWXY1Y2). An S1 nuclease protection assay demonstrated that AlgR repressed both known hcnA promoters in PAO1. Additionally, direct measurement of hydrogen cyanide (HCN) production showed that P. aeruginosa PAO1 produced threefold-less HCN than did its algR deletion strain. AlgR also repressed transcription of two promoters of the uncharacterized open reading frame PA1557. Further, the twitching motility defect of an algR mutant was complemented by the fimTU-pilVWXY1Y2E operon, thus identifying the AlgR-controlled genes responsible for this defect in an algR mutant. This study identified four new roles for AlgR: (i) AlgR can repress gene transcription, (ii) AlgR activates the fimTU-pilVWXY1Y2E operon, (iii) AlgR regulates HCN production, and (iv) AlgR controls transcription of the putative cbb3-type cytochrome PA1557.

Lobner-Olesen, A., M. G. Marinus, et al. (2003). "Role of SeqA and Dam in Escherichia coli gene expression: a global/microarray analysis." Proc Natl Acad Sci U S A **100**(8): 4672-7.

High-density oligonucleotide arrays were used to monitor global transcription patterns in Escherichia coli with various levels of Dam and SeqA proteins. Cells lacking Dam methyltransferase showed a modest increase in transcription of the genes belonging to the SOS regulon. Bacteria devoid of the SeqA protein, which preferentially binds hemimethylated DNA, were found to have a transcriptional profile almost identical to WT bacteria overexpressing Dam methyltransferase. The latter two strains differed from WT in two ways. First, the origin proximal genes were transcribed with increased frequency due to increased gene dosage. Second, chromosomal domains of high transcriptional activity alternate with regions of low activity, and our results indicate that the activity in each domain is modulated in the same way by SeqA deficiency or Dam overproduction. We suggest that the methylation status of the cell is an important factor in forming and/or maintaining chromosome structure.

Long, A. D., H. J. Mangalam, et al. (2001). "Improved statistical inference from DNA microarray data

using analysis of variance and a Bayesian statistical framework. Analysis of global gene expression in Escherichia coli K12." J Biol Chem **276**(23): 19937-44.

We describe statistical methods based on the t test that can be conveniently used on high density array data to test for statistically significant differences between treatments. These t tests employ either the observed variance among replicates within treatments or a Bayesian estimate of the variance among replicates within treatments based on a prior estimate obtained from a local estimate of the standard deviation. The Bayesian prior allows statistical inference to be made from microarray data even when experiments are only replicated at nominal levels. We apply these new statistical tests to a data set that examined differential gene expression patterns in IHF(+) and IHF(-) Escherichia coli cells (Arfin, S. M., Long, A. D., Ito, E. T., Tolleri, L., Riehle, M. M., Paegle, E. S., and Hatfield, G. W. (2000) J. Biol. Chem. 275, 29672-29684). These analyses identify a more biologically reasonable set of candidate genes than those identified using statistical tests not incorporating a Bayesian prior. We also show that statistical tests based on analysis of variance and a Bayesian prior identify genes that are up- or down-regulated following an experimental manipulation more reliably than approaches based only on a t test or fold change. All the described tests are implemented in a simple-to-use web interface called Cyber-T that is located on the University of California at Irvine genomics web site.

Loos, A., C. Glanemann, et al. (2001). "Development and validation of corynebacterium DNA microarrays." Appl Environ Microbiol **67**(5): 2310-8.

We have developed DNA microarray techniques for studying Corynebacterium glutamicum. A set of 52 C. glutamicum genes encoding enzymes from primary metabolism was amplified by PCR and printed in triplicate onto glass slides. Total RNA was extracted from cells harvested during the exponential-growth and lysine production phases of a C. glutamicum fermentation. Fluorescently labeled cDNAs were prepared by reverse transcription using random hexamer primers and hybridized to the microarrays. To establish a set of benchmark metrics for this technique, we compared the variability between replicate spots on the same slide, between slides hybridized with cDNAs from the same labeling reaction, and between slides hybridized with cDNAs prepared in separate labeling reactions. We found that the results were both robust and statistically reproducible. Spot-to-spot variability was 3.8% between replicate spots on a given slide, 5.0% between spots on separate slides (though hybridized with identical, labeled cDNA), and 8.1% between spots from separate slides hybridized with samples from separate reverse transcription reactions yielding an average spot to spot variability of 7.1% across all conditions. Furthermore, when we examined the changes in gene expression that occurred between the two phases of the fermentation, we found that results for the majority of the genes agreed with observations made using other methods. These procedures will be a valuable addition to the metabolic engineering toolbox for the improvement of C. glutamicum amino acid-producing strains.

Lou, Y. and I. T. Baldwin (2004). "Nitrogen supply influences herbivore-induced direct and indirect defenses and transcriptional responses in Nicotiana attenuata." Plant Physiol **135**(1): 496-506.

Although nitrogen (N) availability is known to alter constitutive resistance against herbivores, its influence on herbivore-induced responses, including signaling pathways, transcriptional signatures, and the subsequently elicited chemical defenses is poorly understood. We used the native tobacco, Nicotiana attenuata, which germinates in the postfire environment and copes with large changes in soil N during postfire succession, to compare a suite of Manduca sexta- and elicitor-induced responses in plants grown under high- and low-N (LN) supply rates. LN supply decreased relative growth rates and biomass by 35% at 40 d compared to high-N plants; furthermore, it also attenuated (by 39 and 60%) the elicitor-induced jasmonate and salicylate bursts, two N-intensive direct defenses (nicotine and trypsin proteinase inhibitors, albeit by different mechanisms), and carbon-containing nonvolatile defenses (rutin, chlorogenic acid, and diterpene glycosides), but did not affect the induced release of volatiles (cis-alpha-bergamotene and germacrene A), which function as indirect defenses. M. sexta and methyl jasmonate-induced transcriptional responses measured with a microarray enriched in herbivore-induced genes were

also substantially reduced in plants grown under LN supply rates. In M. sexta-attacked LN plants, only 36 (45%) up-regulated and 46 (58%) down-regulated genes showed the same regulation as those in attacked high-N plants. However, transcriptional responses frequently directly countered the observed metabolic changes. Changes in a leaf's sensitivity to elicitation, an attacked leaf's waning ability to export oxylipin wound signals, and/or resource limitations in LN plants can account for the observed results, underscoring the conclusion that defense activation is a resource-intensive response.

Lu, J., H. H. Chua, et al. (2003). "Regulation of matrix metalloproteinase-1 by Epstein-Barr virus proteins." Cancer Res **63**(1): 256-62.

Matrix metalloproteinases (MMPs) play crucial roles in tumor progression. To investigate the roles of MMPs in the progression of nasopharyngeal carcinoma (NPC), the expression of MMP-1, MMP-2, MMP-3, MMP-7, MMP-12, MMP-13, MMP-14, and MMP-19 was explored by microarray assay. Among them, MMP-1 was significantly up-regulated in NPC biopsies. These results were confirmed further by real-time quantitative PCR in additional NPC biopsies and comparison with normal tissues and other head and neck cancers. Moreover, the use of RNA from different cellular constituents of NPC biopsies revealed that MMP-1 was detected predominantly in epithelial cells. Immunohistochemical staining of paraffin-fixed NPC sections confirmed that MMP-1 protein was expressed in the epithelial tumor cells. Because EBV is strongly associated with NPC formation, we sought a correlation between viral gene expression and MMP-1 up-regulation. The results showed clearly that the amounts of transcripts, proteins, and enzyme activities of MMP-1 were increased in cells expressing EBV proteins, LMP1 (latent membrane protein 1) and Zta (Z transactivator; also named as BZLF1 or ZEBRA) but not EBNA-1 (EBV nuclear antigen-1). Additionally, the mobility of LMP1 and Zta transfectants was increased in scrape-wound migration assays. The invasiveness and ability to survive in a three-dimensional collagen gel also were enhanced in LMP1- and Zta-expressing cells. Furthermore, anti-MMP-1 antibody and peptide inhibitors could block the invasiveness and survival properties of LMP1 and Zta transfectants, suggesting a real contribution of MMP-1 to cell mobility and survival. Taken together, our data show that the viral LMP1 and Zta proteins regulate the expression and activity of MMP-1, and thereby confer the invasive properties of the cells. This study presents the first evidence that viral proteins are capable of regulating MMP-1 and also provides clues for the role of EBV in NPC progression.

Lue, R. Y., G. Y. Chen, et al. (2004). "Versatile protein biotinylation strategies for potential high-throughput proteomics." J Am Chem Soc **126**(4): 1055-62.

We present intein-mediated approaches for efficient biotinylation of proteins site-specifically. The reactive C-terminal thioester generated from intein-assisted protein splicing (either in vitro or in live cells) served as an attractive and exclusive site for attaching cysteine-containing biotin. Using these novel biotinylation strategies, we were able to efficiently biotinylate many proteins from different biological sources in a potentially high-throughput, high-content fashion. Some of these proteins were subsequently immobilized, in a very simple manner, onto different avidin-functionalized solid surfaces for applications such as protein microarray and surface plasmon resonance (SPR) spectroscopy, highlighting the numerous advantages of using biotin over other tags (e.g., GST, His-tag, etc.) as the method of choice in protein purification/immobilization. In addition, our intein-mediated strategies provided critical advantages over other protein biotinylation strategies in a number of ways. For the first time, we also successfully demonstrated that intein-mediated protein biotinylation proceeded adequately inside both bacterial and mammalian living cells, as well as in a cell-free protein synthesis system. Taken together, our results indicate the versatility of these intein-mediated strategies for potential high-throughput proteomics applications. They may also serve as useful tools for various biochemical and biophysical studies of proteins both in vitro and in vivo.

Luke, K., A. Radek, et al. (2002). "Microarray analysis of gene expression during bacteriophage T4

infection." Virology **299**(2): 182-91.

Genomic microarrays were used to examine the complex temporal program of gene expression exhibited by bacteriophage T4 during the course of development. The microarray data confirm the existence of distinct early, middle, and late transcriptional classes during the bacteriophage replicative cycle. This approach allows assignment of previously uncharacterized genes to specific temporal classes. The genomic expression data verify many promoter assignments and predict the existence of previously unidentified promoters.

Ma, Y., R. Croxton, et al. (2002). "Identification of novel E2F1-regulated genes by microarray." Arch Biochem Biophys **399**(2): 212-24.

The E2F pathway has been proposed to regulate genes involved in the transition from quiescence into DNA synthesis. However, this hypothesis has not been rigorously tested on a genomic scale. Toward this end, we have infected quiescent mouse fibroblasts, which do not express E2F1, with an E2F1-expressing adenovirus and examined the expression of more than 6000 genes using high-density microarrays. Microarray results clearly support the current paradigm; however, they suggest that E2F1 may also regulate unanticipated cellular functions including pathways involved in apoptosis, signal transduction, transcriptional control, and membrane biology. Most surprisingly, we identified a number of genes that are repressed by E2F1 expression, suggesting that E2F1 may have the potential to repress transcription of numerous genes through an unknown mechanism.

Maeda, M., H. Sakamoto, et al. (2003). "Changing patterns of gene expression in dictyostelium prestalk cell subtypes recognized by in situ hybridization with genes from microarray analyses." Eukaryot Cell **2**(3): 627-37.

We used microarrays carrying most of the genes that are developmentally regulated in Dictyostelium to discover those that are preferentially expressed in prestalk cells. Prestalk cells are localized at the front of slugs and play crucial roles in morphogenesis and slug migration. Using whole-mount in situ hybridization, we were able to verify 104 prestalk genes. Three of these were found to be expressed only in cells at the very front of slugs, the PstA cell type. Another 10 genes were found to be expressed in the small number of cells that form a central core at the anterior, the PstAB cell type. The rest of the prestalk-specific genes are expressed in PstO cells, which are found immediately posterior to PstA cells but anterior to 80% of the slug that consists of prespore cells. Half of these are also expressed in PstA cells. At later stages of development, the patterns of expression of a considerable number of these prestalk genes changes significantly, allowing us to further subdivide them. Some are expressed at much higher levels during culmination, while others are repressed. These results demonstrate the extremely dynamic nature of cell-type-specific expression in Dictyostelium and further define the changing physiology of the cell types. One of the signals that affect gene expression in PstO cells is the hexaphenone DIF-1. We found that expression of about half of the PstO-specific genes were affected in a mutant that is unable to synthesize DIF-1, while the rest appeared to be DIF independent. These results indicate that differentiation of some aspects of PstO cells can occur in the absence of DIF-1.

Magee, T. R., M. Ferrini, et al. (2002). "Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase." Biol Reprod **67**(3): 1033-41.

Gene transfer to the penile corpora cavernosa of constructs of the inducible and endothelial nitric oxide synthase (NOS) cDNAs ameliorates erectile dysfunction in aged rats. In this study, we investigated whether the neuronal NOS (nNOS) variant responsible for erection, penile nNOS (PnNOS), can exert a similar effect, and whether the combination of electroporation with a helper-dependent adenovirus (AdV) improves gene transfer. PnNOS and beta-galactosidase cDNAs were cloned in plasmid (pCMV-PnNOS; pCMV-beta-gal) and "gutless" AdV (AdV-CMV-PnNOS; AdV-CMV-beta-gal) vectors, and injected into the penis of adult (beta-gal) or aged (PnNOS) rats, with or without electroporation. Penile erection was measured at different times after PnNOS cDNA injection, by electrical field stimulation of the cavernosal

nerve. The expression of beta-galactosidase or PnNOS was estimated in penile tissue by either histochemistry and luminometry or Western blot, and the effects of AdV-CMV-PnNOS on mRNA expression were examined by a DNA microarray. We found that electroporation increased pCMV-beta-gal uptake, and its expression was detectable at 56 days. In the aged rats treated with pCMV-PnNOS and electroporation, the maximal intracavernosal:mean arterial pressure ratios were elevated for 11 and 18 days when compared with those in controls. Electroporation intensified penile uptake of as few as 10(6) viral particles (vp) of AdV-CMV-beta-gal, and with 10(7) vp beta-galactosidase was still detectable at 60 days. Electroporated AdV-CMV-PnNOS (10(7) vp) was effective at 18 days in stimulating the erection of aged rats, without inducing the expression of cytotoxic genes. In conclusion, intracavernosal gene therapy with PnNOS cDNA corrected the aging-related erectile dysfunction for at least 18 days when given by electroporation in a helper-dependent AdV at low viral loads.

Maglova, L. M., W. E. Crowe, et al. (2004). "Perinuclear localization of Na-K-Cl-cotransporter protein after human cytomegalovirus infection." Am J Physiol Cell Physiol **286**(6): C1324-34.

We (41) previously reported that Na-K-Cl-cotransporter (NKCC) function and microsomal protein expression are both dramatically reduced late in human cytomegalovirus (HCMV) infection of a human fibroblast cell line (MRC-5). We now report DNA microarray data showing that no significant HCMV-dependent NKCC gene repression can be detected 30 h postexposure (PE) to the virus. Consequently, we used plasma membrane biotinylation and subsequent subcellular fractionation in combination with semiquantitative immunoblotting and confocal microscopy to investigate the possibility that intracellular redistribution of the NKCC protein after HCMV infection could be a cause of the HCMV-induced loss of NKCC ion transport function. Our results show that the lifetime of plasmalemmal NKCC protein in quiescent, uninfected MRC-5 cells is approximately 48 h, and <20% of the total expressed NKCC protein are in the plasma membrane. The remainder (approximately 80%) was detected as diffusely distributed, small punctate structures in the cytoplasm. Following HCMV infection: 1) NKCC protein expression in the plasmalemma was sharply reduced (approximately 75%) within 24 h PE and thereafter continued to slowly decrease; 2) total cellular NKCC protein content remained unchanged or slightly increased during the course of the viral infection; and 3) HCMV infection caused NKCC protein to accumulate in the perinuclear region late in the HCMV infection (72 h PE). Thus our results imply that, in the process of productive HCMV infection, NKCC protein continues to be synthesized, but, instead of being delivered to the plasma membrane, it is clustered in a large, detergent-soluble perinuclear structure.

Magwene, P. M., P. Lizardi, et al. (2003). "Reconstructing the temporal ordering of biological samples using microarray data." Bioinformatics **19**(7): 842-50.

MOTIVATION: Accurate time series for biological processes are difficult to estimate due to problems of synchronization, temporal sampling and rate heterogeneity. Methods are needed that can utilize multi-dimensional data, such as those resulting from DNA microarray experiments, in order to reconstruct time series from unordered or poorly ordered sets of observations. RESULTS: We present a set of algorithms for estimating temporal orderings from unordered sets of sample elements. The techniques we describe are based on modifications of a minimum-spanning tree calculated from a weighted, undirected graph. We demonstrate the efficacy of our approach by applying these techniques to an artificial data set as well as several gene expression data sets derived from DNA microarray experiments. In addition to estimating orderings, the techniques we describe also provide useful heuristics for assessing relevant properties of sample datasets such as noise and sampling intensity, and we show how a data structure called a PQ-tree can be used to represent uncertainty in a reconstructed ordering. AVAILABILITY: Academic implementations of the ordering algorithms are available as source code (in the programming language Python) on our web site, along with documentation on their use. The artificial 'jelly roll' data set upon which the algorithm was tested is also available from this web site. The publicly available gene expression data may be found at http://genome-www.stanford.edu/cellcycle/ and http://caulobacter.stanford.edu/CellCycle/.

Maleck, K., A. Levine, et al. (2000). "The transcriptome of Arabidopsis thaliana during systemic acquired resistance." Nat Genet **26**(4): 403-10.

Infected plants undergo transcriptional reprogramming during initiation of both local defence and systemic acquired resistance (SAR). We monitored gene-expression changes in Arabidopsis thaliana under 14 different SAR-inducing or SAR-repressing conditions using a DNA microarray representing approximately 25-30% of all A. thaliana genes. We derived groups of genes with common regulation patterns, or regulons. The regulon containing PR-1, a reliable marker gene for SAR in A. thaliana, contains known PR genes and novel genes likely to function during SAR and disease resistance. We identified a common promoter element in genes of this regulon that binds members of a plant-specific transcription factor family. Our results extend expression profiling to definition of regulatory networks and gene discovery in plants.

Manganelli, R., M. I. Voskuil, et al. (2002). "Role of the extracytoplasmic-function sigma factor sigma(H) in Mycobacterium tuberculosis global gene expression." Mol Microbiol **45**(2): 365-74.

Like other bacterial species, Mycobacterium tuberculosis has multiple sigma (sigma) factors encoded in its genome. In previously published work, we and others have shown that mutations in some of these transcriptional activators render M. tuberculosis sensitive to various environmental stresses and, in some cases, cause attenuated virulence phenotypes. In this paper, we characterize a M. tuberculosis mutant lacking the ECF sigma factor sigma(H). This mutant was more sensitive than the wild type to heat shock and to various oxidative stresses, but did not show decreased ability to grow inside macrophages. Using quantitative reverse transcription-PCR and microarray technology, we have started to define the sigma(H) regulon and its involvement in the global regulation of the response to heat shock and the thiol-specific oxidizing agent diamide. We identified 48 genes whose expression increased after exposure of M. tuberculosis to diamide; out of these, 39 were not induced in the sigH mutant, showing their direct or indirect dependence on sigma(H). Some of these genes encode proteins whose predicted function is related to thiol metabolism, such as thioredoxin, thioredoxin reductase and enzymes involved in cysteine and molybdopterine biosynthesis. Other genes under sigma(H) control encode transcriptional regulators such as sigB, sigE, and sigH itself.

Manganelli, R., M. I. Voskuil, et al. (2001). "The Mycobacterium tuberculosis ECF sigma factor sigmaE: role in global gene expression and survival in macrophages." Mol Microbiol **41**(2): 423-37.

In previously published work, we identified three Mycobacterium tuberculosis sigma (sigma) factor genes responding to heat shock (sigB, sigE and sigH). Two of them (sigB and sigE) also responded to SDS exposure. As these responses to stress suggested that the sigma factors encoded by these genes could be involved in pathogenicity, we are studying their role in physiology and virulence. In this work, we characterize a sigE mutant of M. tuberculosis H37Rv. The sigE mutant strain was more sensitive than the wild-type strain to heat shock, SDS and various oxidative stresses. It was also defective in the ability to grow inside both human and murine unactivated macrophages and was more sensitive than the wild-type strain to the killing activity of activated murine macrophages. Using microarray technology and quantitative reverse transcription-polymerase chain reaction (RT-PCR), we started to define the sigmaE regulon of M. tuberculosis and its involvement in the global regulation of the stress induced by SDS. We showed the requirement for a functional sigE gene for full expression of sigB and for its induction after SDS exposure but not after heat shock. We also identified several genes that are no longer induced when sigmaE is absent. These genes encode proteins belonging to different classes including transcriptional regulators, enzymes involved in fatty acid degradation and classical heat shock proteins.

Manna, D., A. M. Breier, et al. (2004). "Microarray analysis of transposition targets in Escherichia coli: the impact of transcription." Proc Natl Acad Sci U S A **101**(26): 9780-5.

Transposable elements have influenced the genetic and physical composition of all modern organisms. Defining how different transposons select target sites is critical for understanding the

biochemical mechanism of this type of recombination and the impact of mobile genes on chromosome structure and function. Phage Mu replicates in Gram-negative bacteria using an extremely efficient transposition reaction. Replicated copies are excised from the chromosome and packaged into virus particles. Each viral genome plus several hundred base pairs of host DNA covalently attached to the prophage right end is packed into a virion. To study Mu transposition preferences, we used DNA microarray technology to measure the abundance of >4,000 Escherichia coli genes in purified Mu phage DNA. Insertion hot- and cold-spot genes were found throughout the genome, reflecting >1,000-fold variation in utilization frequency. A moderate preference was observed for genes near the origin compared to terminus of replication. Large biases were found at hot and cold spots, which often include several consecutive genes. Efficient transcription of genes had a strong negative influence on transposition. Our results indicate that local chromosome structure is more important than DNA sequence in determining Mu target-site selection.

Mansson, R., P. Tsapogas, et al. (2004). "Pearson correlation analysis of microarray data allows for the identification of genetic targets for early B-cell factor." J Biol Chem **279**(17): 17905-13.

B lymphocyte development is a complex biological process critically dependent on the transcription factor early B cell factor (EBF). To deepen understanding of the roles for EBF in this process, we have used Pearson correlation analysis to evaluate microarray data from a set of mouse B lymphoid cell lines representing different stages of development. Comparing the expression pattern of EBF to that of the other genes in the data set revealed that VpreB1, mb-1, and lambda5, all known target genes, presented high correlation values to EBF. High correlations were also seen for the VpreB3 and CD19 genes and biochemical as well as functional data supported that they are target genes for EBF even though the expression of CD19 was critically dependent of Pax-5. We also obtained evidence for extensive collaborative actions of EBF and E47 even though microarray analysis of hematopoetic progenitor cells ectopically expressing these proteins suggested that they activated only a subset of pre-B cell restricted genes.

Marin, K., I. Suzuki, et al. (2003). "Identification of histidine kinases that act as sensors in the perception of salt stress in Synechocystis sp. PCC 6803." Proc Natl Acad Sci U S A **100**(15): 9061-6.

In plants and microorganisms, salt stress regulates the expression of large numbers of genes. However, the machinery that senses salt stress remains to be characterized. In this study we identified sensory histidine kinases that are involved in the perception of salt stress in the cyanobacterium Synechocystis sp. strain PCC 6803. A library of strains with mutations in all 43 histidine kinases was screened by DNA microarray analysis of genomewide gene expression under salt stress. The results suggested that four histidine kinases, namely, Hik16, Hik33, Hik34, and Hik41, perceived and transduced salt signals. However, Hik33, Hik34, and Hik16 acting with Hik41 regulated the expression of different sets of genes. These histidine kinases regulated the expression of approximately 20% of the salt-inducible genes, whereas the induction of the remaining salt-inducible genes was unaffected by mutations in any of the histidine kinases, suggesting that additional sensory mechanisms might operate in the perception of salt stress. We also used DNA microarrays to investigate the effect of various salts on gene expression. Our results indicate that Hik33 responds to sodium salts and not to KCl, whereas the Hik16/Hik41 system responds only to NaCl.

Marokhazi, J., N. Waterfield, et al. (2003). "Using a DNA microarray to investigate the distribution of insect virulence factors in strains of photorhabdus bacteria." J Bacteriol **185**(15): 4648-56.

Photorhabdus is an insect-pathogenic bacterium in which oral toxicity to insects is found in two distinct taxonomic groups. Using a DNA microarray and comparative genomics, we show that oral toxicity is associated with toxin complex genes tcaABC and that this locus can be mobilized or deleted within different strains.

Martell, M., C. Briones, et al. (2004). "Structural analysis of hepatitis C RNA genome using DNA microarrays." Nucleic Acids Res **32**(11): e90.

Many studies have tried to identify specific nucleotide sequences in the quasispecies of hepatitis C virus (HCV) that determine resistance or sensitivity to interferon (IFN) therapy, unfortunately without conclusive results. Although viral proteins represent the most evident phenotype of the virus, genomic RNA sequences determine secondary and tertiary structures which are also part of the viral phenotype and can be involved in important biological roles. In this work, a method of RNA structure analysis has been developed based on the hybridization of labelled HCV transcripts to microarrays of complementary DNA oligonucleotides. Hybridizations were carried out at non-denaturing conditions, using appropriate temperature and buffer composition to allow binding to the immobilized probes of the RNA transcript without disturbing its secondary/tertiary structural motifs. Oligonucleotides printed onto the microarray covered the entire 5' non-coding region (5'NCR), the first three-quarters of the core region, the E2-NS2 junction and the first 400 nt of the NS3 region. We document the use of this methodology to analyse the structural degree of a large region of HCV genomic RNA in two genotypes associated with different responses to IFN treatment. The results reported here show different structural degree along the genome regions analysed, and differential hybridization patterns for distinct genotypes in NS2 and NS3 HCV regions.

Martin, R. G. and J. L. Rosner (2002). "Genomics of the marA/soxS/rob regulon of Escherichia coli: identification of directly activated promoters by application of molecular genetics and informatics to microarray data." Mol Microbiol **44**(6): 1611-24.

Microarray analyses are providing a plethora of data concerning transcriptional responses to specific gene regulators and their inducers but do not distinguish between direct and indirect responses. Here, we identify directly activated promoters of the overlapping marA, soxS and rob regulon(s) of Escherichia coli by applying informatics, genomics and molecular genetics to microarray data obtained by others. Those studies found that overexpression of marA, or the treatment of cells with salicylate to derepress marA, or treatment with paraquat to induce soxS, resulted in elevated transcription of 153 genes. However, only 27 out of the promoters showed increased transcription under at least two of the aforementioned conditions and eight of those were previously known to be directly activated. A computer algorithm was used to identify potential activator binding sites located upstream of the remaining 19 promoters of this subset, and conventional genetic and biochemical approaches were applied to test whether these sites are critical for activation by the homologous MarA, SoxS and Rob transcriptional activators. Only seven out of the 19 promoters were found to be activated when fused to lacZ and tested as single lysogens. All seven contained an essential activator binding site. The remaining promoters were insensitive to stimulation by the inducers suggesting that the great majority of elevated microarray transcripts either were misidentified or resulted from indirect effects requiring sequences outside of the promoter region. We estimate that the total number of directly activated promoters in the regulon is less than 40.

Martin-Galiano, A. J., J. M. Wells, et al. (2004). "Relationship between codon biased genes, microarray expression values and physiological characteristics of Streptococcus pneumoniae." Microbiology **150**(Pt 7): 2313-25.

A codon-profile strategy was used to predict gene expression levels in Streptococcus pneumoniae. Predicted highly expressed (PHE) genes included those encoding glycolytic and fermentative enzymes, sugar-conversion systems and carbohydrate-transporters. Additionally, some genes required for infection that are involved in oxidative metabolism and hydrogen peroxide production were PHE. Low expression values were predicted for genes encoding specific regulatory proteins like two-component systems and competence genes. Correspondence analysis localized 484 ORFs which shared a distinctive codon profile in the right horn. These genes had a mean G+C content (33.4 %) that was lower than the bulk of the genome coding sequences (39.7 %), suggesting that many of them were acquired by

horizontal transfer. Half of these genes (242) were pseudogenes, ORFs shorter than 80 codons or without assigned function. The remaining genes included several virulence factors, such as capsular genes, iga, lytB, nanB, pspA, choline-binding proteins, and functions related to DNA acquisition, such as restriction-modification systems and comDE. In order to compare predicted translation rate with the relative amounts of mRNA for each gene, the codon adaptation index (CAI) values were compared with microarray fluorescence intensity values following hybridization of labelled RNA from laboratory-grown cultures. High mRNA amounts were observed in 32.5 % of PHE genes and in 64 % of the 25 genes with the highest CAI values. However, high relative amounts of RNA were also detected in 10.4 % of non-PHE genes, such as those encoding fatty acid metabolism enzymes and proteases, suggesting that their expression might also be regulated at the level of transcription or mRNA stability under the conditions tested. The effects of codon bias and mRNA amount on different gene groups in S. pneumoniae are discussed.

Mary-Huard, T., J. J. Daudin, et al. (2004). "Spotting effect in microarray experiments." BMC Bioinformatics **5**(1): 63.

BACKGROUND: Microarray data must be normalized because they suffer from multiple biases. We have identified a source of spatial experimental variability that significantly affects data obtained with Cy3/Cy5 spotted glass arrays. It yields a periodic pattern altering both signal (Cy3/Cy5 ratio) and intensity across the array. RESULTS: Using the variogram, a geostatistical tool, we characterized the observed variability, called here the spotting effect because it most probably arises during steps in the array printing procedure. CONCLUSIONS: The spotting effect is not appropriately corrected by current normalization methods, even by those addressing spatial variability. Importantly, the spotting effect may alter differential and clustering analysis.

Mascher, T., D. Zahner, et al. (2003). "The Streptococcus pneumoniae cia regulon: CiaR target sites and transcription profile analysis." J Bacteriol **185**(1): 60-70.

The ciaR-ciaH system is one of 13 two-component signal-transducing systems of the human pathogen Streptococcus pneumoniae. Mutations in the histidine protein kinase CiaH confer increased resistance to beta-lactam antibiotics and interfere with the development of genetic competence. In order to identify the genes controlled by the cia system, the cia regulon, DNA fragments targeted by the response regulator CiaR were isolated from restricted chromosomal DNA using the solid-phase DNA binding assay and analyzed by hybridization to an oligonucleotide microarray representing the S. pneumoniae genome. A set of 18 chromosomal regions containing 26 CiaR target sites were detected and proposed to represent the minimal cia regulon. The putative CiaR target loci included genes important for the synthesis and modification of cell wall polymers, peptide pheromone and bacteriocin production, and the htrA-spo0J region. In addition, the transcription profile of cia loss-of-function mutants and those with an apparent activated cia system representing the off and on states of the regulatory system were analyzed. The transcript analysis confirmed the cia-dependent expression of seven putative target loci and revealed three additional cia-regulated loci. Five putative target regions were silent under all conditions, and for the remaining three regions, no cia-dependent expression could be detected. Furthermore, the competence regulon, including the comCDE operon required for induction of competence, was completely repressed by the cia system.

Masuda, N. and G. M. Church (2002). "Escherichia coli gene expression responsive to levels of the response regulator EvgA." J Bacteriol **184**(22): 6225-34.

To investigate the function of the EvgA response regulator, we compared the genome-wide transcription profile of EvgA-overexpressing and EvgA-lacking Escherichia coli strains by oligonucleotide microarrays. The microarray measurements allowed the identification of at least 37 EvgA-activated genes, including acid resistance-related genes gadABC and hdeAB, efflux pump genes yhiUV and emrK, and 21 genes with unknown function. EvgA overexpression conferred acid resistance

to exponentially growing cells. This acid resistance was abolished by deletion of ydeP, ydeO, or yhiE, which was induced by EvgA overexpression. These results suggest that ydeP, ydeO, and yhiE are novel genes related to acid resistance and that EvgA regulates several acid resistance genes. Furthermore, the deletion of yhiE completely abolished acid resistance in stationary-phase cells, suggesting that YhiE plays a critical role in stationary-phase acid resistance. The multidrug resistance in an acrB deletion mutant caused by EvgA overexpression was completely abolished by deletion of yhiUV, while the emrKY deletion had no effect on the increase in resistance by EvgA overexpression. In addition, EvgA overexpression did not confer resistance in a tolC-deficient strain. These results suggest that YhiUV induced by EvgA overexpression is functionally associated with TolC and contributes to multidrug resistance.

Masuda, N. and G. M. Church (2003). "Regulatory network of acid resistance genes in Escherichia coli." Mol Microbiol **48**(3): 699-712.

Overexpression of the response regulator EvgA confers an acid-resistant phenotype to exponentially growing Escherichia coli. This acid resistance is partially abolished by deletion of ydeP, yhiE or ydeO, genes induced by EvgA overexpression. Microarray analysis identified two classes of operons (genes). The first class contains seven operons induced by EvgA overexpression in the absence of ydeO, an AraC/XylS regulator gene. The second class contains 12 operons induced by YdeO overexpression. Operons in the second class were induced by EvgA overexpression only in the presence of ydeO. EvgA is likely to directly upregulate operons in the first class, and indirectly upregulate operons in the second class via YdeO. Analysis using the motif-finding program alignace identified an 18 bp inverted repeat motif in six upstream regions of all seven operons directly regulated by EvgA. Gel mobility shift assays showed the specific binding of EvgA to the six sequences. Introduction of mutations into the inverted repeats upstream of ydeP and b1500-ydeO resulted in reduction in EvgA-induced ydeP and ydeO expression and acid resistance. These results suggest that EvgA binds to the inverted repeats and upregulates the downstream genes. Overexpression of YdeP, YdeO and YhiE conferred acid resistance to exponentially growing cells, whereas GadX overexpression did not. Microarray analysis also identified several GadX-activated genes. Several genes induced by overexpression of YdeO and GadX overlapped; however, yhiE was induced only by YdeO. The acid resistance induced by YdeO overexpression was abolished by deletion of yhiE, gadC, slp-yhiF, hdeA or hdeD, genes induced by YdeO overexpression, suggesting that several genes orchestrate YdeO-induced acid resistance. We propose a model of the regulatory network of the acid resistance genes.

Mathai, J. P., M. Germain, et al. (2002). "Induction and endoplasmic reticulum location of BIK/NBK in response to apoptotic signaling by E1A and p53." Oncogene **21**(16): 2534-44.

A DNA microarray analysis identified the BH3-only BCL-2 family member, BIK/NBK, as a transcript that is upregulated during induction of apoptosis by oncogenic E1A. E1A depended on wild-type p53 to induce BIK and activate the death program. Further, p53 independently induced BIK RNA and protein, and BIK alone stimulated cell death in p53-null cells, dependent on the activation of caspases. BIK function, however, was abrogated by a disabling point mutation within the BH3 domain. Collectively, these results argue that BIK is a downstream apoptotic effector of p53 in response to a physiological p53-mediated death stimulus provided by E1A. Elevated BCL-2 functioned downstream of p53 and BIK induction to inhibit the E1A death pathway, with the ratio of anti-apoptotic BCL-2 and pro-apoptotic BIK determining cell death or survival in E1A-expressing cells. Cells expressing BCL-2 or treated with the pan caspase inhibitor, zVAD-fmk, allowed accumulation of high levels of cytotoxic BIK compared to control cells. Of note, a significant fraction of either ectopic or endogenous BIK was found associated with the endoplasmic reticulum, suggesting that this organelle, in addition to mitochondria, may be a target of BIK function.

Matrajt, M., R. G. Donald, et al. (2002). "Identification and characterization of differentiation mutants in

the protozoan parasite Toxoplasma gondii." Mol Microbiol **44**(3): 735-47.

Two forms of the protozoan parasite Toxoplasma gondii are associated with intermediate hosts such as humans: rapidly growing tachyzoites are responsible for acute illness, whereas slowly dividing encysted bradyzoites can remain latent within the tissues for the life of the host. In order to identify genetic factors associated with parasite differentiation, we have used a strong bradyzoite-specific promoter (identified by promoter trapping) to drive the expression of T. gondii hypoxanthine-xanthine-guanine phosphoribosyltransferase (HXGPRT) in stable transgenic parasites, providing a stage-specific positive/negative selectable marker. Insertional mutagenesis has been carried out on this parental line, followed by bradyzoite induction in vitro and selection in 6-thioxanthine to identify misregulation mutants. Two different mutants fail to induce the HXGPRT gene efficiently during bradyzoite differentiation. These mutants are also defective in other aspects of differentiation: they replicate well under bradyzoite growth conditions, lysing the host cell monolayer as effectively as tachyzoites. Expression of the major bradyzoite antigen BAG1 is reduced, and staining with Dolichos biflorus lectin shows reduced cyst wall formation. Microarray hybridizations show that these mutants behave more like tachyzoites at a global level, even under bradyzoite differentiation conditions.

Matsubara, Y., M. Kobayashi, et al. (2002). "Application of a microchamber array for DNA amplification using a novel dispensing method." Arch Histol Cytol **65**(5): 481-8.

We recently developed a microchamber array chip for DNA amplification by adopting semiconductor microfabrication technology; a polymerase chain reaction (PCR) was performed in the microchamber array, and the amplified DNA was detected using a fluorescent dye. In order to manipulate a single cell or sample into each microchamber individually in this system, the chip was directly sealed with a cover glass slip which impeded the retrieval of the products from each chamber. The present study was therefore carried out to improve the system by developing methods for covering the microchambers and introducing the reaction solution. First, we fabricated a microchamber array chip, and the oil layer was coated on the whole chip instead of the cover glass slip. The solution for DNA amplification was introduced into each chamber through an oil layer using a nano-liter dispenser. Following this, the microarray chip was placed onto the thermal cycling system for DNA amplification, and the amplified DNA was subsequently detected by fluorescence microscopy. In this system, the products were easily retrieved using a micromanipulator for further analysis.

Mauser, A., E. Holley-Guthrie, et al. (2002). "The Epstein-Barr virus immediate-early protein BZLF1 induces expression of E2F-1 and other proteins involved in cell cycle progression in primary keratinocytes and gastric carcinoma cells." J Virol **76**(24): 12543-52.

The Epstein-Barr virus (EBV) immediate-early protein BZLF1 mediates the switch between the latent and lytic forms of EBV infection and has been previously shown to induce a G(1)/S block in cell cycle progression in some cell types. To examine the effect of BZLF1 on cellular gene expression, we performed microarray analysis on telomerase-immortalized human keratinocytes that were mock infected or infected with a control adenovirus vector (AdLacZ) or a vector expressing the EBV BZLF1 protein (AdBZLF1). Cellular genes activated by BZLF1 expression included E2F-1, cyclin E, Cdc25A, and a number of other genes involved in cell cycle progression. Immunoblot analysis confirmed that BZLF1 induced expression of E2F-1, cyclin E, Cdc25A, and stem loop binding protein (a protein known to be primarily expressed during S phase) in telomerase-immortalized keratinocytes. Similarly, BZLF1 increased expression of E2F-1, cyclin E, and stem loop binding protein (SLBP) in primary tonsil keratinocytes. In contrast, BZLF1 did not induce E2F-1 expression in normal human fibroblasts. Cell cycle analysis revealed that while BZLF1 dramatically blocked G(1)/S progression in normal human fibroblasts, it did not significantly affect cell cycle progression in primary human tonsil keratinocytes. Furthermore, in EBV-infected gastric carcinoma cells, the BZLF1-positive cells had an increased number of cells in S phase compared to the BZLF1-negative cells. Thus, in certain cell types (but not others), BZLF1 enhances expression of cellular proteins associated with cell cycle progression, which suggests

that an S-phase-like environment may be advantageous for efficient lytic EBV replication in some cell types.

Maxwell, S. A. and G. E. Davis (2000). "Biological and molecular characterization of an ECV-304-derived cell line resistant to p53-mediated apoptosis." Apoptosis **5**(3): 277-90.

Upregulation of the p53 tumor suppressor protein by infection with a recombinant p53 adenovirus resulted in extensive apoptosis in ECV-304 cells and the eventual death of almost all the cells. To establish a system to elucidate the molecular mechanisms involved in p53-mediated apoptosis of these cells, we established a variant of ECV-304 that is resistant to p53-induced apoptosis by repeated infections with a recombinant p53 adenovirus. We have designated this variant as the DECV cell line (Differentiated ECV-304). DECV cells expressed similar amounts of nuclear-localized p53 as ECV-304 cells when infected with recombinant p53 adenovirus, but in contrast to ECV-304 cells, greater than 95% of DECV cells survived and remained viable after 24 hours of infection. In further contrast to ECV-304 cells, DECV cells grew less efficiently in soft agar and exhibited contact inhibition in growth assays. Moreover, DECV cells formed unusual lattice or cyst-like structures in culture and formed lumenal structures indicative of epithelial differentiation in three-dimensional collagen matrices, while parental ECV-304 cells showed minimal evidence of these cellular behaviors. A comparative molecular analysis of gene expression in DECV and ECV-304 cells was conducted by cDNA microarray technology. Protocadherin-1 was found to be expressed in DECV cells but not in ECV-304 cells, while the Id-3 gene was observed expressed in ECV-304 cells but not in DECV cells. Moreover, upregulated expression of p53 in ECV-304 cells induced the EPHB2 (Ephrin) receptor tyrosine kinase and the ephrin-B1 ligand mRNAs compared to DECV cells treated in the same manner. These data demonstrate that a new variant of the ECV-304 cell line, which is resistant to p53-mediated apoptosis, exhibits differential gene expression as well as distinct cell behaviors as compared to the parental ECV-304 cell line. DECV cells should prove to be a useful tool in future studies to elucidate mechanisms of p53-mediated apoptosis and differentiation.

Maxwell, S. A. and G. E. Davis (2000). "Differential gene expression in p53-mediated apoptosis-resistant vs. apoptosis-sensitive tumor cell lines." Proc Natl Acad Sci U S A **97**(24): 13009-14.

Induction of wild-type p53 in the ECV-304 bladder carcinoma cell line by infection with a p53 recombinant adenovirus (Ad5CMV-p53) resulted in extensive apoptosis and eventual death of nearly all of the cells. As a strategy to determine the molecular events important to p53-mediated apoptosis in these transformed cells, ECV-304 cells were selected for resistance to p53 by repeated infections with Ad5CMV-p53. We compared the expression of 5,730 genes in p53-resistant (DECV) and p53-sensitive ECV-304 cells by reverse transcription-PCR, Northern blotting, and DNA microarray analysis. The expression of 480 genes differed by 2-fold or more between the two p53-infected cell lines. A number of potential targets for p53 were identified that play roles in cell cycle regulation, DNA repair, redox control, cell adhesion, apoptosis, and differentiation. Proline oxidase, a mitochondrial enzyme involved in the proline/pyrroline-5-carboxylate redox cycle, was up-regulated by p53 in ECV but not in DECV cells. Pyrroline-5-carboxylate (P5C), a proline-derived metabolite generated by proline oxidase, inhibited the proliferation and survival of ECV-304 and DECV cells and induced apoptosis in both cell lines. A recombinant proline oxidase protein tagged with a green fluorescent protein at the amino terminus localized to mitochondria and induced apoptosis in p53-null H1299 non-small cell lung carcinoma cells. The results directly implicate proline oxidase and the proline/P5C pathway in p53-induced growth suppression and apoptosis.

May, B. J., Q. Zhang, et al. (2001). "Complete genomic sequence of Pasteurella multocida, Pm70." Proc Natl Acad Sci U S A **98**(6): 3460-5.

We present here the complete genome sequence of a common avian clone of Pasteurella multocida, Pm70. The genome of Pm70 is a single circular chromosome 2,257,487 base pairs in length

and contains 2,014 predicted coding regions, 6 ribosomal RNA operons, and 57 tRNAs. Genome-scale evolutionary analyses based on pairwise comparisons of 1,197 orthologous sequences between P. multocida, Haemophilus influenzae, and Escherichia coli suggest that P. multocida and H. influenzae diverged approximately 270 million years ago and the gamma subdivision of the proteobacteria radiated about 680 million years ago. Two previously undescribed open reading frames, accounting for approximately 1% of the genome, encode large proteins with homology to the virulence-associated filamentous hemagglutinin of Bordetella pertussis. Consistent with the critical role of iron in the survival of many microbial pathogens, in silico and whole-genome microarray analyses identified more than 50 Pm70 genes with a potential role in iron acquisition and metabolism. Overall, the complete genomic sequence and preliminary functional analyses provide a foundation for future research into the mechanisms of pathogenesis and host specificity of this important multispecies pathogen.

McCallum, N., M. Bischoff, et al. (2004). "TcaR, a putative MarR-like regulator of sarS expression." J Bacteriol **186**(10): 2966-72.

TcaR, which shares sequence homology with MarR-like transcriptional regulators, has been identified as a novel Staphylococcus aureus regulator affecting the expression of the global regulatory element SarS (SarH1), as well as that of the cell surface-associated protein SasF (N315-SA2439). Microarray analysis, confirmatory Northern blots, and genetic complementation experiments showed that TcaR upregulates sarS and thus spa transcription. In addition, it attenuates whole-length transcription of sasF, thereby producing a truncated transcript lacking the 3' terminus, which codes for the cell wall anchor motif. Hence, in strains containing an intact tcaR gene, TcaR is likely to decrease the amount of the surface-associated protein SasF and to increase that of the surface-associated protein A. The widely used laboratory strains derived from NCTC8325 were found to be natural, truncated mutants of tcaR, harboring an inactive TcaR and therefore expressing very low levels of sarS. The data presented here identified TcaR as a further activator of sarS, and a modulator of sasF expression that has to be taken into account in studies of virulence gene expression in S. aureus.

McCluskey, J., J. Hinds, et al. (2004). "A two-component system that controls the expression of pneumococcal surface antigen A (PsaA) and regulates virulence and resistance to oxidative stress in Streptococcus pneumoniae." Mol Microbiol **51**(6): 1661-75.

Recent genomic-based studies have identified 13 two-component signal transduction systems (TCS) in Streptococcus pneumoniae. Bacterial TCSs are important for regulating expression of bacterial genes, including those which are important to the virulence of pathogenic bacteria. We have used virulence assays together with microarray analysis to investigate the importance of pneumococcal TCS04 in the virulence and gene regulation of this pathogen. Deletion mutants of the response regulator of TCS04, rr04, were examined in three independent pneumococcal strains representing three different pneumococcal serotypes. Analysis of the virulence of the three strains enabled us to identify a serotype-specific attenuation of virulence due to deletion of rr04. Microarray comparison of the transcriptional profiles of the wild-type strains with the rr04 mutants allowed us to determine which transcriptional changes were occurring in the rr04 mutants. Virulence-associated changes were demonstrated in the attenuated strain with significant downregulation of a previously determined virulence locus, psaB, psaC and psaA.

McDaniel, L. S., J. A. Thornton, et al. (2004). "Use of cDNA microarrays to analyze responses to pneumococcal virulence factors." Indian J Med Res **119 Suppl**: 99-103.

BACKGROUND & OBJECTIVES: The complex interactions that occur between host and pathogen during bacteraemia caused by Streptococcus pneumoniae are not well understood. Upon entering the blood stream the pneumococcus intiates responses through contact with naive monocytes and macrophages resulting in an inflammatory response. To elucidate the role of microbial virulence factors in the host response to the pneumococcus, cDNA microarray analysis was used to identify genes in THP-

1 cells, a human monocytic cell line, that are responsive to pneumococcal virulence factors. METHODS: S. pneumoniae D39, a serotype 2 pneumococcus, and PLN an isogenic mutant of D39 that does not express pneumolysin were used. Gene expression profiles elicited by both wild-type and mutant were compared with that of THP-1 cells not exposed to pneumococci. Results obtained from microarray analysis were confirmed and further characterized using reverse transcriptase (RT)-PCR, real-time RT-PCR, and ELISA. RESULTS: Genes in THP-1 cells that were responsive to the pneumococcus independent of the presence of the specific virulence factor, pneumolysin, were identified. THP-1 cell genes that were differentially expressed independent of pneumolysin included the ones involved in cell-to-cell signaling and antipathogen responses. Those that were responsive to pneumolysin included genes encoding adhesion molecules, chemokines, cytokine receptors, and cell cycle and apoptosis proteins. INTERPRETATION & CONCLUSION: The global transcriptional response of naive monocytes to contact with the pneumococcus was characterized and the utility of cDNA microarray analysis in elucidating the role of specific factors in host-pathogen interactions were demonstrated.

McLaren, P. J., M. Mayne, et al. (2004). "Antigen-specific gene expression profiles of peripheral blood mononuclear cells do not reflect those of T-lymphocyte subsets." Clin Diagn Lab Immunol **11**(5): 977-82.

Advances in microarray technology have allowed for the monitoring of thousands of genes simultaneously. This technology is of particular interest to immunologists studying infectious diseases, because it provides tremendous potential for investigating host-pathogen interactions at the level of immune gene expression. To date, many studies have focused either on cell lines, where the physiological relevance is questionable, or on mixed cell populations, where the contributions of individual subpopulations are unknown. In the present study, we perform an intrasubject comparison of antigen-stimulated immune gene expression profiles between a mixed population of peripheral blood mononuclear cells (PBMC) and the two predominant cell types found in PBMC, CD4+ and CD8+ T lymphocytes. We show that the microarray profiles of CD4+ and CD8+ T lymphocytes differ from each other as well as from that of the mixed cell population. The independence of the gene expression profiles of different cell types is demonstrated with a ubiquitous antigen (Candida albicans) as well as with a disease-specific antigen (human immunodeficiency virus p24). This study has important implications for microarray studies of host immunity and underscores the importance of profiling the expression of specific cell types.

Merrell, D. S., L. J. Thompson, et al. (2003). "Growth phase-dependent response of Helicobacter pylori to iron starvation." Infect Immun **71**(11): 6510-25.

Iron is an essential nutrient that is often found in extremely limited available quantities within eukaryotic hosts. Because of this, many pathogenic bacteria have developed regulated networks of genes important for iron uptake and storage. In addition, it has been shown that many bacteria use available iron concentrations as a signal to regulate virulence gene expression. We have utilized DNA microarray technology to identify genes of the human pathogen Helicobacter pylori that are differentially regulated on a growth-inhibiting shift to iron starvation conditions. In addition, the growth phase-dependent expression of these genes was investigated by examining both exponential and stationary growth phase cultures. We identified known iron-regulated genes, as well as a number of genes whose regulation by iron concentration was not previously appreciated. Included in the list of regulated factors were the known virulence genes cagA, vacA, and napA. We examined the effect of iron starvation on the motility of H. pylori and found that exponential- and stationary-phase cultures responded differently to the stress. We further found that while growing cells are rapidly killed by iron starvation, stationary-phase cells show a remarkable ability to survive iron depletion. Finally, bioinformatic analysis of the predicted promoter regions of the differentially regulated genes led to identification of several putative Fur boxes, suggesting a direct role for Fur in iron-dependent regulation of these genes.

Mhashilkar, A. M., A. L. Stewart, et al. (2003). "MDA-7 negatively regulates the beta-catenin and PI3K

signaling pathways in breast and lung tumor cells." Mol Ther **8**(2): 207-19.

mda-7 is a novel tumor suppressor with cytokine properties. Adenoviral mda-7 (Ad-mda7) induces apoptosis and cell death selectively in tumor cells. The molecular mechanisms underlying the anti-tumor activity of Ad-mda7 in breast and lung cancer lines were investigated. Microarray analyses implicated both the beta-catenin and the PI3K signaling pathways. Ad-mda7 treatment increased protein expression from tumor suppressor genes, including E-cadherin, APC, GSK-3beta, and PTEN, and decreased expression of proto-oncogenes involved in beta-catenin and PI3K signaling. Ad-mda7 caused a redistribution of cellular beta-catenin from the nucleus to the plasma membrane, resulting in reduced TCF/LEF transcriptional activity, and upregulated the E-cadherin-beta-catenin adhesion complex in a tumor cell-specific manner. Expression of the PI3K pathway members (p85 PI3K, FAK, ILK-1, Akt, and PLC-gamma) was downregulated and expression of the PI3K antagonist PTEN was increased. Consistent with this result, pharmacological inhibition of PI3K by wortmannin did not abrogate killing by Ad-mda7. Killing of breast cancer cells by Ad-mda7 required both MAPK and MEK1/2 signaling pathways, whereas these pathways were not essential for MDA-7-mediated killing in lung cancer cells. Thus, in breast and lung tumor cells MDA-7 protein expression modulates cell-cell adhesion and intracellular signaling via coordinate regulation of the beta-catenin and PI3K pathways.

Mikami, K., Y. Kanesaki, et al. (2002). "The histidine kinase Hik33 perceives osmotic stress and cold stress in Synechocystis sp PCC 6803." Mol Microbiol **46**(4): 905-15.

The stress imposed on living organisms by hyperosmotic conditions and low temperature appears to be perceived via changes in the physical state of membrane lipids. We compared genome-wide patterns of transcription between wild-type Synechocystis sp. PCC 6803 and cells with a mutation in the histidine kinase Hik33 using a DNA microarray. Our results indicated that Hik33 regulated the expression of both osmostress-inducible and cold-inducible genes. The respective genes that were regulated by Hik33 under hyperosmotic and low-temperature conditions were, for the most part, different from one another. However, Hik33 also regulated the expression of a set of genes whose expression was induced both by osmotic stress and by cold stress. These results indicate that Hik33 is involved in responses to osmotic stress and low-temperature stress but that the mechanisms of the responses differ.

Miller, E. D., J. A. Smith, et al. (2003). "Activation of the signal transducer and activator of transcription 1 signaling pathway in thymocytes from HIV-1-infected human thymus." Aids **17**(9): 1269-77.

OBJECTIVE: To identify HIV-induced host factors in the severe combined immunodeficient (SCID)-hu Thy/Liv mouse that may contribute to HIV pathogenesis in the thymus. DESIGN: To identify genes specifically altered by HIV-1 infection using the cDNA microarray assay, SCID-hu Thy/Liv organs derived from the same donors were used. Therefore, no genetic variations existed between HIV and mock-infected samples. In addition, the 12-14 day post-infection timepoint was chosen because no significant thymocyte depletion was detected in HIV-infected Thy/Liv organs, so mRNA from the same cell types could be compared. METHODS: Using SCID-hu Thy/Liv mice constructed from the same donor tissues, we analysed the expression of 9183 host genes in response to HIV infection with cDNA microarrays. Expression of selected genes with more than threefold induction was confirmed by measuring RNA (reverse transcriptase-polymerase chain reaction; RT-PCR) and proteins. RESULTS: HIV-1 (JD or NL4-3) infection of the SCID-hu Thy/Liv mouse led to more than threefold induction of 19 genes, 12 of which were IFN-inducible and six were unknown EST clones. We confirmed induction by RT-PCR and protein blots. Both signal transducer and activator of transcription (STAT)1 and STAT2 proteins were induced, and STAT1 was also activated by phosphorylation at the Tyr701 and Ser727 sites in human thymus infected with HIV-JD or NL4-3. Treatment of human fetal thymus organ culture or human thymocytes with recombinant HIV-1 gp120 proteins also led to induction or activation of STAT1. CONCLUSION: HIV-1 infection of the thymus led to activation of the STAT1 signaling pathway in thymocytes, which may contribute to HIV-1 pathogenesis in the thymus.

Miller, S. K., R. T. Good, et al. (2002). "A subset of Plasmodium falciparum SERA genes are expressed and appear to play an important role in the erythrocytic cycle." J Biol Chem **277**(49): 47524-32.

The Plasmodium falciparum serine repeat antigen (SERA) has shown considerable promise as a blood stage vaccine for the control of malaria. A related protein, SERPH, has also been described in P. falciparum. Whereas their biological role remains unknown, both proteins possess papain-like protease domains that may provide attractive targets for therapeutic intervention. Genomic sequencing has recently shown that SERA and SERPH are the fifth and sixth genes, respectively, in a cluster of eight SERA homologues present on chromosome 2. In this paper, the expression and functional relevance of these eight genes and of a ninth SERA homologue found on chromosome 9 were examined in blood stage parasites. Using reverse transcriptase-PCR and microarray approaches, we demonstrate that whereas mRNA to all nine SERA genes is synthesized late in the erythrocytic cycle, it is those genes in the central region of the chromosome 2 cluster that are substantially up-regulated at this time. Using antibodies specific to each SERA, it was apparent that SERA4 to -6, and possibly also SERA9, are synthesized in blood stage parasites. The reactivity of antibodies from malaria-immune individuals with the SERA recombinant proteins suggested that SERA2 and SERA3 are also expressed at least in some parasite populations. To examine whether SERA genes are essential to blood stage growth, each of the eight chromosome 2 SERA genes was targeted for disruption. Whereas genes at the periphery of the cluster were mostly dispensable (SERA2 and -3 and SERA7 and -8), those in the central region (SERA4 to -6) could not be disrupted. The inability to disrupt SERA4, -5, and -6 is consistent with their apparent dominant expression and implies an important role for these genes in maintenance of the erythrocytic cycle.

Minagawa, S., H. Ogasawara, et al. (2003). "Identification and molecular characterization of the Mg2+ stimulon of Escherichia coli." J Bacteriol **185**(13): 3696-702.

Transcription profile microarray analysis in Escherichia coli was performed to identify the member genes of the Mg(2+) stimulon that respond to the availability of external Mg(2+) in a PhoP/PhoQ two-component system-dependent manner. The mRNA levels of W3110 in the presence of 30 mM MgCl(2), WP3022 (phoP defective), and WQ3007 (phoQ defective) were compared with those of W3110 in the absence of MgCl(2). The expression ratios of a total of 232 genes were <0.75 in all three strains (the supplemental data are shown at http://www.nara.kindai.ac.jp/nogei/seiken/array.html), suggesting that the PhoP/PhoQ system is involved directly or indirectly in the transcription of these genes. Of those, 26 contained the PhoP box-like sequences with the direct repeats of (T/G)GTTTA within 500 bp upstream of the initiation codon. Furthermore, S1 nuclease assays of 26 promoters were performed to verify six new Mg(2+) stimulon genes, hemL, nagA, rstAB, slyB, vboR, and yrbL, in addition to the phoPQ, mgrB, and mgtA genes reported previously. In gel shift and DNase I footprinting assays, all of these genes were found to be regulated directly by PhoP. Thus, we concluded that the phoPQ, mgrB, mgtA, hemL, nagA, rstAB, slyB, vboR, and yrbL genes make up the Mg(2+) stimulon in E. coli.

Minning, T. A., J. Bua, et al. (2003). "Microarray profiling of gene expression during trypomastigote to amastigote transition in Trypanosoma cruzi." Mol Biochem Parasitol **131**(1): 55-64.

Trypanosoma cruzi, the causative agent of Chagas disease, remains a significant public health concern throughout South and Central America. Although much is known about immune control of T. cruzi and in particular the importance of recognition of parasite-infected cells, relatively little is known about the target antigens of these protective immune responses. For instance, few of the genes expressed in the intracellular amastigote stage have been identified. To gain insight into the molecular events, at the level of mRNA abundance, involved in this critical point in the parasite life-cycle, we used DNA microarrays of 4400 sequences from T. cruzi ORF-selected and random, genomic sequencing libraries to determine relative mRNA abundances in trypomastigotes and developing amastigotes. Results from six hybridizations using independently generated parasite samples consistently identified 60 probes that detected genes upregulated within 2h after extracellular trypomastigotes were induced, in vitro, to

differentiate into amastigotes. Sequence analysis from these 60 probes identified 14 known and 25 novel T. cruzi genes. The general direction of regulation was confirmed by quantitative RT-PCR for seven of the array-identified, amastigote upregulated, known genes. This work demonstrates the feasibility of computational and microarray approaches to gene discovery in T. cruzi, an organism for which a fully assembled and annotated genome sequence is not yet available and in which control of transcription initiation is believed to be absent. Moreover, this work is the first report of amastigote up regulation for 38 genes, thus expanding considerably the pool of genes known to be upregulated in this important yet poorly-studied stage of the T. cruzi life-cycle.

Mirza, A., Q. Wu, et al. (2003). "Global transcriptional program of p53 target genes during the process of apoptosis and cell cycle progression." Oncogene **22**(23): 3645-54.

The temporal gene expression profile during the entire process of apoptosis and cell cycle progression in response to p53 in human ovarian cancer cells was explored with cDNA microarrays representing 33 615 individual human genes. A total of 1501 genes (4.4%) were found to respond to p53 (approximately 80% of these were repressed by p53) using 2.5-fold change as a cutoff. It was anticipated that most of p53 responsive genes resulted from the secondary effect of p53 expression at late stage of apoptosis. To delineate potential p53 direct and indirect target genes during the process of apoptosis and cell cycle progression, microarray data were combined with global p53 DNA-binding site analysis. Here we showed that 361 out of 1501 p53 responsive genes contained p53 consensus DNA-binding sequence(s) in their regulatory region, approximately 80% of which were repressed by p53. This is the first time that a large number of p53-repressed genes have been identified to contain p53 consensus DNA-binding sequence(s) in their regulatory region. Hierarchical cluster analysis of these genes revealed distinct temporal expression patterns of transcriptional activation and repression by p53. More genes were activated at early time points, while more repressed genes were found after the onset of apoptosis. A small-scale quantitative chromatin immunoprecipitation analysis indicated that in vivo p53-DNA interaction was detected in eight out of 10 genes, most of which were repressed by p53 at the early onset of apoptosis, suggesting that a portion of p53 target genes in the human genome could be negatively regulated by p53 via sequence-specific DNA binding. The approaches and genes described here should aid the understanding of global gene regulatory network of p53.

Mitchell, R., C. Y. Chiang, et al. (2003). "Global analysis of cellular transcription following infection with an HIV-based vector." Mol Ther **8**(4): 674-87.

We have examined the changes in cellular transcription resulting from infection with HIV-based vectors. Previous work suggested that the incoming viral genome may under some circumstances be detected as DNA damage, so to explore this possibility, we compared the transcriptional response to infection with an HIV-based vector to the response to treatment with the DNA-damaging agent etoposide. Expression levels of about 12,000 cellular RNA transcripts were determined in a human B-cell line at different times after either treatment. Statistical analysis revealed that the infection with the lentivirus vector resulted in quite modest changes in gene expression. Treatment with etoposide, in contrast, caused drastic changes in expression of genes known or inferred to be involved in apoptosis. Statistically significant though subtle parallels in the cellular transcriptional responses to etoposide treatment and HIV-vector infection could be detected. Several further data sets analyzing infections with HIV-based vectors or wild-type HIV-1 showed similar modest effects on cellular transcription and very modest parallels among different data sets. These findings establish that HIV-vector or HIV-1 infection has remarkably little effect on cellular transcription. The statistical methods described here may be of wide use in mining microarray data sets. Our observations support the idea that gene therapy with HIV-based vectors should not be particularly toxic to cells due to disruption of cellular transcription.

Mitra, R. M., S. L. Shaw, et al. (2004). "Six nonnodulating plant mutants defective for Nod factor-induced transcriptional changes associated with the legume-rhizobia symbiosis." Proc Natl Acad Sci U S

A **101**(27): 10217-22.

As the legume-rhizobia symbiosis is established, the plant recognizes bacterial-signaling molecules, Nod factors (NFs), and initiates transcriptional and developmental changes within the root to allow bacterial invasion and the construction of a novel organ, the nodule. Plant mutants defective in nodule initiation (Nod(-)) are thought to have defects in NF-signal transduction. However, it is unknown whether WT plants respond to NF-independent bacterial-derived signals or whether Nod(-) plant mutants show defects in global symbiosis-associated gene expression. To characterize plant gene expression in the establishment of the symbiosis, we used an Affymetrix oligonucleotide microarray representing 9,935 Medicago truncatula expressed sequences. We identified 46 sequences that are differentially expressed in plants exposed for 24 h to WT Sinorhizobium meliloti or to the invasion defective S. meliloti mutant, exoA. Eight of these genes encode nucleolar proteins, which are implicated in ribosome biogenesis. We also identified differentially expressed transcription factors, signaling components, defense response proteins, stress response proteins, and several previously uncharacterized genes. NF appears both necessary and sufficient to induce most changes. Six of seven Nod(-) M. truncatula mutants (nfp, dmi1, dmi2, dmi3, nsp1, and nsp2) showed no transcriptional response to S. meliloti, suggesting that the encoded proteins are required for initiating new transcription. The Nod(-) mutant hcl, however, exhibits a reduced transcriptional response to S. meliloti, indicating that the machinery responsible for initiating new transcription is at least partially functional in this mutant.

Molle, V., M. Fujita, et al. (2003). "The Spo0A regulon of Bacillus subtilis." Mol Microbiol **50**(5): 1683-701.

The master regulator for entry into sporulation in Bacillus subtilis is the DNA-binding protein Spo0A, which has been found to influence, directly or indirectly, the expression of over 500 genes during the early stages of development. To search on a genome-wide basis for genes under the direct control of Spo0A, we used chromatin immunoprecipitation in combination with gene microarray analysis to identify regions of the chromosome at which an activated form of Spo0A binds in vivo. This information in combination with transcriptional profiling using gene microarrays, gel electrophoretic mobility shift assays, using the DNA-binding domain of Spo0A, and bioinformatics enabled us to assign 103 genes to the Spo0A regulon in addition to 18 previously known members. Thus, in total, 121 genes, which are organized as 30 single-gene units and 24 operons, are likely to be under the direct control of Spo0A. Forty of these genes are under the positive control of Spo0A, and 81 are under its negative control. Among newly identified members of the regulon with transcription that was stimulated by Spo0A are genes for metabolic enzymes and genes for efflux pumps. Among members with transcription that was inhibited by Spo0A are genes encoding components of the DNA replication machinery and genes that govern flagellum biosynthesis and chemotaxis. Also in-cluded in the regulon are many (25) genes with products that are direct or indirect regulators of gene transcription. Spo0A is a master regulator for sporulation, but many of its effects on the global pattern of gene transcription are likely to be mediated indirectly by regulatory genes under its control.

Molle, V., Y. Nakaura, et al. (2003). "Additional targets of the Bacillus subtilis global regulator CodY identified by chromatin immunoprecipitation and genome-wide transcript analysis." J Bacteriol **185**(6): 1911-22.

Additional targets of CodY, a GTP-activated repressor of early stationary-phase genes in Bacillus subtilis, were identified by combining chromatin immunoprecipitation, DNA microarray hybridization, and gel mobility shift assays. The direct targets of CodY newly identified by this approach included regulatory genes for sporulation, genes that are likely to encode transporters for amino acids and sugars, and the genes for biosynthesis of branched-chain amino acids.

Moore, R. A., S. Reckseidler-Zenteno, et al. (2004). "Contribution of gene loss to the pathogenic evolution of Burkholderia pseudomallei and Burkholderia mallei." Infect Immun **72**(7): 4172-87.

Burkholderia pseudomallei is the causative agent of melioidosis. Burkholderia thailandensis is a closely related species that can readily utilize l-arabinose as a sole carbon source, whereas B. pseudomallei cannot. We used Tn5-OT182 mutagenesis to isolate an arabinose-negative mutant of B. thailandensis. Sequence analysis of regions flanking the transposon insertion revealed the presence of an arabinose assimilation operon consisting of nine genes. Analysis of the B. pseudomallei chromosome showed a deletion of the operon from this organism. This deletion was detected in all B. pseudomallei and Burkholderia mallei strains investigated. We cloned the B. thailandensis E264 arabinose assimilation operon and introduced the entire operon into the chromosome of B. pseudomallei 406e via homologous recombination. The resultant strain, B. pseudomallei SZ5028, was able to utilize l-arabinose as a sole carbon source. Strain SZ5028 had a significantly higher 50% lethal dose for Syrian hamsters compared to the parent strain 406e. Microarray analysis revealed that a number of genes in a type III secretion system were down-regulated in strain SZ5028 when cells were grown in l-arabinose, suggesting a regulatory role for l-arabinose or a metabolite of l-arabinose. These results suggest that the ability to metabolize l-arabinose reduces the virulence of B. pseudomallei and that the genes encoding arabinose assimilation may be considered antivirulence genes. The increase in virulence associated with the loss of these genes may have provided a selective advantage for B. pseudomallei as these organisms adapted to survival in animal hosts.

Moore-Lai, D. and E. Rowland (2004). "Microarray data demonstrate that Trypanosoma cruzi downregulates the expression of apoptotic genes in BALB/c fibroblasts." J Parasitol **90**(4): 893-5.

Parasites have been shown to up- and downregulate host apoptosis, most likely facilitating their ability to successfully establish an infection in the host. It has been demonstrated that pathogens modulate well-established pathways, leading to cell death, including induction of the Fas-FasL system to promote apoptosis. In contrast, it has also been shown that in other instances a decrease in host cell apoptosis results after the upregulation of genes in the Bcl-2 family. The present study examined the ability of Trypanosoma cruzi to modulate expression of host cell genes of the TNFR1 apoptotic pathway. Using microarray technology, gene expression was compared between uninfected BALB/c fibroblasts and T. cruzi-infected BALB/c fibroblasts. After comparing expression patterns between uninfected and T. cruzi-infected BALB/c fibroblasts, it was concluded that genetic expression of genes in the TNFR1 apoptotic pathway is downregulated in T. cruzi-infected cells, indicating that in BALB/c fibroblasts the parasite decreases the expression of genes, leading to host cell apoptosis.

Moran, N. A., G. R. Plague, et al. (2003). "A genomic perspective on nutrient provisioning by bacterial symbionts of insects." Proc Natl Acad Sci U S A **100 Suppl 2**: 14543-8.

Many animals show intimate interactions with bacterial symbionts that provision hosts with limiting nutrients. The best studied such association is that between aphids and Buchnera aphidicola, which produces essential amino acids that are rare in the phloem sap diet. Genomic studies of Buchnera have provided a new means for inferring metabolic capabilities of the symbionts and their likely contributions to hosts. Despite evolutionary reduction of genome size, involving loss of most ancestral genes, Buchnera retains capabilities for biosynthesis of all essential amino acids. In contrast, most genes duplicating amino acid biosynthetic capabilities of hosts have been eliminated. In Buchnera of many aphids, genes for biosynthesis of leucine and tryptophan have been transferred from the chromosome to distinctive plasmids, a feature interpreted as a mechanism for overproducing these amino acids through gene amplification. However, the extent of plasmid-associated amplification varies between and within species, and plasmid-borne genes are sometimes fewer in number than single copy genes on the (polyploid) main chromosome. This supports the broader interpretation of the plasmid location as a means of achieving regulatory control of gene copy number and/or transcription. Buchnera genomes have eliminated most regulatory sequences, raising the question of the extent to which gene expression is moderated in response to changing demands imposed by host nutrition or other factors. Microarray analyses of the Buchnera transcriptome reveal only slight changes in expression of nutrition-related genes

in response to shifts in host diet, with responses less dramatic than those observed for the related nonsymbiotic species, Escherichia coli.

Moran, P. J., Y. Cheng, et al. (2002). "Gene expression profiling of Arabidopsis thaliana in compatible plant-aphid interactions." Arch Insect Biochem Physiol **51**(4): 182-203.

Phloem feeding involves unique biological interactions between the herbivore and its host plant. The economic importance of aphids, whiteflies, and other phloem-feeding insects as pests has prompted research to isolate sources of resistance to piercing-sucking insects in crops. However, little information exists about the molecular nature of plant sensitivity to phloem feeding. Recent discoveries involving elicitation by plant pathogens and chewing insects and limited studies on phloem feeders suggest that aphids are capable of inducing responses in plants broadly similar to those associated with pathogen infection and wounding. Our past work showed that compatible aphid feeding on leaves of Arabidopsis thaliana induces localized changes in levels of transcripts of genes that are also associated with infection, mechanical damage, chewing herbivory, or resource allocation shifts. We used microarray and macroarray gene expression analyses of infested plants to better define the response profile of A. thaliana to M. persicae feeding. The results suggest that genes involved in oxidative stress, calcium-dependent signaling, pathogenesis-related responses, and signaling are key components of this profile in plants infested for 72 or 96 h. The use of plant resistance to aphids in crops will benefit from a better understanding of induced responses. The establishment of links between insect elicitation, plant signaling associated with phloem feeding, and proximal resistance mechanisms is critical to further research progress in this area.

Mori, T., Y. Anazawa, et al. (2002). "Cyclin K as a direct transcriptional target of the p53 tumor suppressor." Neoplasia **4**(3): 268-74.

Cyclin K, a newly recognized member of the "transcription" cyclin family, may play a dual role by regulating CDK and transcription. Using cDNA microarray technology, we found that cyclin K mRNA was dramatically increased in U373MG, a glioblastoma cell line deficient in wild-type p53, in the presence of exogenous p53. An electrophoretic mobility-shift assay showed that a potential p53-binding site (p53BS) in intron 1 of the cyclin K gene could indeed bind to p53 protein. Moreover, a heterologous reporter assay revealed that the p53BS possessed p53-dependent transcriptional activity. Colony-formation assays indicated that overexpression of cyclin K suppressed growth of T98G, U373MG and SW480 cells. The results suggested that cyclin K may play a role in regulating the cell cycle or apoptosis after being targeted for transcription by p53.

Morimoto, M., D. Zarlenga, et al. (2003). "Ascaris suum: cDNA microarray analysis of 4th stage larvae (L4) during self-cure from the intestine." Exp Parasitol **104**(3-4): 113-21.

There is spontaneous cure of a large portion of Ascaris suum 4th-stage larvae (L4) from the jejunum of infected pigs between 14 and 21 days after inoculation (DAI). Those L4 that remain in the jejunum continue to develop while those that have moved to the ileum are eventually expelled from the intestines. Although increases in intestinal mucosal mast cells and changes in local host immunity are coincidental with spontaneous cure, the population of L4 that continue to develop in the jejunum may counteract host protective mechanisms by the differential production of factors related to parasitism. To this end, a cDNA library was constructed from L4 isolated from pig jejunum at 21 DAI, and 93% of 1920 original clones containing a single amplicon in the range 400-1500 bp were verified by gel electrophoresis and printed onto glass slides for microarray analysis. Fluorescent probes were prepared from total RNA isolated from: (1) 3rd stage-larvae from lung at 7 DAI, (L3); (2) L4 from jejunum at 14 DAI (L4-14-J); (3) L4 from jejunum at 21 DAI (L4-21-J); (4) L4 from ileum at 21 DAI (L4-21-I, and; (5) adults (L5). Cy3-labeled L3, L4-14-J, L4-21-I and L5 cDNA, and Cy5-labeled L4-21-J cDNA were simultaneously used to screen the printed arrays containing the L4-21-J-derived cDNA library. Several clones showed consistent differential gene expression over two separate experiments and were grouped

into 3 distinct transcription patterns. The data showed that sequences from muscle actin and myosin, ribosomal protein L11, glyceraldehyde-3-phosphate dehydrogenase and the flavoprotein subunit of succinate dehydrogenase were highly expressed in L4-21-J, but not in L4-21-I; as were a collection of un-annotated genes derived from a worm body wall-hypodermis library, and a testes germinal zone tissue library. These results suggest that only actively developing A. suum L4 are destined to parasitize the host and successfully neutralize host protective responses.

Morrison, D. A. and J. T. Ellis (2003). "The design and analysis of microarray experiments: applications in parasitology." DNA Cell Biol **22**(6): 357-94.

Microarray experiments can generate enormous amounts of data, but large datasets are usually inherently complex, and the relevant information they contain can be difficult to extract. For the practicing biologist, we provide an overview of what we believe to be the most important issues that need to be addressed when dealing with microarray data. In a microarray experiment we are simply trying to identify which genes are the most "interesting" in terms of our experimental question, and these will usually be those that are either overexpressed or underexpressed (upregulated or downregulated) under the experimental conditions. Analysis of the data to find these genes involves first preprocessing of the raw data for quality control, including filtering of the data (e.g., detection of outlying values) followed by standardization of the data (i.e., making the data uniformly comparable throughout the dataset). This is followed by the formal quantitative analysis of the data, which will involve either statistical hypothesis testing or multivariate pattern recognition. Statistical hypothesis testing is the usual approach to "class comparison," where several experimental groups are being directly compared. The best approach to this problem is to use analysis of variance, although issues related to multiple hypothesis testing and probability estimation still need to be evaluated. Pattern recognition can involve "class prediction," for which a range of supervised multivariate techniques are available, or "class discovery," for which an even broader range of unsupervised multivariate techniques have been developed. Each technique has its own limitations, which need to be kept in mind when making a choice from among them. To put these ideas in context, we provide a detailed examination of two specific examples of the analysis of microarray data, both from parasitology, covering many of the most important points raised.

Mossman, K. L., P. F. Macgregor, et al. (2001). "Herpes simplex virus triggers and then disarms a host antiviral response." J Virol **75**(2): 750-8.

Virus infection induces an antiviral response that is predominantly associated with the synthesis and secretion of soluble interferon. Here, we report that herpes simplex virus type 1 virions induce an interferon-independent antiviral state in human embryonic lung cells that prevents plaquing of a variety of viruses. Microarray analysis of 19,000 human expressed sequence tags revealed induction of a limited set of host genes, the majority of which are also induced by interferon. Genes implicated in controlling the intracellular spread of virus and eliminating virally infected cells were among those induced. Induction of the cellular response occurred in the absence of de novo cellular protein synthesis and required viral penetration. In addition, this response was only seen when viral gene expression was inhibited, suggesting that a newly synthesized viral protein(s) may function as an inhibitor of this response.

Mueller, A., J. O'Rourke, et al. (2003). "Distinct gene expression profiles characterize the histopathological stages of disease in Helicobacter-induced mucosa-associated lymphoid tissue lymphoma." Proc Natl Acad Sci U S A **100**(3): 1292-7.

Long-term colonization of humans with Helicobacter pylori can cause the development of gastric B cell mucosa-associated lymphoid tissue lymphoma, yet little is known about the sequence of molecular steps that accompany disease progression. We used microarray analysis and laser microdissection to identify gene expression profiles characteristic and predictive of the various histopathological stages in a mouse model of the disease. The initial step in lymphoma development is marked by infiltration of reactive lymphocytes into the stomach and the launching of a mucosal immune response. Our analysis

uncovered molecular markers of both of these processes, including genes coding for the immunoglobulins and the small proline-rich protein Sprr 2A. The subsequent step is characterized histologically by the antigen-driven proliferation and aggregation of B cells and the gradual appearance of lymphoepithelial lesions. In tissues of this stage, we observed increased expression of genes previously associated with malignancy, including the laminin receptor-1 and the multidrug-resistance channel MDR-1. Finally, we found that the transition to destructive lymphoepithelial lesions and malignant lymphoma is marked by an increase in transcription of a single gene encoding calgranulin Amrp-8.

Mukherjee, S., T. J. Belbin, et al. (2003). "Microarray analysis of changes in gene expression in a murine model of chronic chagasic cardiomyopathy." Parasitol Res **91**(3): 187-96.

Chagas' disease, caused by infection with Trypanosoma cruzi, is a major cause of cardiomyopathy in endemic regions. Infection leads to cardiac remodeling associated with congestive heart failure and dilated cardiomyopathy. In order to study the changes in the gene expression profile due to infection, C57BL/6 x 129sv male mice were infected with 1 x 10(3) trypomastigotes of the Brazil strain of T. cruzi. Histopathological examination of the myocardium revealed chronic inflammation, vasculitis and fibrosis 100 days post-infection. Cardiac magnetic resonance imaging revealed a significantly dilated heart compared with uninfected mice. The relative abundance or depletion of myocardial mRNAs was evaluated using high-density microarrays consisting of 27,400 mouse cDNAs, which were hybridized with fluorescent probes generated from mRNAs of T. cruzi infected and uninfected hearts. Differentially expressed genes were sorted according to their normalized expression patterns and functional groups including those involved in transcription, intracellular transport, structure/junction/adhesion or extracellular matrix, signaling, host defense, energetics, metabolism, cell shape and death. The regulated genes are interpreted in the pathogenesis of chagasic heart disease.

Muljo, S. A. and M. S. Schlissel (2003). "A small molecule Abl kinase inhibitor induces differentiation of Abelson virus-transformed pre-B cell lines." Nat Immunol **4**(1): 31-7.

Abelson murine leukemia virus-transformed cell lines have provided a critical model system for studying the regulation of B cell development. However, transformation by v-Abl blocks B cell development, resulting in the arrest of these transformants in an early pre-B cell-like state. We report here that treatment of Abelson virus-transformed pre-B cell lines with the small molecule Abl kinase inhibitor (STI571) results in their differentiation to a late pre-B cell-like state characterized by induction of immunoglobulin (Ig) light chain gene rearrangement. DNA microarray analyses enabled us to identify two genes inhibited by v-Abl that encode the Igk 3' enhancer-binding transcription factors Spi-B and IRF-4. We show that enforced expression of these two factors is sufficient to induce germline Igk transcription in Abelson-transformed pro-B cell lines. This suggests a key role for these factors, and perhaps for c-Abl itself, in the regulated activation of Ig light chain gene rearrangement.

Munir, S. and V. Kapur (2003). "Regulation of host cell transcriptional physiology by the avian pneumovirus provides key insights into host-pathogen interactions." J Virol **77**(8): 4899-910.

Infection with a viral pathogen triggers several pathways in the host cell that are crucial to eliminating infection, as well as those that are used by the virus to enhance its replication and virulence. We have here used suppression subtractive hybridization and cDNA microarray analyses to characterize the host transcriptional response in an avian pneumovirus model of infection. The results of our investigations reveal a dynamic host response that includes the regulation of genes with roles in a vast array of cellular functions as well as those that have not been described previously. The results show a considerable upregulation in transcripts representing the interferon-activated family of genes, predicted to play a role in virus replication arrest. The analysis also identified transcripts for proinflammatory leukocyte chemoattractants, adhesion molecules, and complement that were upregulated and may account for the inflammatory pathology that is the hallmark of viral respiratory infection. Interestingly, alterations in the transcription of several genes in the ubiquitin and endosomal protein trafficking pathways were

observed, suggesting a role for these pathways in virus maturation and budding. Taken together, the results of our investigations provide key insights into individual genes and pathways that constitute the host cell's response to avian pneumovirus infection, and they have enabled the development of resources and a model of host-pathogen interaction for an important avian respiratory tract pathogen.

Munir, S., S. Singh, et al. (2004). "Suppression subtractive hybridization coupled with microarray analysis to examine differential expression of genes in virus infected cells." Biol Proced Online **6**: 94-104.

High throughput detection of differential expression of genes is an efficient means of identifying genes and pathways that may play a role in biological systems under certain experimental conditions. There exist a variety of approaches that could be used to identify groups of genes that change in expression in response to a particular stimulus or environment. We here describe the application of suppression subtractive hybridization (SSH) coupled with cDNA microarray analysis for isolation and identification of chicken transcripts that change in expression on infection of host cells with a paramyxovirus. SSH was used for initial isolation of differentially expressed transcripts, a large-scale validation of which was accomplished by microarray analysis. The data reveals a large group of regulated genes constituting many biochemical pathways that could serve as targets for future investigations to explore their role in paramyxovirus pathogenesis. The detailed methods described herein could be useful and adaptable to any biological system for studying changes in gene expression.

Munson, R. S., Jr., A. Harrison, et al. (2004). "Partial analysis of the genomes of two nontypeable Haemophilus influenzae otitis media isolates." Infect Immun **72**(5): 3002-10.

In 1995, The Institute for Genomic Research completed the genomic sequence of a rough derivative of Haemophilus influenzae serotype d, strain KW20. This sequence, though extremely useful in understanding the basic biology of H. influenzae, has yet to provide significant insight into our understanding of disease caused by nontypeable H. influenzae (NTHI), because serotype d strains are not generally pathogens. In contrast, NTHI strains are frequently mucosal pathogens and are the primary pathogens of chronic otitis media as well as a significant cause of acute otitis media in children. Thus, it is of great importance to further understand their biology. We used a DNA-based microarray approach to identify genes present in a clinical isolate of NTHI that were absent from strain Rd. We also sequenced the genome of a second NTHI isolate from a child with chronic otitis media to threefold coverage and then used an array of bioinformatics tools to identify genes present in this NTHI strain but absent from strain Rd. These methods were complementary in approach and results. We identified, in both strains, homologues of H. influenzae lav, an autotransported protein of unknown function; tnaA, which encodes tryptophanase; as well as a homologue of Pasteurella multocida tsaA, which encodes an alkyl peroxidase that may play a role in protection against reactive oxygen species. We also identified a number of putative restriction-modification systems, bacteriophage genes and transposon-related genes. These data provide new insight that complements and extends our ongoing analysis of NTHI virulence determinants.

Nagpal, S., M. W. Karaman, et al. (2004). "Improving the sensitivity and specificity of gene expression analysis in highly related organisms through the use of electronic masks." Nucleic Acids Res **32**(5): e51.

DNA microarrays are powerful tools for comparing gene expression profiles from closely related organisms. However, a single microarray design is frequently used in these studies. Therefore, the levels of certain transcripts can be grossly underestimated due to sequence differences between the transcripts and the arrayed DNA probes. Here, we seek to improve the sensitivity and specificity of oligonucleotide microarray-based gene expression analysis by using genomic sequence information to predict the hybridization efficiency of orthologous transcripts to a given microarray. To test our approach, we examine hybridization patterns from three Escherichia coli strains on E.coli K-12 MG1655 gene expression microarrays. We create electronic mask files to discard data from probes predicted to have poor hybridization sensitivity and specificity to cDNA targets from each strain. We increased the

accuracy of gene expression analysis and identified genes that cannot be accurately interrogated in each strain using these microarrays. Overall, these studies provide guidelines for designing effective electronic masks for gene expression analysis in organisms where substantial genome sequence information is available.

Naiki, T., M. Nagaki, et al. (2002). "Analysis of gene expression profile induced by hepatocyte nuclear factor 4alpha in hepatoma cells using an oligonucleotide microarray." J Biol Chem **277**(16): 14011-9.

Hepatocyte nuclear factor 4alpha (HNF-4alpha), a liver-specific transcription factor, plays a significant role in many liver-specific functions, including lipid, glucose, drug, and ammonia metabolism, and also in embryonal liver development. However, its functions and regulation are not yet clearly understood. In this study, we constructed an adenovirus vector carrying rat HNF-4alpha cDNA and transfected the adenovirus to human hepatoma cells, HuH-7, to enforce expression of the exogenous HNF-4alpha gene. We analyzed HNF-4alpha-induced genes using cDNA microarray technology, which included over 9000 genes. As a result, 62 genes showed a greater than 2.0-fold change in expression level after the viral transfection. Fifty-six genes were consistently induced by HNF-4alpha overexpression, and six genes were repressed. To assess HNF-4alpha function, we attempted to classify the genes, which had been classified by their encoding protein functions in a previous report. We could classify 45 genes. The rest of the HNF-4alpha-sensitive genes were unclassified (4 genes) or not identified (13 genes). Among the classified genes, almost half of the induced genes (26 of 40) were related to metabolism genes and particularly to lipid metabolism-related genes. This cDNA microarray analysis showed that HNF-4alpha is one of the central liver metabolism regulators.

Nair, S., S. Alokam, et al. (2004). "Salmonella enterica serovar Typhi strains from which SPI7, a 134-kilobase island with genes for Vi exopolysaccharide and other functions, has been deleted." J Bacteriol **186**(10): 3214-23.

Salmonella enterica serovar Typhi has a 134-kb island of DNA identified as salmonella pathogenicity island 7 (SPI7), inserted between pheU and 'pheU (truncated), two genes for tRNA(Phe). SPI7 has genes for Vi exopolysaccharide, for type IVB pili, for putative conjugal transfer, and for sopE bacteriophage. Pulsed-field gel electrophoresis following digestion with the endonuclease I-CeuI, using DNA from a set of 120 wild-type strains of serovar Typhi assembled from several sources, identified eight strains in which the I-CeuI G fragment, which contains SPI7, had a large deletion. In addition, agglutination tests with Vi antiserum and phage typing with Vi phages show that all eight strains are Vi negative. We therefore tested these strains for deletion of SPI7 by multiplex PCR, by microarray analysis, and by sequencing of PCR amplicons. Data show that seven of the eight strains are precise deletions of SPI7: a primer pair flanking SPI7 results in a PCR amplicon containing a single pheU gene; microarrays show that all SPI7 genes are deleted. Two of the strains produce amplicons which have A derived from pheU at bp 27, while five have C derived from 'pheU at this position; thus, the position of the crossover which results in the deletion can be inferred. The deletion in the eighth strain, TYT1669, removes 175 kb with junction points in genes STY4465 and STY4664; the left junction of SPI7 and adjacent genes, as well as part of SPI7 including the viaB operon for Vi exopolysaccharide, was removed, while the right junction of SPI7 was retained. We propose that these deletions occurred during storage following isolation.

Nakagomi, S., Y. Suzuki, et al. (2003). "Expression of the activating transcription factor 3 prevents c-Jun N-terminal kinase-induced neuronal death by promoting heat shock protein 27 expression and Akt activation." J Neurosci **23**(12): 5187-96.

Activating transcription factor 3 (ATF3) is induced and functions both as a cellular response to stress and to stimulate proliferation in multiple tissues. However, in the nervous system ATF3 is expressed only in injured neurons. Here we reveal a function of ATF3 in neurons under death stress. Overexpression of ATF3 by adenovirus inhibits the mitogen-activated kinase kinase kinase 1 (MEKK1)-

c-Jun N-Terminal Kinase (JNK)-induced apoptosis and induces neurite elongation via Akt activation in PC12 cells and superior nerve ganglion neurons. A DNA microarray study reveals that ATF3 expression and JNK activation induce expression of the heat shock protein 27 (Hsp27). Immunoprecipitation analysis and promoter assay for Hsp27 expression suggest that both ATF3 and c-Jun are necessary for transcriptional activation of Hsp27. Hsp27 expression significantly inhibits JNK-induced apoptosis as well as Akt activation in PC12 cells and superior cervical ganglion neurons. We conclude that the combination of ATF3 and c-Jun induces the anti-apoptotic factor Hsp27, which directly or indirectly activates Akt, and thereby possibly inhibits apoptosis and induces nerve elongation. Our results suggest that ATF3- and c-Jun-induced Hsp27 expression is a novel survival response in neurons under death stress such as nerve injury.

Nakahigashi, K., N. Kubo, et al. (2002). "HemK, a class of protein methyl transferase with similarity to DNA methyl transferases, methylates polypeptide chain release factors, and hemK knockout induces defects in translational termination." Proc Natl Acad Sci U S A **99**(3): 1473-8.

HemK, a universally conserved protein of unknown function, has high amino acid similarity with DNA-(adenine-N6) methyl transferases (MTases). A certain mutation in hemK gene rescues the photosensitive phenotype of a ferrochelatase-deficient (hemH) mutant in Escherichia coli. A hemK knockout strain of E. coli not only suffered severe growth defects, but also showed a global shift in gene expression to anaerobic respiration, as determined by microarray analysis, and this shift may lead to the abrogation of photosensitivity by reducing the oxidative stress. Suppressor mutations that abrogated the growth defects of the hemK knockout strain were isolated and shown to be caused by a threonine to alanine change at codon 246 of polypeptide chain release factor (RF) 2, indicating that hemK plays a role in translational termination. Consistent with such a role, the hemK knockout strain showed an enhanced rate of read-through of nonsense codons and induction of transfer-mRNA-mediated tagging of proteins within the cell. By analysis of the methylation of RF1 and RF2 in vivo and in vitro, we showed that HemK methylates RF1 and RF2 in vitro within the tryptic fragment containing the conserved GGQ motif, and that hemK is required for the methylation within the same fragment of, at least, RF1 in vivo. This is an example of a protein MTase containing the DNA MTase motif and also a protein-(glutamine-N5) Mtase.

Nakano, S., E. Kuster-Schock, et al. (2003). "Spx-dependent global transcriptional control is induced by thiol-specific oxidative stress in Bacillus subtilis." Proc Natl Acad Sci U S A **100**(23): 13603-8.

The Spx protein of Bacillus subtilis represses activator-stimulated transcription by interacting with the C-terminal domain of RNA polymerase (RNAP) alpha subunit. Its concentration increases in cells lacking the ATP-dependent protease, ClpXP, resulting in severe effects on growth and developmental processes. Microarray analysis was undertaken to identify genes that are induced or repressed when Spx interacts with RNAP. The induced genes included those encoding products known to function in maintaining thiol homeostasis. Two genes, thioredoxin (trxA) and thioredoxin reductase (trxB), are transcriptionally induced under conditions of thiol-specific oxidative (disulfide) stress by a mechanism involving Spx-RNAP interaction. Disulfide stress also results in an increase in Spx-dependent transcriptional repression. The increase in Spx activity in cells encountering disulfide stress is due in part to a posttranscriptional mechanism of spx control resulting in an increase in Spx concentration. An spx null mutant and a strain bearing an allele of rpoA that prevents Spx-RNAP interaction show hypersensitivity to disulfide stress. From these results, it is proposed that Spx is an activator that mobilizes the operations necessary to reverse the effects of oxidative damage, but it also serves as a negative regulator that causes the postponement of developmental programs and energy-consuming growth-related functions while the cell copes with the period of stress.

Nakaya, A., S. Goto, et al. (2001). "Extraction of correlated gene clusters by multiple graph comparison." Genome Inform Ser Workshop Genome Inform **12**: 44-53.

This paper presents a new method to extract a set of correlated genes with respect to multiple biological features. Relationships among genes on a specific feature are encoded as a graph structure whose nodes correspond to genes. For example, the genome is a graph representing positional correlations of genes on the chromosome, the pathway is a graph representing functional correlations of gene products, and the expression profile is a graph representing gene expression similarities. When a set of genes are localized in a single graph, such as a gene cluster on the chromosome, an enzyme cluster in the metabolic pathway, or a set of coexpressed genes in the microarray gene expression profile, this may suggest a functional link among those genes. The functional link would become stronger when the clusters are correlated; namely, when a set of corresponding genes form clusters in multiple graphs. The newly introduced heuristic algorithm extracts such correlated gene clusters as isomorphic subgraphs in multiple graphs by using inter-graph links that are defined based on biological relevance. Using the method, we found E.coli correlated gene clusters in which genes are related with respect to the positions in the genome and the metabolic pathway, as well as the 3D structural similarity. We also analyzed protein-protein interaction data by two-hybrid experiments and gene coexpression data by microarrays in S.cerevisiae, and estimated the possibility of utilizing our method for screening the datasets that are likely to contain many false positive relations.

Nam, J. H., C. H. Yu, et al. (2002). "Application of cDNA microarray technique to detection of gene expression in host cells infected with viruses." Acta Virol **46**(3): 141-6.

cDNA microarray technique was used to monitor changes in mRNA levels in cells after Hantaan virus (HTNV) infection. The values of the ratio of medians for HTNV and Japanese encephalitis virus (JEV) at the early stage of infection were compared and found similar, suggesting that the same or similar genes are associated with the early events of infection with either virus. The reproducibility of values of the "ratio of medians" for HTNV was examined. We found that applying cluster analysis to the gene expression data groups efficiently together genes with the same function. Therefore, in analyzing the effects of viral infection on host cells by the cDNA microarray technique, clustering data appear to be necessary for gaining biological meaning from a dump of gene expression profiles obtained from virus-infected cells.

Narezkina, A., K. D. Taganov, et al. (2004). "Genome-wide analyses of avian sarcoma virus integration sites." J Virol **78**(21): 11656-63.

The chromosomal features that influence retroviral integration site selection are not well understood. Here, we report the mapping of 226 avian sarcoma virus (ASV) integration sites in the human genome. The results show that the sites are distributed over all chromosomes, and no global bias for integration site selection was detected. However, RNA polymerase II transcription units (protein-encoding genes) appear to be favored targets of ASV integration. The integration frequency within genes is similar to that previously described for murine leukemia virus but distinct from the higher frequency observed with human immunodeficiency virus type 1. We found no evidence for preferred ASV integration sites over the length of genes and immediate flanking regions. Microarray analysis of uninfected HeLa cells revealed that the expression levels of ASV target genes were similar to the median level for all genes represented in the array. Although expressed genes were targets for integration, we found no preference for integration into highly expressed genes. Our results provide a more detailed description of the chromosomal features that may influence ASV integration and support the idea that distinct, virus-specific mechanisms mediate integration site selection. Such differences may be relevant to viral pathogenesis and provide utility in retroviral vector design.

Narusaka, Y., M. Narusaka, et al. (2003). "The cDNA microarray analysis using an Arabidopsis pad3 mutant reveals the expression profiles and classification of genes induced by Alternaria brassicicola attack." Plant Cell Physiol **44**(4): 377-87.

The hypersensitive response (HR) was induced in a wild-type Arabidopsis thaliana plant

(Columbia) (Col-wt) by inoculation with Alternaria brassicicola that causes the development of small brown necrotic lesions on the leaves. By contrast, pad3-1 mutants challenged with A. brassicicola produced spreading lesions. The cell death in pad3-1 mutants could not inhibit the pathogen growth and development, although both production of H(2)O(2) and localized cell death were similar in Col-wt and pad3-1 plants after the inoculation. The difference between Col-wt and pad3-1 plants is defense responses after the occurrence of cell death. In other words, PAD3 is necessary for defense response to A. brassicicola. Therefore, we examined the changes in the expression patterns of ca. 7,000 genes by cDNA microarray analysis after inoculation with A. brassicicola. The cDNA microarrays were also done to analyze Arabidopsis responses after treatment with signal molecules, reactive oxygen species (ROS)-inducing compounds and UV-C. The results suggested that the pad3-1 mutation altered not only the accumulation of camalexin but also the timing of expression of many defense-related genes in response to the challenge with A. brassicicola. Furthermore, the plants integrate two or more signals that act together for promoting the induction of multiple defense pathways.

Nasr, R., A. Rosenwald, et al. (2003). "Arsenic/interferon specifically reverses 2 distinct gene networks critical for the survival of HTLV-1-infected leukemic cells." Blood **101**(11): 4576-82.

Adult T-cell leukemia (ATL) is a severe chemotherapy-resistant malignancy associated with prolonged infection by the human T cell-lymphotropic virus 1 (HTLV-1) retrovirus. Although the Tax viral transactivator is clearly an oncogene, the role of its continuous expression in the maintenance of the transformed phenotype is controversial. Because arsenic trioxide (As) and interferon alpha (IFN) synergize to induce cell cycle arrest and apoptosis of ATL cells both ex vivo and in vitro, we investigated the effects of As alone and As/IFN combination on gene networks in HTLV-1-infected leukemic cells. The As/IFN combination reduced Tax expression and, accordingly, reversed the Tax-induced constitutive nuclear factor kappaB (NF-kappaB) activation. Using DNA microarray analyses, we demonstrated that As rapidly and selectively blocks the transcription of NF-kappaB-dependent genes in HTLV-1-infected cells only. Reversal of NF-kappaB activation by As alone resulted from dramatic stabilization of IkappaB-alpha and IkappaB-epsilon, independently of IkappaB kinase (IKK) activity modulation or Tax degradation. In contrast, only the As/IFN combination induced late and massive down-regulation of cell cycle-regulated genes, concomitantly with Tax degradation by the proteasome and cell death induction, indicating the importance of continuous Tax expression for ATL cell survival. These 2 successive events likely account for the potent and specific effects of the As/IFN combination in ATL.

Natarajan, K., M. S. Rajala, et al. (2003). "Corneal IL-8 expression following adenovirus infection is mediated by c-Src activation in human corneal fibroblasts." J Immunol **170**(12): 6234-43.

Emerging evidence indicates that intracellular signaling cascades mediate entry of pathogenic adenoviruses into target host cells as well as some of the undesirable inflammatory responses to adenoviral gene vectors. We found that Ad19 infection of cultured human corneal fibroblasts induced IL-8 gene transcription independently of IL-1beta, TNF-alpha, and viral gene expression, suggesting that intracellular signaling events might mediate early inflammatory events in adenovirus keratitis. Heat but not UV light inactivation of the virus abrogated the effect of infection on IL-8 mRNA and protein levels, consistent with a viral binding-mediated mechanism. The tyrosine kinase inhibitor herbimycin blocked Ad19-induced IL-8 expression. Western blot analysis revealed tyrosine phosphorylation of the functionally related kinases c-Src and extracellular signal-regulated kinase (ERK) 1/2 in corneal fibroblasts within 15 min after infection. Respective inhibitors of these kinases, PP2 and PD98059, also blocked Ad19-induced IL-8 mRNA and protein expression. Application of inhibitors to Src and ERK kinase assays suggested an upstream relationship of c-Src to ERK. Finally, DNA microarray studies performed 1 h after Ad19 or mock infection of corneal fibroblasts in the presence or absence of the Src-specific inhibitor PP2 confirmed a relationship between c-Src and IL-8 expression in Ad19-infected corneal cells. c-Src may act as a global regulator of early proinflammatory host responses to Ad19 infection of the human cornea.

Neilsen, P. O., G. A. Zimmerman, et al. (2001). "Escherichia coli Braun lipoprotein induces a lipopolysaccharide-like endotoxic response from primary human endothelial cells." J Immunol **167**(9): 5231-9.

All bacteria contain proteins in which their amino-terminal cysteine residue is modified with N-acyl S-diacylglycerol functions, and peptides and proteins bearing this modification are immunomodulatory. The major outer membrane lipoprotein of Escherichia coli, the Braun lipoprotein (BLP), is the prototypical triacylated cysteinyl-modified protein. We find it is as active as LPS in stimulating human endothelial cells to an inflammatory phenotype, and a BLP-negative mutant of E. coli was less inflammatory than its parental strain. While the lipid modification was essential, the lipidated protein was more potent than a lipid-modified peptide. BLP associates with CD14, but this interaction, unlike that with LPS, was not required to elicit endothelial cell activation. BLP stimulated endothelial cell E-selectin surface expression, IL-6 secretion, and up-regulation of the same battery of cytokine mRNAs induced by LPS. Quantitative microarray analysis of 4400 genes showed the same 30 genes were induced by BLP and LPS, and that there was near complete concordance in the level of gene induction. We conclude that the lipid modification of at least one abundant Gram-negative protein is essential for endotoxic activity, but that the protein component also influences activity. The equivalent potency of BLP and LPS, and their complete concordance in the nature and extent of endothelial cell activation show that E. coli endotoxic activity is not due to just LPS. The major outer membrane protein of E. coli is a fully active endotoxic agonist for endothelial cells.

Neiman, P. E., J. J. Grbic, et al. (2003). "Functional genomic analysis reveals distinct neoplastic phenotypes associated with c-myb mutation in the bursa of Fabricius." Oncogene **22**(7): 1073-86.

Avian retroviral integration into the c-myb locus is casually associated with the development of lymphomas in the bursa of Farbricius of chickens; these arise with a shorter latency than bursal lymphomas caused by deregulation of c-myc. This study indicates that c-myb mutation in embryonic bursal precursors leads to an oligoclonal population of developing bursal follicles, showing a variable propensity to form a novel lesion, the neoplastic follicle (NF). About half of such bursas rapidly developed lymphomas. Detection of changes in gene expression, during the development of neoplasms, was carried out by cDNA microarray analysis. The transcriptional signature of lymphomas with mutant c-myb was more limited than, and only partially shared with, those of bursal lymphomas caused by Myc or Rel oncogenes. The c-myb-associated lymphomas frequently showed overexpression of c-myc and altered expression of other genes involved in cell cycle control and proliferation-related signal transduction. Oligoclonal, NF-containing bursas lacked detectable c-myc overexpression and demonstrated a pattern of gene expression distinct from that of normal bursa and partially shared with the short-latency lymphomas. This functional genomic analysis uncovered several different pathways of lymphomagenesis by oncogenic transcription factors acting in a B-cell lineage.

Nelson, P. J., V. D. D'Agati, et al. (2003). "Amelioration of nephropathy in mice expressing HIV-1 genes by the cyclin-dependent kinase inhibitor flavopiridol." J Antimicrob Chemother **51**(4): 921-9.

Cumulative evidence suggests that human immunodeficiency virus-associated nephropathy (HIVAN), the third leading cause of end-stage renal disease in African-Americans, may respond to therapeutic strategies that interrupt HIV-1 expression in infected renal epithelium. We recently demonstrated that suppression of HIV-1 transcription in infected glomerular visceral epithelial cells by flavopiridol, a small-molecule inhibitor of the cyclin-dependent kinases required for HIV-1 promoter activity, reversed HIV-induced proliferation and dedifferentiation in vitro. To address whether flavopiridol could ameliorate HIV-induced renal disease, we utilized a well-established HIV-1 NL4-3 transgenic mouse model of HIVAN. HIV-1 proviral transgene expression in whole kidney was markedly suppressed by a 20 day treatment with flavopiridol. Following treatment, histopathological, serological and urinary indices of nephrosis were normalized in flavopiridol-treated but not in vehicle-treated transgenics. Microarray analysis showed that 82% of the dysregulated genes in HIVAN kidney were

normalized to control levels by flavopiridol, whereas continued dysregulation of most of the remaining 18% was attributable to an effect from flavopiridol alone. These results demonstrate for the first time that targeting the cyclin-dependent kinases that support HIV-1 expression can ameliorate HIV-induced disease in an animal model.

Nene, V., R. Bishop, et al. (2000). "Theileria parva genomics reveals an atypical apicomplexan genome." Int J Parasitol **30**(4): 465-74.

The discipline of genomics is setting new paradigms in research approaches to resolving problems in human and animal health. We propose to determine the genome sequence of Theileria parva, a pathogen of cattle, using the random shotgun approach pioneered at The Institute for Genomic Research (TIGR). A number of features of the T. parva genome make it particularly suitable for this approach. The G+C content of genomic DNA is about 31%, non-coding repetitive DNA constitutes less than 1% of total DNA and a framework for the 10-12 Mbp genome is available in the form of a physical map for all four chromosomes. Minisatellite sequences are the only dispersed repetitive sequences identified so far, but they are limited in distribution to 13 of 33 SfiI fragments. Telomere and sub-telomeric non-coding sequences occupy less than 10 kbp at each chromosomal end and there are only two units encoding cytoplasmic rRNAs. Three sets of distinct multicopy sequences encoding ORFs have been identified but it is not known if these are associated with expression of parasite antigenic diversity. Protein coding genes exhibit a bias in codon usage and introns when present are unusually short. Like other apicomplexan organisms, T. parva contains two extrachromosomal DNAs, a mitochondrial DNA and a plastid DNA molecule. By annotating the genome sequence, in combination with the use of microarray technology and comparative genomics, we expect to gain significant insights into unique aspects of the biology of T. parva. We believe that the data will underpin future research to aid in the identification of targets of protective CD8+ cell mediated immune responses, and parasite molecules involved in inducing reversible host leukocyte transformation and tumour-like behaviour of transformed parasitised cells.

Netzer, R., M. Krause, et al. (2004). "Roles of pyruvate kinase and malic enzyme in Corynebacterium glutamicum for growth on carbon sources requiring gluconeogenesis." Arch Microbiol **182**(5): 354-63.

In many bacteria, pyruvate kinase serves a well-defined function in glycolysis, catalyzing an ATP-generating reaction. However, its role during growth on carbon sources requiring glucoeneogenesis is less well investigated. We analyzed a defined pyruvate kinase gene (pyk) deletion mutant of Corynebacterium glutamicum, which is unable to grow on ribose as sole carbon source. Unexpectedly, the pyk deletion mutant was also unable to grow on acetate or citrate as sole carbon sources unless low amounts of pyruvate were added to the growth medium. A spontaneous suppressor mutant of the pyk deletion strain that regained the ability to grow on acetate was isolated. DNA microarray experiments revealed increased expression of the malic enzyme gene malE. The point mutation upstream of malE identified in this mutant was responsible for the loss of carbon-source-dependent regulation, as revealed by transcriptional fusion analysis. Overexpression of malE was sufficient to restore growth of the pyk deletion strain on acetate or citrate. The requirement of increased malic enzyme levels to re-route the carbon flux at the interface between glycolysis, gluconeogenesis and the tricarboxylic acid cycle in order to compensate for the absence of pyruvate kinase indicates a metabolic flux bifurcation at the metabolic node phosphoenolpyruvate. Whereas during growth of C. glutamicum on acetate or citrate most of the phosphoenolpyruvate generated from oxaloacetate is metabolized in gluconeogenesis, a fraction is converted by pyruvate kinase in the glycolytic direction to sustain proper pyruvate availability for biomass synthesis.

Newton, M. A., C. M. Kendziorski, et al. (2001). "On differential variability of expression ratios: improving statistical inference about gene expression changes from microarray data." J Comput Biol **8**(1): 37-52.

We consider the problem of inferring fold changes in gene expression from cDNA microarray

data. Standard procedures focus on the ratio of measured fluorescent intensities at each spot on the microarray, but to do so is to ignore the fact that the variation of such ratios is not constant. Estimates of gene expression changes are derived within a simple hierarchical model that accounts for measurement error and fluctuations in absolute gene expression levels. Significant gene expression changes are identified by deriving the posterior odds of change within a similar model. The methods are tested via simulation and are applied to a panel of Escherichia coli microarrays.

Ng, L. C., O. Forslund, et al. (2003). "The response of murine macrophages to infection with Yersinia pestis as revealed by DNA microarray analysis." Adv Exp Med Biol **529**: 155-60.

Macrophages play a crucial role in recognition and phagocytosis of pathogens and in the induction of response, immunity and immunopathology. A key strategy employed by numerous pathogens such as Yersinia pestis is to circumvent the immune response of the host via actively down-regulating the activation of macrophages. The study on host-pathogen interaction and gene expression is imperative for the development of alternative therapeutics. We have combined Suppression Subtractive Hybridisation (SSH), Microarray techniques, Northern blot analysis and quantitative reverse transcription coupled PCR (RT-PCR) to gain a view of differential host gene expression in response to Y. pestis-26 degrees C infection. In our study, a total of 22 different genes were identified as up-regulated in response to the Y. pestis infection. These genes include unknown EST's, cytokines, enzyme of cytokine, receptors, ligands, transcriptional factors, inhibitor of transcriptional factor, and proteins involved with cytoskeleton. More interestingly, among them are 7 genes that encode for factors known to be associated with cell cycling and cell proliferation, with 3 of them playing a role in apoptosis. Our data also indicate that macrophage cells undergo apoptosis during an infection with Y. pestis-37 degrees C, however an infection with 26 degrees C cultures results in a delayed apoptosis. The correlation between the delayed apoptosis and the up-regulation of anti-apoptotic gene is currently being studied.

Ng, R. K., C. Y. Lau, et al. (2004). "cDNA microarray analysis of early gene expression profiles associated with hepatitis B virus X protein-mediated hepatocarcinogenesis." Biochem Biophys Res Commun **322**(3): 827-35.

Chronic hepatitis B virus (HBV) infection is one of the major causes of hepatocellular carcinoma. HBV encodes an oncogenic hepatitis B virus X protein (HBx), which can transactivate host cell transcriptional machinery and mediate cellular transformation. To disclose the early genetic response in HBx-mediated transformation process, we constructed a conditional HBx-expressing hepatocyte cell line, which allows us to compare the gene expression profiles under controllable HBx induction. A cDNA microarray containing more than 8700 mouse genes and ESTs was utilized to examine the gene expression profiles. We identified 260 candidate genes and 259 ESTs which have shown aberrant expression under HBx induction. Most of them are involved in signal transduction pathway, cell cycle control, metastasis, transcriptional regulation, immune response, and metabolism. These results provide additional insight into early cellular targets of HBx, which could give us a better understanding of the function of HBx and their progressive changes during HBx-mediated hepatocarcinogenesis.

Nguyen, T. N., A. D. Ejaz, et al. (2004). "Whole-genome expression profiling of Thermotoga maritima in response to growth on sugars in a chemostat." J Bacteriol **186**(14): 4824-8.

To provide data necessary to study catabolite-linked transcriptional networks in Thermotoga maritima, we used full-genome DNA microarray analysis of global transcriptional responses to growth on glucose, lactose, and maltose in a chemostat. A much larger number of genes changed expression in cells grown on lactose than on maltose, each relative to genes expressed in cells grown on glucose. Genes encoding putative oligopeptide transporters were often coregulated with adjacent glycosidase-encoding genes. Genes encoding enzymes catalyzing NADH oxidation were up-regulated on both lactose and maltose. Genes involved in iron and sulfur metabolism were differentially expressed in response to lactose. These data help define the sets of coregulated genes and suggest possible functions for their

encoded products.

Nicholson, T. L., K. Chiu, et al. (2004). "Chlamydia trachomatis lacks an adaptive response to changes in carbon source availability." Infect Immun **72**(7): 4286-9.

Most bacteria coordinately regulate gene expression as an adaptive response to a variety of environmental changes. One key environmental cue is the carbon source necessary for central metabolism. We used microarray analysis to monitor the global transcriptional response of the obligate intracellular pathogen Chlamydia trachomatis to the presence of glycolytic and gluconeogenic carbon sources. In contrast to free-living bacteria, changing the carbon source from glucose to glutamate or alpha-ketoglutarate had little effect on the global gene transcription of C. trachomatis.

Nicholson, T. L., L. Olinger, et al. (2003). "Global stage-specific gene regulation during the developmental cycle of Chlamydia trachomatis." J Bacteriol **185**(10): 3179-89.

Distinct morphological changes associated with the complex development cycle of the obligate intracellular bacterial pathogen Chlamydia trachomatis have been historically well characterized by microscopy. A number of temporally regulated genes have been characterized previously, suggesting that the chlamydial developmental cycle is regulated at the transcriptional level. This hypothesis was tested by microarray analysis in which the entire C. trachomatis genome was analyzed, providing a comprehensive assessment of global gene regulation throughout the chlamydial developmental cycle. Seven temporally cohesive gene clusters were identified, with 22% (189 genes) of the genome differentially expressed during the developmental cycle. The correlation of these gene clusters with hallmark morphological events of the chlamydial developmental cycle suggests three global stage-specific networks of gene regulation.

Niehus, E., H. Gressmann, et al. (2004). "Genome-wide analysis of transcriptional hierarchy and feedback regulation in the flagellar system of Helicobacter pylori." Mol Microbiol **52**(4): 947-61.

The flagellar system of Helicobacter pylori, which comprises more than 40 mostly unclustered genes, is essential for colonization of the human stomach mucosa. In order to elucidate the complex transcriptional circuitry of flagellar biosynthesis in H. pylori and its link to other cell functions, mutants in regulatory genes governing flagellar biosynthesis (rpoN, flgR, flhA, flhF, HP0244) and whole-genome microarray technology were used in this study. The regulon controlled by RpoN, its activator FlgR (FleR) and the cognate histidine kinase HP0244 (FleS) was characterized on a genome-wide scale for the first time. Seven novel genes (HP1076, HP1233, HP1154/1155, HP0366/367, HP0869) were identified as belonging to RpoN-associated flagellar regulons. The hydrogenase accessory gene HP0869 was the only annotated non-flagellar gene in the RpoN regulon. Flagellar basal body components FlhA and FlhF were characterized as functional equivalents to master regulators in H. pylori, as their absence led to a general reduction of transcripts in the RpoN (class 2) and FliA (class 3) regulons, and of 24 genes newly attributed to intermediate regulons, under the control of two or more promoters. FlhA- and FlhF-dependent regulons comprised flagellar and non-flagellar genes. Transcriptome analysis revealed that negative feedback regulation of the FliA regulon was dependent on the antisigma factor FlgM. FlgM was also involved in FlhA- but not FlhF-dependent feedback control of the RpoN regulon. In contrast to other bacteria, chemotaxis and flagellar motor genes were not controlled by FliA or RpoN. A true master regulator of flagellar biosynthesis is absent in H. pylori, consistent with the essential role of flagellar motility and chemotaxis for this organism.

Nomura, S., T. Baxter, et al. (2004). "Spasmolytic polypeptide expressing metaplasia to preneoplasia in H. felis-infected mice." Gastroenterology **127**(2): 582-94.

BACKGROUND & AIMS: The emergence of oxyntic atrophy and metaplastic cell lineages in response to chronic Helicobacter pylori infection predisposes to gastric neoplasia. We have described a trefoil factor family 2 (TFF2; spasmolytic polypeptide) expressing metaplasia (SPEM) associated with

gastric neoplasia in both rodent and human fundus. To examine the relationship of SPEM to the neoplastic process in the H. felis -infected C57BL/6 mouse, we have now studied the association of SPEM-related transcripts with preneoplasia. METHODS: SPEM-related transcripts were identified by microarray analysis of amplified cRNA from SPEM, and surface mucous cells were isolated by laser capture microdissection from the same gastric sections from male C57BL/6 mice infected with H. felis for 6 months. Expression of SPEM-related transcripts was assessed by in situ hybridization and quantitative RT-PCR, as well as immunohistochemistry for prothymosin alpha. RESULTS: Eleven SPEM-related transcripts were identified as detectable only in SPEM. The expression of the SPEM-related transcripts was validated by in situ hybridization and quantitative PCR. One transcript, the noncoding RNA Xist, was only identified in SPEM cells from the infected male mice. Ten of the 11 transcripts as well as TFF2 were also expressed in regions of gastritis cystica profunda. Immunocytochemistry for one of the identified proteins, prothymosin alpha, demonstrated prominent nuclear staining in SPEM and gastritis cystica profunda. CONCLUSIONS: The expression of SPEM-related transcripts in regions of gastritis cystica profunda suggests that SPEM represents a precursor lineage for the development of dysplasia in this animal model of gastric carcinogenesis.

Nunes, L. R., Y. B. Rosato, et al. (2003). "Microarray analyses of Xylella fastidiosa provide evidence of coordinated transcription control of laterally transferred elements." Genome Res **13**(4): 570-8.

Genetically distinct strains of the plant bacterium Xylella fastidiosa (Xf) are responsible for a variety of plant diseases, accounting for severe economic damage throughout the world. Using as a reference the genome of Xf 9a5c strain, associated with citrus variegated chlorosis (CVC), we developed a microarray-based comparison involving 12 Xf isolates, providing a thorough assessment of the variation in genomic composition across the group. Our results demonstrate that Xf displays one of the largest flexible gene pools characterized to date, with several horizontally acquired elements, such as prophages, plasmids, and genomic islands (GIs), which contribute up to 18% of the final genome. Transcriptome analysis of bacteria grown under different conditions shows that most of these elements are transcriptionally active, and their expression can be influenced in a coordinated manner by environmental stimuli. Finally, evaluation of the genetic composition of these laterally transferred elements identified differences that may help to explain the adaptability of Xf strains to infect such a wide range of plant species.

Ogura, M. and T. Tanaka (2002). "Recent progress in Bacillus subtilis two-component regulation." Front Biosci **7**: d1815-24.

Two-component regulatory systems serve to control gene expression in response to environmental and physiological changes. They are widespread among a variety of organisms and most often found in prokaryotes. One of the gram-positive microorganisms Bacillus subtilis is a well-studied bacterium whose complete nucleotide sequence has been determined. Thus, it is now possible to study transcription of the whole genome with microarray analysis. In this review we summarize the recent progress in B. subtilis two-component regulatory systems by describing the known systems and those for which the function was recently assigned. Also included is an attempt to construct a partial transcriptional network involving several two-component systems. The studies described here are based on the data from traditional genetics and biochemistry, and from microarray analysis of 29 two-component systems.

Ogura, M., H. Yamaguchi, et al. (2002). "Whole-genome analysis of genes regulated by the Bacillus subtilis competence transcription factor ComK." J Bacteriol **184**(9): 2344-51.

The Bacillus subtilis competence transcription factor ComK is required for establishment of competence for genetic transformation. In an attempt to study the ComK factor further, we explored the genes regulated by ComK using the DNA microarray technique. In addition to the genes known to be dependent on ComK for expression, we found many genes or operons whose ComK dependence was not known previously. Among these genes, we confirmed the ComK dependence of 16 genes by using lacZ

fusions, and three genes were partially dependent on ComK. Transformation efficiency was significantly reduced in an smf disruption mutant, although disruption of the other ComK-dependent genes did not result in significant decreases in transformation efficiency. Nucleotide sequences similar to that of the ComK box were found for most of the newly discovered genes regulated by ComK.

Ogura, M., H. Yamaguchi, et al. (2001). "DNA microarray analysis of Bacillus subtilis DegU, ComA and PhoP regulons: an approach to comprehensive analysis of B.subtilis two-component regulatory systems." Nucleic Acids Res **29**(18): 3804-13.

We have analyzed the regulons of the Bacillus subtilis two-component regulators DegU, ComA and PhoP by using whole genome DNA microarrays. For these experiments we took the strategy that the response regulator genes were cloned downstream of an isopropyl-beta-D-thiogalactopyranoside-inducible promoter on a multicopy plasmid and expressed in disruptants of the cognate sensor kinase genes, degS, comP and phoR, respectively. The feasibility of this experimental design to detect target genes was demonstrated by the following two results. First, expression of lacZ fusions of aprE, srfA and ydhF, the target genes of DegU, ComA and PhoP, respectively, was stimulated in their cognate sensor kinase-deficient mutants upon overproduction of the regulators. Secondly, by microarray analysis most of the known target genes for the regulators were detected and, where unknown genes were found, the regulator dependency of several of them was demonstrated. As the mutants used were deficient in the kinase genes, these results show that target candidates can be detected without signal transduction. Using this experimental design, we identified many genes whose dependency on the regulators for expression had not been known. These results suggest the applicability of the strategy to the comprehensive transcription analysis of the B.subtilis two-component systems.

Oh, M. K. and J. C. Liao (2000). "DNA microarray detection of metabolic responses to protein overproduction in Escherichia coli." Metab Eng **2**(3): 201-9.

It has been commonly observed that gratuitous overexpression of proteins in Escherichia coli causes growth retardation. However, the molecular events involved in the metabolic response to the over-expression of proteins are still unclear. Here we used DNA microarray technology to characterize the changes in transcriptional patterns of selected host genes during protein overexpression. A nontoxic, soluble protein, LuxA (coded by luxA), which is the alpha-subunit of the luciferase heterodimer, was overexpressed for this purpose. A total of 132 E. coli genes, including those in the central metabolism, key biosynthetic pathways, and selected regulatory functions, were used as probes for detecting the level of mRNA transcripts in E. coli strains JM109, MC4100, and VJS676A during protein overexpression. Upon induction, these strains shared several common responses, such as the upregulation of glk and the heat shock genes as well as the downregulation of fba, ppc, atpA, and gnd. In addition, the biosynthesis genes glnA, glyA, and leuA were downregulated in all three strains. Media-dependent responses were also observed in our study. For example, many respiratory genes that were upregulated in defined media showed an opposite effect in complex media under protein-overproducing conditions. These results demonstrate that gratuitous overexpression of proteins triggers a complex global response that involves several metabolic and regulatory systems. Explanations based on either existing knowledge of global regulations such as the heat shock response and the stringent response or stoichiometric analysis without regulatory considerations cannot account for the response induced by protein overexpression.

Oh, M. K., L. Rohlin, et al. (2002). "Global expression profiling of acetate-grown Escherichia coli." J Biol Chem **277**(15): 13175-83.

This study characterized the transcript profile of Escherichia coli in acetate cultures using DNA microarray on glass slides. Glucose-grown cultures were used as a reference. At the 95% confidence level, 354 genes were up-regulated in acetate, while 370 genes were down-regulated compared with the glucose-grown culture. Generally, more metabolic genes were up-regulated in acetate than other gene groups, while genes involved in cell replication, transcription, and translation machinery tended to be

down-regulated. It appears that E. coli commits more resources to metabolism at the expense of growth when cultured in the poor carbon source. The expression profile confirms many known features in acetate metabolism such as the induction of the glyoxylate pathway, tricarboxylic acid cycle, and gluconeogenic genes. It also provided many previously unknown features, including induction of malic enzymes, ppsA, and the glycolate pathway and repression of glycolytic and glucose phosphotransferase genes in acetate. The carbon flux delivered from the malic enzymes and PpsA in acetate was further confirmed by deletion mutations. In general, the gene expression profiles qualitatively agree with the metabolic flux changes and may serve as a predictor for gene function and metabolic flux distribution.

Ohashi, Y., T. Inaoka, et al. (2003). "Expression profiling of translation-associated genes in sporulating Bacillus subtilis and consequence of sporulation by gene inactivation." Biosci Biotechnol Biochem **67**(10): 2245-53.

A DNA microarray technique was used to demonstrate global changes in the transcription pattern of translation-associated genes that encode fifty-four ribosomal proteins including a putative ribosomal gene, and eleven translation factors in sporulating B. subtilis. We found that the mRNA levels of nine genes involved in the translation system, which include the genes for three ribosomal proteins (rpmA, rpmGB, and ctc) and two translation factors (efp, and frr), were maintained at a high level at the onset of sporulation. The ypfD gene, which encodes the ribosomal protein S1 homologue, was also found to be expressed significantly during the early sporulation stage. In order to demonstrate the significance of these genes for sporulation, mutants were constructed using the pMutinT3 disruption vector. We detected an impaired sporulation in the mutants of rpmA (gene for the ribosomal protein L27), efp (elongation factor P), frr (ribosome recycling factor), and ypfD. The effect was especially pronounced in the efp mutant, sporulation of which was entirely abolished without affecting growth. The reduced expression of rpmGB (ribosomal protein L33) resulted in an impaired sporulation only at a high temperature (47 degrees C). Only the rplI mutant, which encodes the ribosomal protein L9, could not be obtained, implying that its function is essential for viability. Thus, we successfully demonstrated the significance of several translation-associated genes in sporulation by using the results of the gene expression profiling.

Ohdate, H., C. R. Lim, et al. (2003). "Impairment of the DNA binding activity of the TATA-binding protein renders the transcriptional function of Rvb2p/Tih2p, the yeast RuvB-like protein, essential for cell growth." J Biol Chem **278**(17): 14647-56.

In Saccharomyces cerevisiae, two highly conserved proteins, Rvb1p/Tih1p and Rvb2p/Tih2p, have been demonstrated to be major components of the chromatin-remodeling INO80 complex. The mammalian orthologues of these two proteins have been shown to physically associate with the TATA-binding protein (TBP) in vitro but not clearly in vivo. Here we show that yeast proteins interact with TBP under both conditions. To assess the functional importance of these interactions, we examined the effect of mutating both TIH2/RVB2 and SPT15, which encodes TBP, on yeast cell growth. Intriguingly, only those spt15 mutations that affected the ability of TBP to bind to the TATA box caused synthetic growth defects in a tih2-ts160 background. This suggests that Tih2p might be important in recruiting TBP to the promoter. A DNA microarray technique was used to identify genes differentially expressed in the tih2-ts160 strain grown at the restrictive temperature. Only 34 genes were significantly and reproducibly affected; some up-regulated and others down-regulated. We compared the transcription of several of these Tih2p target genes in both wild type and various mutant backgrounds. We found that the transcription of some genes depends on functions possessed by both Tih2p and TBP and that these functions are substantially impaired in the spt15/tih2-ts160 double mutants that confer synthetic growth defects.

Ohno, H., G. Zhu, et al. (2003). "The effects of reactive nitrogen intermediates on gene expression in Mycobacterium tuberculosis." Cell Microbiol **5**(9): 637-48.

Nitric oxide (NO) and related reactive nitrogen intermediates (RNI) are effective antimycobacterial agents and signal-transducing molecules. The present study uses microarray analysis to

examine the effects of RNI on Mycobacterium tuberculosis gene expression. A common set of 53 genes was regulated by two chemically distinct nitric oxide donors. For a subset of the RNI-inducible genes, evidence exists suggesting that they may play a role in promoting survival of the tubercle bacillus in the host. Results obtained from studies based on a murine experimental tuberculosis model involving nos2-deficient mice suggest that RNI could regulate M. tuberculosis gene expression in vivo. Finally, there is a remarkable overlap between the RNI-inducible regulon and that previously reported to be regulated by hypoxia; and both reactive nitrogen species and anaerobicity upregulate the expression of one and the same putative two-component regulatory response system. Together, the results of this study provide evidence suggesting that (i) RNI play a role in regulating M. tuberculosis gene expression in vivo; (ii) the reactive nitrogen species upregulate genes that may be conducive to the survival of the tubercle bacillus in the infected host; and (iii) RNI and hypoxia may regulate mycobacterial gene expression via overlapping signal transduction pathways.

Ohta, S., H. Fuse, et al. (2002). "DNA microarray analysis of genes involved in the process of differentiation in mouse Leydig cell line TTE1." Arch Androl **48**(3): 203-8.

A Leydig cell line, TTE1, was established from the temperature-sensitive simian virus 40 large T-antigen transgenic mice. The cells showed temperature-sensitive growth characteristics and a differentiated phenotype at a nonpermissive temperature. To identify differentially expressed genes in the process of Leydig cell differentiation, the authors carried out microarray analysis of TTE1 cells cultured at permissive and nonpermissive temperatures. The resulting fluorescence-labeled cDNAs synthesized from mRNAs were hybridized with Clontech's Atlas glass mouse 1.0 microarrays. Of the 1081 genes analyzed, the levels of 31 genes were changed, with 24 genes showing increased levels of expression and the remaining 7 genes showing decreased levels. Tie2 was the most changed transcript, with a 13.5-fold upregulation under the differentiated condition. The authors believe this to be the first report of broadscale gene expression in Leydig cell differentiation using the microarray technology. The ability to analyze broadscale gene expression in this manner provides a powerful tool for investigating the molecular mechanisms of Leydig cell functions.

Okada, T., N. Iizuka, et al. (2003). "Gene expression profile linked to p53 status in hepatitis C virus-related hepatocellular carcinoma." FEBS Lett **555**(3): 583-90.

To clarify the role of p53 in 22 hepatitis C virus (HCV)-infected hepatocellular carcinomas (HCCs), we compared the gene expression profiles of HCCs with wild-type p53 (wt-p53) (n=17) and those with mutant-type p53 (mt-p53) (n=5) by oligonucleotide microarray analysis. Among 83 p53-related genes identified by a supervised learning method, 25 were underexpressed, and 58 were overexpressed in mt-p53 HCCs compared with wt-p53 HCCs. With a computer search, we identified consensus p53-binding sequences in the 3-kb region upstream of the translation initiation site in 59 of the 83 genes, suggesting that the in vivo p53-associated transcription system is very complicated. These data will provide additional insights into p53-related pathogenesis in HCV-infected HCC.

Okinaka, Y., C. H. Yang, et al. (2002). "Microarray profiling of Erwinia chrysanthemi 3937 genes that are regulated during plant infection." Mol Plant Microbe Interact **15**(7): 619-29.

Microarray technology was used to identify genes in Erwinia chrysanthemi 3937 that are specifically up- or down-regulated in a plant host compared with growth in laboratory culture medium. Several genes were plant down-regulated, and almost all of them were homologues of well-known housekeeping genes, such as those encoding metabolic functions, oxidative phosphorylation components, and transcription or translation processes. On the other hand, almost all of the plant up-regulated genes were involved with specialized functions, including already known or new putative virulence factors, anaerobiosis, iron uptake, transporters or permeases, xenobiotic resistance, chemotaxis, and stress responses to reactive oxygen species and heat. A substantial number of the plant up-regulated genes do not appear to be directly involved in damaging the host, but are probably important in adapting the

pathogen to the host environment. We constructed insertion mutations in several of the plant up-regulated E. chrysanthemi 3937 genes. Among these, mutations of Bacillus subtilis pps1, Escherichia coli purU, and Pseudomonas aeruginosa pheC homologues reduced virulence on African violet leaves. Thus, new insights were obtained into genes important in bacterial virulence.

Orihuela, C. J., J. N. Radin, et al. (2004). "Microarray analysis of pneumococcal gene expression during invasive disease." Infect Immun **72**(10): 5582-96.

Streptococcus pneumoniae is a leading cause of invasive bacterial disease. This is the first study to examine the expression of S. pneumoniae genes in vivo by using whole-genome microarrays available from The Institute for Genomic Research. Total RNA was collected from pneumococci isolated from infected blood, infected cerebrospinal fluid, and bacteria attached to a pharyngeal epithelial cell line in vitro. Microarray analysis of pneumococcal genes expressed in these models identified body site-specific patterns of expression for virulence factors, transporters, transcription factors, translation-associated proteins, metabolism, and genes with unknown function. Contributions to virulence predicted for several unknown genes with enhanced expression in vivo were confirmed by insertion duplication mutagenesis and challenge of mice with the mutants. Finally, we cross-referenced our results with previous studies that used signature-tagged mutagenesis and differential fluorescence induction to identify genes that are potentially required by a broad range of pneumococcal strains for invasive disease.

Oshikawa, M., N. Sugano, et al. (2004). "Differential gene induction in macrophage-like human cells by two types of Porphyromonas gingivalis: a microarray study." J Oral Sci **46**(1): 9-14.

Several studies have provided clinical evidence that FimA clonal variation may contribute to the periodontopathogenicity of Porphyromonas gingivalis (P.g.). We studied the gene expression profiling of the macrophage-like human cell line U937 after infection of two types of P.g. (fimA type I; Pg-I and fimA type II; Pg-II) using microarray. Of 1088 genes examined, 394 genes were detectable. Bioinformatics algorithms were used to analyze the detectable genes. Hierarchical clustering analysis showed that gene expression patterns of Pg-II and the control (no infection) were grouped together. K-means clustering grouped 79 genes into Pg-II dominance and 88 genes into Pg-I dominance. A large number of genes related to cell signaling, extracellular communication proteins, cell receptors (by ligands), protein turnover and cell adhesion receptors/proteins were grouped into clusters of Pg-I dominance. Our results indicate that compared with Pg-I, Pg-II induces a low host response as measured by its weak induction of gene expression.

Oshima, T., H. Aiba, et al. (2002). "Transcriptome analysis of all two-component regulatory system mutants of Escherichia coli K-12." Mol Microbiol **46**(1): 281-91.

We have systematically examined the mRNA profiles of 36 two-component deletion mutants, which include all two-component regulatory systems of Escherichia coli, under a single growth condition. DNA microarray results revealed that the mutants belong to one of three groups based on their gene expression profiles in Luria-Bertani broth under aerobic conditions: (i) those with no or little change; (ii) those with significant changes; and (iii) those with drastic changes. Under these conditions, the anaeroresponsive ArcB/ArcA system, the osmoresponsive EnvZ/OmpR system and the response regulator UvrY showed the most drastic changes. Cellular functions such as flagellar synthesis and expression of the RpoS regulon were affected by multiple two-component systems. A high correlation coefficient of expression profile was found between several two-component mutants. Together, these results support the view that a network of functional interactions, such as cross-regulation, exists between different two-component systems. The compiled data are avail-able at our website (http://ecoli.aist-nara.ac.jp/xp_analysis/ 2_components).

Oshima, T., C. Wada, et al. (2002). "Genome-wide analysis of deoxyadenosine methyltransferase-mediated control of gene expression in Escherichia coli." Mol Microbiol **45**(3): 673-95.

Deoxyadenosine methyltransferase (Dam) methylates the deoxyadenine residues in 5'-GATC-3' sequences and is important in many cellular processes in Escherichia coli. We performed a computational analysis of the entire E. coli genome and confirmed that GATC sequences are distributed unevenly in regulatory regions, which suggests that Dam might regulate gene transcription. To test this, a high-density DNA microarray of 4097 E. coli genes was constructed and used to assess the gene expression profiles of the wild type and the dam-16::kam mutant strain grown under four different conditions. We also used two-dimensional electrophoretic analysis of the proteome to assess the protein profiles. The expression of a large number of genes was affected by the dam deficiency. Genes involved in aerobic respiration, stress and SOS responses, amino acid metabolism and nucleotide metabolism were expressed at higher levels in the mutant cells, especially in aerobic conditions. In contrast, transcription of genes participating in anaerobic respiration, flagella biosynthesis, chemotaxis and motility was decreased in the dam mutant strain under both aerobic and low aerobic conditions. Thus, Dam-controlled genes are involved in adjusting the metabolic and respiratory pathways and bacterial motility to suit particular environmental conditions. The promoters of most of these Dam-controlled genes were also found to contain GATC sequences that overlap with recognition sites for two global regulators, fumarate nitrate reduction (Fnr) and catabolite activator protein (CRP). We propose that Dam-mediated methylation plays an important role in the global regulation of genes, particularly those with Fnr and CRP binding sites.

Otsuka, M., H. Aizaki, et al. (2003). "Differential cellular gene expression induced by hepatitis B and C viruses." Biochem Biophys Res Commun **300**(2): 443-7.

Hepatitis B virus (HBV) is a hepatotropic virus that causes acute and chronic hepatocellular injury and hepatocellular carcinoma. To clarify how HBV proteins regulate host cellular gene expression, we used our in-house cDNA microarray and HepG2.2.15 cells, which are derived from HepG2 cells and produce all HBV proteins. Of 2304 genes investigated, several genes were differentially expressed in HepG2.2.15 cells compared with HepG2 cells. These genes included insulin-like growth factor II and alpha-fetoprotein, consistent with previous reports. Furthermore, we previously performed similar microarray analyses to clarify the effects of hepatitis C virus (HCV) proteins on host cells, using a HepG2-derivative cell line, which produces all HCV proteins. Using these two microarray results, we compared the differences in cellular gene expression induced by HBV and HCV proteins. The expression of the majority of genes investigated differed only slightly between HBV and HCV protein-producing cells. However, HBV and HCV proteins clearly regulated several genes in a reciprocal manner. Combined, these microarray results shed new light on the effects of HBV proteins on cellular gene expression and on the differences in the pathogenic activities of these two hepatitis viruses.

Otsuki, S., A. Ikeda, et al. (2003). "Novel gene encoding a Ca2+-binding protein and under hexokinase-dependent sugar regulation." Biosci Biotechnol Biochem **67**(2): 347-53.

A cDNA encoding a predicted 15-kDa protein was earlier isolated from sugar-induced genes in rice embryos (Oryza sativa L.) by cDNA microarray analysis. Here we report that this cDNA encodes a novel Ca2+-binding protein, named OsSUR1 (for Oryza sativa sugar-up-regulated-1). The recombinant OsSUR1 protein expressed in Escherichia coli had 45Ca2+-binding activity. Northern analysis showed that the OsSUR1 gene was expressed mainly in the internodes of mature plants and in embryos at an early stage of germination. Expression of the OsSUR1 gene was induced by sugars that could serve as substrates of hexokinase, but expression was not repressed by Ca2+ signaling inhibitors, calmodulin antagonists and inhibitors of protein kinase or protein phosphatase. These results suggested that Os-SUR1 gene expression was stimulated by a hexokinase-dependent pathway not mediated by Ca2+.

Palyada, K., D. Threadgill, et al. (2004). "Iron acquisition and regulation in Campylobacter jejuni." J Bacteriol **186**(14): 4714-29.

Iron affects the physiology of bacteria in two different ways: as a micronutrient for bacterial growth and as a catalyst for the formation of hydroxyl radicals. In this study, we used DNA microarrays

to identify the C. jejuni genes that have their transcript abundance affected by iron availability. The transcript levels of 647 genes were affected after the addition of iron to iron-limited C. jejuni cells. Several classes of affected genes were revealed within 15 min, including immediate-early response genes as well as those specific to iron acquisition and metabolism. In contrast, only 208 genes were differentially expressed during steady-state experiments comparing iron-rich and iron-limited growth conditions. As expected, genes annotated as being involved in either iron acquisition or oxidative stress defense were downregulated during both time course and steady-state experiments, while genes encoding proteins involved in energy metabolism were upregulated. Because the level of protein glycosylation increased with iron limitation, iron may modulate the level of C. jejuni virulence by affecting the degree of protein glycosylation. Since iron homeostasis has been shown to be Fur regulated in C. jejuni, an isogenic fur mutant was used to define the Fur regulon by transcriptome profiling. A total of 53 genes were Fur regulated, including many genes not previously associated with Fur regulation. A putative Fur binding consensus sequence was identified in the promoter region of most iron-repressed and Fur-regulated genes. Interestingly, a fur mutant was found to be significantly affected in its ability to colonize the gastrointestinal tract of chicks, highlighting the importance of iron homeostasis in vivo. Directed mutagenesis of other genes identified by the microarray analyses allowed the characterization of the ferric enterobactin receptor, previously named CfrA. Chick colonization assays indicated that mutants defective in enterobactin-mediated iron acquisition were unable to colonize the gastrointestinal tract. In addition, a mutation in a receptor (Cj0178) for an uncharacterized iron source also resulted in reduced colonization potential. Overall, this work documents the complex response of C. jejuni to iron availability, describes the genetic network between the Fur and iron regulons, and provides insight regarding the role of iron in C. jejuni colonization in vivo.

Pan, W., J. Lin, et al. (2002). "How many replicates of arrays are required to detect gene expression changes in microarray experiments? A mixture model approach." Genome Biol **3**(5): research0022.

BACKGROUND: It has been recognized that replicates of arrays (or spots) may be necessary for reliably detecting differentially expressed genes in microarray experiments. However, the often-asked question of how many replicates are required has barely been addressed in the literature. In general, the answer depends on several factors: a given magnitude of expression change, a desired statistical power (that is, probability) to detect it, a specified Type I error rate, and the statistical method being used to detect the change. Here, we discuss how to calculate the number of replicates in the context of applying a nonparametric statistical method, the normal mixture model approach, to detect changes in gene expression. RESULTS: The methodology is applied to a data set containing expression levels of 1,176 genes in rats with and without pneumococcal middle-ear infection. We illustrate how to calculate the power functions for 2, 4, 6 and 8 replicates. CONCLUSIONS: The proposed method is potentially useful in designing microarray experiments to discover differentially expressed genes. The same idea can be applied to other statistical methods.

Pan, W., J. Lin, et al. (2002). "Model-based cluster analysis of microarray gene-expression data." Genome Biol **3**(2): RESEARCH0009.

BACKGROUND: Microarray technologies are emerging as a promising tool for genomic studies. The challenge now is how to analyze the resulting large amounts of data. Clustering techniques have been widely applied in analyzing microarray gene-expression data. However, normal mixture model-based cluster analysis has not been widely used for such data, although it has a solid probabilistic foundation. Here, we introduce and illustrate its use in detecting differentially expressed genes. In particular, we do not cluster gene-expression patterns but a summary statistic, the t-statistic. RESULTS: The method is applied to a data set containing expression levels of 1,176 genes of rats with and without pneumococcal middle-ear infection. Three clusters were found, two of which contain more than 95% genes with almost no altered gene-expression levels, whereas the third one has 30 genes with more or less differential gene-expression levels. CONCLUSIONS: Our results indicate that model-based clustering of t-statistics (and

possibly other summary statistics) can be a useful statistical tool to exploit differential gene expression for microarray data.

Pan, W., J. Lin, et al. (2003). "A mixture model approach to detecting differentially expressed genes with microarray data." Funct Integr Genomics **3**(3): 117-24.

An exciting biological advancement over the past few years is the use of microarray technologies to measure simultaneously the expression levels of thousands of genes. The bottleneck now is how to extract useful information from the resulting large amounts of data. An important and common task in analyzing microarray data is to identify genes with altered expression under two experimental conditions. We propose a nonparametric statistical approach, called the mixture model method (MMM), to handle the problem when there are a small number of replicates under each experimental condition. Specifically, we propose estimating the distributions of a t -type test statistic and its null statistic using finite normal mixture models. A comparison of these two distributions by means of a likelihood ratio test, or simply using the tail distribution of the null statistic, can identify genes with significantly changed expression. Several methods are proposed to effectively control the false positives. The methodology is applied to a data set containing expression levels of 1,176 genes of rats with and without pneumococcal middle ear infection.

Pappas, C. T., J. Sram, et al. (2004). "Construction and validation of the Rhodobacter sphaeroides 2.4.1 DNA microarray: transcriptome flexibility at diverse growth modes." J Bacteriol **186**(14): 4748-58.

A high-density oligonucleotide DNA microarray, a genechip, representing the 4.6-Mb genome of the facultative phototrophic proteobacterium, Rhodobacter sphaeroides 2.4.1, was custom-designed and manufactured by Affymetrix, Santa Clara, Calif. The genechip contains probe sets for 4,292 open reading frames (ORFs), 47 rRNA and tRNA genes, and 394 intergenic regions. The probe set sequences were derived from the genome annotation generated by Oak Ridge National Laboratory after extensive revision, which was based primarily upon codon usage characteristic of this GC-rich bacterium. As a result of the revision, numerous missing ORFs were uncovered, nonexistent ORFs were deleted, and misidentified start codons were corrected. To evaluate R. sphaeroides transcriptome flexibility, expression profiles for three diverse growth modes--aerobic respiration, anaerobic respiration in the dark, and anaerobic photosynthesis--were generated. Expression levels of one-fifth to one-third of the R. sphaeroides ORFs were significantly different in cells under any two growth modes. Pathways involved in energy generation and redox balance maintenance under three growth modes were reconstructed. Expression patterns of genes involved in these pathways mirrored known functional changes, suggesting that massive changes in gene expression are the major means used by R. sphaeroides in adaptation to diverse conditions. Differential expression was observed for genes encoding putative new participants in these pathways (additional photosystem genes, duplicate NADH dehydrogenase, ATP synthases), whose functionality has yet to be investigated. The DNA microarray data correlated well with data derived from quantitative reverse transcription-PCR, as well as with data from the literature, thus validating the R. sphaeroides genechip as a powerful and reliable tool for studying unprecedented metabolic versatility of this bacterium.

Parish, T., D. A. Smith, et al. (2003). "The senX3-regX3 two-component regulatory system of Mycobacterium tuberculosis is required for virulence." Microbiology **149**(Pt 6): 1423-35.

Two-component regulatory systems have been widely implicated in bacterial virulence. To investigate the role of one such system in Mycobacterium tuberculosis, a strain was constructed in which the senX3-regX3 system was deleted by homologous recombination. The mutant strain (Tame15) showed a growth defect after infection of macrophages and was attenuated in both immunodeficient and immunocompetent mice. Competitive hybridization of total RNA from the wild-type and mutant strains to a whole-genome microarray was used to identify changes in gene expression resulting from the deletion. One operon was highly up-regulated in the mutant, indicating that regX3 probably has a role as a

repressor of this operon. Other genes which were up- or down-regulated were also identified. Many of the genes showing down-regulation are involved in normal growth of the bacterium, indicating that the mutant strain is subject to some type of growth slow-down or stress. Genes showing differential expression were further grouped according to their pattern of gene expression under other stress conditions. From this analysis 50 genes were identified which are the most likely to be controlled by RegX3. Most of these genes are of unknown function and no obvious motifs were found upstream of the genes identified. Thus, it has been demonstrated that the senX3-regX3 two-component system is involved in the virulence of M. tuberculosis and a number of genes controlled by this system have been identified.

Park, Y. H., Y. S. Joo, et al. (2004). "Characterization of lymphocyte subpopulations and major histocompatibility complex haplotypes of mastitis-resistant and susceptible cows." J Vet Sci **5**(1): 29-39.

Bovine mastitis is an infectious disease with a major economic influence on the dairy industry worldwide. Many factors such as environment, pathogen, and host affect susceptibility or resistance of an individual cow to bovine mastitis. Recently, there has been considerable interest in defining genetic and immunological markers that could be used to select for improved disease resistance. In this study we have analyzed the lymphocyte subpopulations of mastitis-resistant and susceptible cows using monoclonal antibodies specific for bovine leukocyte differentiation antigens and flow cytometry. We have also used a microarray typing technique to define the bovine leukocyte antigen (BoLA) class I and class II haplotypes associated with resistance or susceptibility to bovine mastitis. A striking finding of the present study is that susceptibility to mastitis was associated with major histocompatibility complex (MHC) haplotypes that have only a single set of DQ genes. The study also revealed that susceptible cows had CD4:CD8 ratios of less than one in both their mammary gland secretions and peripheral blood. These results raise the possibility that the number of DQ genes that a cow has and/or a cow's CD4:CD8 ratio could be used as indicators of susceptibility to bovine mastitis.

Patel, C. A., M. Mukhtar, et al. (2002). "Lentiviral expression of HIV-1 Vpr induces apoptosis in human neurons." J Neurovirol **8**(2): 86-99.

Our recent studies have demonstrated that extracellular, recombinant human immunodeficiency virus type I (HIV-1) Vpr protein is highly neurotoxic in the microenvironment of differentiated mature human neurons and undifferentiated neuronal precursors. Although most of the direct neurotoxic effects of HIV-1 have been attributed previously to the envelope gene product, gp120, and the Tat regulatory protein, it was demonstrated that Vpr protein caused apoptosis comparable to that induced by gp120 protein in a dose-dependent manner in the neuronal system. Having observed the neurocytopathic effects of extracellular Vpr protein previously, the effects of virally expressed Vpr on nondividing, terminally differentiated human neurons were investigated. An HIV-1-based three-plasmid expression vector system was utilized to study the effects of intracellularly expressed Vpr. These virion preparations were then used to transduce neurons generated from the human neuronal precursor NT2 cell-line. Intracellularly expressed Vpr induced apoptosis within terminally differentiated neurons, as demonstrated by TUNEL assays. Additionally, virions lacking Vpr expression did not significantly induce apoptosis within these neurons. These results suggest that HIV-1 Vpr may also be leading directly to selective neurotoxicity through intracellular expression. Furthermore, human apoptosis gene microarray comparisons exhibited an up-regulation of Bcl-2-related mRNA, as well as other apoptosis genes involved in the mitochondrial apoptotic pathway, for the Vpr-transduced neuronal cells, when compared to Vpr-negative controls. Thus, Vpr delivered intracellularly, as well as extracellularly, is involved in the induction of significant neuronal apoptosis and may be one of the molecular mechanisms in HIV-1-induced encephalopathy.

Patel, V. A., M. J. Dunn, et al. (2002). "Regulation of MDR-1 (P-glycoprotein) by cyclooxygenase-2." J Biol Chem **277**(41): 38915-20.

Cyclooxygenase-2 (Cox-2), an inducible form of the enzyme that catalyzes the first step in the synthesis of prostanoids, has been shown to be overexpressed in a wide range of tumors and possesses

proangiogenic and antiapoptotic properties. To understand the molecular mechanism of Cox-2 action we used adenovirus-mediated transfer of rat Cox-2 cDNA into renal rat mesangial cells and determined the differential gene expression using cDNA microarrays. One of the several genes that were highly up-regulated by over expressed Cox-2 was MDR1. MDR1 or P-glycoprotein (P-gp), the product of the MDR1 gene, is implicated as the primary cause of multidrug resistance (MDR) in tumors where it acts as an efflux pump for chemotherapeutic agents. It is also expressed in normal tissues of the liver and kidney where it functions to actively transport lipophilic xenobiotics. Reverse transcriptase-PCR analysis confirmed the results of the microarray, showing increased mRNA levels for MDR1 in Cox-2 overexpressing cells. This increase in mRNA translated to an increase in MDR1 protein expression, which was dose-dependent on Cox-2 expression. Furthermore, using rhodamine 123 efflux assay we observed a significant increase in P-gp activity in Cox-2 overexpressing renal mesangial cells. The specific Cox-2 inhibitor NS398 was able to block the Cox-2-mediated increase in MDR1 expression and activity, suggesting that Cox-2 products may be implicated in this response. These results prove the existence of a causal link between Cox-2 and P-gp activity, which would have implications for kidney function and multidrug resistance in tumors where Cox-2 is overexpressed.

Paustian, M. L., B. J. May, et al. (2001). "Pasteurella multocida gene expression in response to iron limitation." Infect Immun **69**(6): 4109-15.

Pasteurella multocida is the causative agent of a wide range of diseases in avian and mammalian hosts. Gene expression in response to low iron conditions was analyzed in P. multocida using whole-genome microarrays. The analysis shows that the expression of genes involved in energy metabolism and electron transport generally decreased 2.1- to 6-fold while that of genes used for iron binding and transport increased 2.1- to 7.7-fold in P. multocida during the first 2 h of growth under iron-limiting conditions compared with controls. Notably, 27% of the genes with significantly altered expression had no known function, illustrating the limitations of using publicly available databases to identify genes involved in microbial metabolism and pathogenesis. Taken together, the results of our investigations demonstrate the utility of whole-genome microarray analyses for the identification of genes with altered expression profiles during varying growth conditions and provide a framework for the detailed analysis of the molecular mechanisms of iron acquisition and metabolism in P. multocida and other gram-negative bacteria.

Pavlickova, P., A. Knappik, et al. (2003). "Microarray of recombinant antibodies using a streptavidin sensor surface self-assembled onto a gold layer." Biotechniques **34**(1): 124-30.

We have developed a sensitive method for the detection of recombinant antibody-antigen interactions in a microarray format. The biochip sensor platform used in this study is based on an oriented streptavidin monolayer that provides a biological interface with well-defined surface architecture that dramatically reduces nonspecific binding interactions. All the antibody or antigen probes were biotinylated and coupled onto streptavidin-coated biochip surfaces (1 microL total volume). The detection limits for the immobilized probes on the microarray surface were 0.5 microgram/mL (200 fmol/spot) for the peptide antigen and 0.1 microgram/mL (3 fmol/spot) for the recombinant antibodies. Optimal concentrations for the detection of the Cy5-labeled protein target were in the range of 20 micrograms/mL. Protein microchips were used to measure antibody-antigen kinetics, to find optimal temperature conditions, and to establish the shelf life of recombinant antibodies immobilized on the streptavidin surface. For recombinant antibody fragments with a kDa of 10-100 nM, we have established an easy and direct immunoassay. In addition, we developed an indirect method for antibody detection with no need for expensive and time-consuming antibody purifications and modifications. Such a method was shown to be useful for large-scale screening of recombinant antibody fragments directly after their functional expression in bacteria. Our data demonstrate that recombinant antibody fragments are suitable components in the construction of antibody chips.

Peng, Y., Q. Kang, et al. (2003). "Transcriptional characterization of bone morphogenetic proteins (BMPs)-mediated osteogenic signaling." J Cell Biochem **90**(6): 1149-65.

Bone formation is presumably a complex and well-orchestrated process of osteoblast lineage-specific differentiation. As members of the TGFbeta superfamily, bone morphogenetic proteins (BMPs) play an important role in regulating osteoblast differentiation and subsequent bone formation. Several BMPs are able to induce de novo bone formation. Although significant progress has recently been made about the transcriptional control of osteoblast differentiation, detailed molecular events underlying the osteogenic process remain to be elucidated. In order to identify potentially important signaling mediators activated by osteogenic BMPs but not by non-osteogenic BMPs, we sought to determine the transcriptional differences between three osteogenic BMPs (i.e., BMP-2, BMP-6, and BMP-9) and two inhibitory/non-osteogenic BMPs (i.e., BMP-3 and BMP-12). Through the microarray analysis of approximately 12,000 genes in pre-osteoblast progenitor cells, we found that expression level of 203 genes (105 up-regulated and 98 down-regulated) was altered >2-fold upon osteogenic BMP stimulation. Gene ontology analysis revealed that osteogenic BMPs, but not inhibitory/non-osteogenic BMPs, activate genes involved in the proliferation of pre-osteoblast progenitor cells towards osteoblastic differentiation, and simultaneously inhibit myoblast-specific gene expression. BMP-regulated expression of the selected target genes was confirmed by RT-PCR, as well as by the CodeLink Bioarray analysis. Our findings are consistent with the notion that osteogenesis and myogenesis are two divergent processes. Further functional characterization of these downstream target genes should provide important insights into the molecular mechanisms behind BMP-mediated bone formation.

Peplies, J., F. O. Glockner, et al. (2003). "Optimization strategies for DNA microarray-based detection of bacteria with 16S rRNA-targeting oligonucleotide probes." Appl Environ Microbiol **69**(3): 1397-407.

The usability of the DNA microarray format for the specific detection of bacteria based on their 16S rRNA genes was systematically evaluated with a model system composed of six environmental strains and 20 oligonucleotide probes. Parameters such as secondary structures of the target molecules and steric hindrance were investigated to better understand the mechanisms underlying a microarray hybridization reaction, with focus on their influence on the specificity of hybridization. With adequate hybridization conditions, false-positive signals could be almost completely prevented, resulting in clear data interpretation. Among 199 potential nonspecific hybridization events, only 1 false-positive signal was observed, whereas false-negative results were more common (17 of 41). Subsequent parameter analysis revealed that this was mainly an effect of reduced accessibility of probe binding sites caused by the secondary structures of the target molecules. False-negative results could be prevented and the overall signal intensities could be adjusted by introducing a new optimization strategy called directed application of capture oligonucleotides. The small number of false-positive signals in our data set is discussed, and a general optimization approach is suggested. Our results show that, compared to standard hybridization formats such as fluorescence in situ hybridization, a large number of oligonucleotide probes with different characteristics can be applied in parallel in a highly specific way without extensive experimental effort.

Petersen, M., P. Brodersen, et al. (2000). "Arabidopsis map kinase 4 negatively regulates systemic acquired resistance." Cell **103**(7): 1111-20.

Transposon inactivation of Arabidopsis MAP kinase 4 produced the mpk4 mutant exhibiting constitutive systemic acquired resistance (SAR) including elevated salicylic acid (SA) levels, increased resistance to virulent pathogens, and constitutive pathogenesis-related gene expression shown by Northern and microarray hybridizations. MPK4 kinase activity is required to repress SAR, as an inactive MPK4 form failed to complement mpk4. Analysis of mpk4 expressing the SA hydroxylase NahG and of mpk4/npr1 double mutants indicated that SAR expression in mpk4 is dependent upon elevated SA levels but is independent of NPR1. PDF1.2 and THI2.1 gene induction by jasmonate was blocked in mpk4 expressing NahG, suggesting that MPK4 is required for jasmonic acid-responsive gene expression.

Pfister, T., H. Feng, et al. (2002). "Synchrotron radiation-induced X-ray emission to identify metal ions in preparations of purified protein." Biotechniques **32**(1): 134-6, 138, 140-1.

The suitability of synchrotron radiation-induced X-ray emission (SRIXE) for the detection and identification of metal ions in preparations of purified, soluble proteins was tested. Glutathione S-transferase fused to the proximal zinc finger motif of human transcription factor IIIA or to the cysteine-rich motif of poliovirus protein 2C was expressed in bacteria and purified by affinity chromatography. Aqueous samples containing the purified proteins were analyzed with SRIXE, and trace amounts of zinc and iron were detected. Mutation of the zinc-coordinating residues in the cysteine-rich motif of poliovirus protein 2C resulted in the loss of the zinc-binding ability. Relative quantities of metal in the protein preparations as determined by SRIXE corresponded well with the metal:protein ratios calculated by using a 4-(2-pyridylazo)resorcinol-based assay. We conclude that SRIXE is an accurate, sensitive, and simple method for the detection and identification of protein-bound metal ions in small amounts of sample. Thus, SRIXE may have wide use as a particularly effective method for rapidly determining trace metals in microarray samples.

Pfunder, M., O. Holzgang, et al. (2004). "Development of microarray-based diagnostics of voles and shrews for use in biodiversity monitoring studies, and evaluation of mitochondrial cytochrome oxidase I vs. cytochrome b as genetic markers." Mol Ecol **13**(5): 1277-86.

Molecular methods are widely used for species identification of mammals. In particular, the mitochondrial cytochrome b gene sequence has proven helpful for this purpose. Microarray technology can now open up new perspectives for biodiversity monitoring. With microarrays, many thousands of genetically based characteristics can be tested on one microscopic glass slide called a 'chip'. A 'Mammalia-Chip', for example, could include redundant diagnostic markers to unambiguously identify all European mammal species. Of broader use, and therefore economically more relevant, could be a 'Biodiversity-Chip', containing diagnostic features to distinguish key species in the taxa of bacteria, lichen, molluscs, insects, fungi, mammals, etc. An important prerequisite for any mixed-phyla chip is a standardization of methods. One of the most promising genes as a universal marker for all eukaryotes is cytochrome oxidase I. We show that cytochrome oxidase I is adequate for the discrimination of different species of voles and shrews with cluster analysis. Based on these results we present a diagnostic microarray-chip using cytochrome oxidase I sequences for the identification of three species of Sorex (Soricidae, Insectivora) and four species of Microtus (Arvicolinae, Rodentia). We conclude that cytochrome oxidase I can be used as an alternative marker to cytochrome b in a mixed-phyla chip, or both genes can be used in combination to enhance redundance and thus robustness of a specific chip including small mammals.

Philpott, D. J., D. Belaid, et al. (2002). "Reduced activation of inflammatory responses in host cells by mouse-adapted Helicobacter pylory isolates." Cell Microbiol **4**(5): 285-96.

Helicobacter pylori strains that harbour the Cag pathogenicity island (Cag PAI) induce interleukin (IL)-8 secretion in gastric epithelial cells, via the activation of NF- kappa B, and are associated with severe inflammation in humans. To investigate the influence of Cag PAI-mediated inflammatory responses on H. pylori adaptation to mice, a selection of H. pylori clinical isolates (n = 12) was cag PAI genotyped and tested in co-culture assays with AGS gastric epithelial cells, and in mouse colonization studies. Six isolates were shown to harbour a complete cag PAI and to induce NF- kappa B activation and IL-8 secretion in AGS cells. Of the eight isolates that spontaneously colonized mice, six had a cag PAI(-) genotype and did not induce pro-inflammatory responses in these cells. Mouse-to-mouse passage of the two cag PAI(+) -colonizing strains yielded host-adapted variants that infected mice with bacterial loads 100-fold higher than those of the respective parental strains (P= 0.001). These mouse-adapted variants were affected in their capacity to induce pro-inflammatory responses in host cells, yet no changes in cag PAI gene content were detected between the strains by DNA microarray analysis. This work provides evidence for in vivo selection of H. pylori bacteria with a reduced capacity to induce inflammatory

responses and suggests that such bacteria are better adapted to colonize mice.

Pierer, M., J. Rethage, et al. (2004). "Chemokine secretion of rheumatoid arthritis synovial fibroblasts stimulated by Toll-like receptor 2 ligands." J Immunol **172**(2): 1256-65.

To analyze the role of Toll-like receptors (TLR) in the pathogenesis of rheumatoid arthritis, we have assessed the effects of stimulation of cultured synovial fibroblasts by the TLR-2 ligand bacterial peptidoglycan. By using high density oligonucleotide microarray analysis we identified 74 genes that were up-regulated >2.5-fold. Fourteen CC and CXC chemokine genes were among the genes with the highest up-regulation. Quantitative real-time PCR analysis confirmed up-regulation of granulocyte chemotactic protein (GCP)-2, RANTES, monocyte chemoattractant protein (MCP)-2, IL-8, growth-related oncogene-2, and to a lesser extent, macrophage-inflammatory protein 1alpha, MCP-1, EXODUS, and CXCL-16. GCP-2, RANTES, and MCP-2 were detected in culture supernatants of synovial fibroblasts stimulated with peptidoglycan. Chemokine secretion induced by stimulation of rheumatoid arthritis synovial fibroblasts via TLR-2 was functionally relevant as demonstrated by chemotaxis assays. GCP-2 and MCP-2 expression, which have not been reported previously in rheumatoid arthritis, was demonstrated in synovial tissue sections of patients diagnosed with rheumatoid arthritis but not in those with osteoarthritis. Correspondingly, synovial fluid levels were significantly higher in patients diagnosed with rheumatoid arthritis as compared with osteoarthritis. Thus, we present evidence for an induction of chemokine secretion by activation of synovial fibroblasts via TLR-2, possibly contributing to the formation of inflammatory infiltrates characteristically found in rheumatoid arthritis joints.

Pirttila, A. M., L. M. McIntyre, et al. (2004). "Expression profile analysis of wild-type and fcc1 mutant strains of Fusarium verticillioides during fumonisin biosynthesis." Fungal Genet Biol **41**(6): 647-56.

Fusarium verticillioides produces a group of mycotoxins known as fumonisins that are associated with a variety of mycotoxicoses in humans and animals. In this study, DNA microarrays were constructed with expressed sequence tags (ESTs) from F. verticillioides. To identify genes with patterns of expression similar to the fumonisin biosynthetic (FUM) genes, the microarray was probed with labeled cDNAs originating from a wild-type strain and a fcc1 mutant grown on maize and in a defined medium adjusted to either pH 3 or pH 8. The comparative analyses revealed differential expression of genes corresponding to 116 ESTs when the fungal strains were grown on maize. Under different pH conditions, 166 ESTs were differentially expressed, and 19 ESTs were identified that displayed expression patterns similar to the FUM ESTs. These results provide candidate genes with potential roles in fumonisin biosynthesis.

Plate, J. M., K. S. Petersen, et al. (2000). "Gene expression in chronic lymphocytic leukemia B cells and changes during induction of apoptosis." Exp Hematol **28**(11): 1214-24.

Our studies in chronic lymphocytic leukemia (CLL) are directed at understanding which signals maintain viability in vivo and become lost upon removal of leukemic cells from the body, such that they immediately begin to undergo apoptosis ex vivo. In this report, we examine changes in gene expression observed between freshly isolated CLL B cells and after maintenance in vitro with and without Fludara. We compare these effects with an Epstein-Barr virus (EBV)-transformed cell line treated similarly. Kinetic effects of drug treatment on apoptosis and cell division were examined with DNA laddering, radioisotopic labeling, and flow cytometry using the fluorescent dye carboxyfluorescein diacetate succinimidyl ester. Reverse transcriptase polymerase chain reaction and hybridization blots of microarray cDNA analyses were performed to examine gene expression. We demonstrate that many genes, especially cyclin D1, were downregulated after culture of CLL cells. Anti-apoptotic genes BAG-1 and Akt2 were upregulated. The greatest positive effect with Fludara was the upregulation of JNK1. The EBV-transformed cell line was resistant to classic DNA laddering induced with Fludara. Although DNA synthesis was blocked, the EBV-transformed cell line had some ability to recover from treatment following drug washout. CLL cells express cell cycle regulatory genes that are specific for activated cells in the G(1)-S phase of the cell cycle. Growth regulatory signals are lost when the leukemic cells are

isolated from the body. Fludara enhances kinetics of apoptosis and induces expression of a gene responsive to stress that regulates expression of a kinase involved in initiation of the apoptotic pathway.

Poggioli, G. J., R. L. DeBiasi, et al. (2002). "Reovirus-induced alterations in gene expression related to cell cycle regulation." J Virol **76**(6): 2585-94.

Mammalian reovirus infection results in perturbation of host cell cycle progression. Since reovirus infection is known to activate cellular transcription factors, we investigated alterations in cell cycle-related gene expression following HEK293 cell infection by using the Affymetrix U95A microarray. Serotype 3 reovirus infection results in differential expression of 10 genes classified as encoding proteins that function at the G(1)-to-S transition, 11 genes classified as encoding proteins that function at G(2)-to-M transition, and 4 genes classified as encoding proteins that function at the mitotic spindle checkpoint. Serotype 1 reovirus infection results in differential expression of four genes classified as encoding proteins that function at the G(1)-to-S transition and three genes classified as encoding proteins that function at G(2)-to-M transition but does not alter any genes classified as encoding proteins that function at the mitotic spindle checkpoint. We have previously shown that serotype 3, but not serotype 1, reovirus infection induces a G(2)-to-M transition arrest resulting from an inhibition of cdc2 kinase activity. Of the differentially expressed genes encoding proteins regulating the G(2)-to-M transition, chk1, wee1, and GADD45 are known to inhibit cdc2 kinase activity. A hypothetical model describing serotype 3 reovirus-induced inhibition of cdc2 kinase is presented, and reovirus-induced perturbations of the G(1)-to-S, G(2)-to-M, and mitotic spindle checkpoints are discussed.

Polen, T., D. Rittmann, et al. (2003). "DNA microarray analyses of the long-term adaptive response of Escherichia coli to acetate and propionate." Appl Environ Microbiol **69**(3): 1759-74.

In its natural environment, Escherichia coli is exposed to short-chain fatty acids, such as acetic acid or propionic acid, which can be utilized as carbon sources but which inhibit growth at higher concentrations. DNA microarray experiments revealed expression changes during exponential growth on complex medium due to the presence of sodium acetate or sodium propionate at a neutral external pH. The adaptive responses to acetate and propionate were similar and involved genes in three categories. First, the RNA levels for chemotaxis and flagellum genes increased. Accordingly, the expression of chromosomal fliC'-'lacZ and flhDC'-'lacZ fusions and swimming motility increased after adaptation to acetate or propionate. Second, the expression of many genes that are involved in the uptake and utilization of carbon sources decreased, indicating some kind of catabolite repression by acetate and propionate. Third, the expression of some genes of the general stress response increased, but the increases were more pronounced after short-term exposure for this response than for the adaptive response. Adaptation to propionate but not to acetate involved increased expression of threonine and isoleucine biosynthetic genes. The gene expression changes after adaptation to acetate or propionate were not caused solely by uncoupling or osmotic effects but represented specific characteristics of the long-term response of E. coli to either compound.

Polen, T. and V. F. Wendisch (2004). "Genomewide expression analysis in amino acid-producing bacteria using DNA microarrays." Appl Biochem Biotechnol **118**(1-3): 215-32.

DNA microarray technology has become an important research tool for biotechnology and microbiology. It is now possible to characterize genetic diversity and gene expression in a genomewide manner. DNA microarrays have been applied extensively to study the biology of many bacteria including Escherichia coli, but only recently have they been developed for the Gram-positive Corynebacterium glutamicum. Both bacteria are widely used for biotechnological amino acid production. In this article, in addition to the design and generation of microarrays as well as their use in hybridization experiments and subsequent data analysis, we describe recent applications of DNA microarray technology regarding amino acid production in C. glutamicum and E. coli. We also discuss the impact of functional genomics studies on fundamental as well as applied aspects of amino acid production with C. glutamicum and E. coli.

Politi, K., M. Szabolcs, et al. (2004). "A mouse model of uterine leiomyosarcoma." Am J Pathol **164**(1): 325-36.

We are using an approach that is based on the cre/loxP recombination process and involves a binary system of Cre-producing and Cre-responding transgenic mice to achieve ubiquitous or tissue-specific expression of oncoproteins. To develop mouse models of tumorigenesis, Cre-producers are mated with responder animals carrying a dormant oncogene targeted into the 3' untranslated region of the locus encoding cytoplasmic beta-actin (actin cassette). Production of oncoprotein from a bicistronic message is accomplished in bitransgenic progeny by Cre-mediated excision of a segment flanked by loxP sites that is located upstream from the oncogenic sequence. Widespread Cre-dependent activation and expression of an actin-cassette transgene encoding the T antigens of the SV40 early region (SVER) commencing in embryos was compatible with normal development and did not impair viability. However, at approximately 3 months of age, all female animals developed massive uterine leiomyosarcomas, whereas practically all males exhibited enormously enlarged seminal vesicles because of pronounced hyperplasia of the smooth muscle layers. In addition, because of smooth muscle hyperproliferation, marked dilation of the gallbladder was observed in mice of both sexes. To begin exploring aberrant signaling events in the SVER-triggered tumorigenic pathways, we analyzed the expression profile of leiomyosarcomas by DNA microarray analysis.

Poly, F., D. Threadgill, et al. (2004). "Identification of Campylobacter jejuni ATCC 43431-specific genes by whole microbial genome comparisons." J Bacteriol **186**(14): 4781-95.

This study describes a novel approach to identify unique genomic DNA sequences from the unsequenced strain C. jejuni ATCC 43431 by comparison with the sequenced strain C. jejuni NCTC 11168. A shotgun DNA microarray was constructed by arraying 9,600 individual DNA fragments from a C. jejuni ATCC 43431 genomic library onto a glass slide. DNA fragments unique to C. jejuni ATCC 43431 were identified by competitive hybridization to the array with genomic DNA of C. jejuni NCTC 11168. The plasmids containing unique DNA fragments were sequenced, allowing the identification of up to 130 complete and incomplete genes. Potential biological roles were assigned to 66% of the unique open reading frames. The mean G+C content of these unique genes (26%) differs significantly from the G+C content of the entire C. jejuni genome (30.6%). This suggests that they may have been acquired through horizontal gene transfer from an organism with a G+C content lower than that of C. jejuni. Because the two C. jejuni strains differ by Penner serotype, a large proportion of the unique ATCC 43431 genes encode proteins involved in lipooligosaccharide and capsular biosynthesis, as expected. Several unique open reading frames encode enzymes which may contribute to genetic variability, i.e., restriction-modification systems and integrases. Interestingly, many of the unique C. jejuni ATCC 43431 genes show identity with a possible pathogenicity island from Helicobacter hepaticus and components of a potential type IV secretion system. In conclusion, this study provides a valuable resource to further investigate Campylobacter diversity and pathogenesis.

Pomati, F., B. P. Burns, et al. (2004). "Identification of an Na(+)-dependent transporter associated with saxitoxin-producing strains of the cyanobacterium Anabaena circinalis." Appl Environ Microbiol **70**(8): 4711-9.

Blooms of the freshwater cyanobacterium Anabaena circinalis are recognized as an important health risk worldwide due to the production of a range of toxins such as saxitoxin (STX) and its derivatives. In this study we used HIP1 octameric-palindrome repeated-sequence PCR to compare the genomic structure of phylogenetically similar Australian isolates of A. circinalis. STX-producing and nontoxic cyanobacterial strains showed different HIP1 (highly iterated octameric palindrome 1) DNA patterns, and characteristic interrepeat amplicons for each group were identified. Suppression subtractive hybridization (SSH) was performed using HIP1 PCR-generated libraries to further identify toxic-strain-specific genes. An STX-producing strain and a nontoxic strain of A. circinalis were chosen as testers in two distinct experiments. The two categories of SSH putative tester-specific sequences were characterized

by different families of encoded proteins that may be representative of the differences in metabolism between STX-producing and nontoxic A. circinalis strains. DNA-microarray hybridization and genomic screening revealed a toxic-strain-specific HIP1 fragment coding for a putative Na(+)-dependent transporter. Analysis of this gene demonstrated analogy to the mrpF gene of Bacillus subtilis, whose encoded protein is involved in Na(+)-specific pH homeostasis. The application of this gene as a molecular probe in laboratory and environmental screening for STX-producing A. circinalis strains was demonstrated. The possible role of this putative Na(+)-dependent transporter in the toxic cyanobacterial phenotype is also discussed, in light of recent physiological studies of STX-producing cyanobacteria.

Pomati, F. and B. A. Neilan (2004). "PCR-based positive hybridization to detect genomic diversity associated with bacterial secondary metabolism." Nucleic Acids Res **32**(1): e7.

A PCR-based positive hybridization (PPH) method was developed to explore toxic-specific genes in common between toxigenic strains of Anabaena circinalis, a cyanobacterium able to produce saxitoxin (STX). The PPH technique is based on the same principles of suppression subtractive hybridization (SSH), although with the former no driver DNA is required and two tester genomic DNAs are hybridized at high stringency. The aim was to obtain genes associated with cyanobacterial STX production. The genetic diversity within phylogenetically similar strains of A.circinalis was investigated by comparing the results of the standard SSH protocol to the PPH approach by DNA-microarray analysis. SSH allowed the recovery of DNA libraries that were mainly specific for each of the two STX-producing strains used. Several candidate sequences were found by PPH to be in common between both the STX-producing testers. The PPH technique performed using unsubtracted genomic libraries proved to be a powerful tool to identify DNA sequences possibly transferred laterally between two cyanobacterial strains that may be candidate(s) in STX biosynthesis. The approach presented in this study represents a novel and valid tool to study the genetic basis for secondary metabolite production in microorganisms.

Porwollik, S., J. Frye, et al. (2003). "A non-redundant microarray of genes for two related bacteria." Nucleic Acids Res **31**(7): 1869-76.

A microarray with sequences from the annotated open reading frames (ORFs) in Salmonella enterica subspecies 1, serovar Typhimurium was supplemented with annotated chromosomal ORFs from serovar Typhi that are divergent from Typhimurium (>10% DNA sequence divergence). This non-redundant array was used to (i) measure changes in gene copy number in DNA from actively growing versus stationary Typhi and (ii) to reveal the transcriptional response of Typhi to peroxide, a stress similar to that experienced when they are phagocytosed by macrophages. In S.enterica subspecies 1, pairs of genomes differ in the presence or absence of approximately 10% of their genes. An array twice the size of that needed to cover all ORFs for one genome could carry close homologs of all the ORFs for 10 genomes. Non-redundant DNA arrays could be constructed for any group of closely related organisms that differ by the presence and absence of a few genes.

Porwollik, S., R. M. Wong, et al. (2004). "DNA amplification and rearrangements in archival Salmonella enterica serovar Typhimurium LT2 cultures." J Bacteriol **186**(6): 1678-82.

Variations in genome size and gene order were observed in archival Salmonella enterica serovar Typhimurium cultures stored for over 40 years. In one strain, microarray analysis revealed a large, stable amplification. PCR analysis of the same strain revealed a genomic duplication that underwent a translocation. Other strains had smaller duplications and deletions. These results demonstrate that storage in stabs over time at room temperature not only allows for further bacterial growth but also may produce an environment that selects for a variety of mutations, including genomic rearrangements.

Porwollik, S., R. M. Wong, et al. (2002). "Evolutionary genomics of Salmonella: gene acquisitions revealed by microarray analysis." Proc Natl Acad Sci U S A **99**(13): 8956-61.

The presence of homologues of Salmonella enterica sv. Typhimurium LT2 genes was assessed in

22 other Salmonella including members of all seven subspecies and Salmonella bongori. Genomes were hybridized to a microarray of over 97% of the 4,596 annotated ORFs in the LT2 genome. A phylogenetic tree based on homologue content, relative to LT2, was largely concordant with previous studies using sequence information from several loci. Based on the topology of this tree, homologues of genes in LT2 acquired by various clades were predicted including 513 homologues acquired by the ancestor of all Salmonella, 111 acquired by S. enterica, 105 by diphasic Salmonella, and 216 by subspecies 1, most of which are of unknown function. Because this subspecies is responsible for almost all Salmonella infections of mammals and birds, these genes will be of particular interest for further mechanistic studies. Overall, a high level of gene gain, loss, or rapid divergence was predicted along all lineages. For example, at least 425 close homologues of LT2 genes may have been laterally transferred into Salmonella and then between Salmonella lineages.

Porwollik, S., R. M. Wong, et al. (2001). "The DeltauvrB mutations in the Ames strains of Salmonella span 15 to 119 genes." Mutat Res **483**(1-2): 1-11.

The DeltauvrB mutations present in strains of Salmonella enterica Typhimurium used commonly in the Salmonella (Ames) mutagenicity assay were isolated independently for at least five different his mutants. These deletions all involved the galactose operon, biotin operon, nucleotide-excision-repair uvrB gene, and chlorate-resistance genes. Beyond this, the size of the deletions and the number and type of genes deleted have remained unknown for nearly 30 years. Here, we have used genomic hybridization to a Typhimurium microarray to characterize these five DeltauvrB deletions. The number of genes (and amount of DNA) deleted due to the DeltauvrB mutations are 15 (16kb) each in TA97 and TA104, 47 (50kb) in TA100, 87 (96kb) in TA1537, and 119 (125kb) in TA98, accounting for 0.3, 0.3, 1.0, 1.9, and 2.6% of the genome, respectively. In addition, TA97 and TA104 contain an identical three-gene deletion elsewhere in their genomes, and, most remarkably, TA104 contains a 282-gene amplification, representing 7% of the genome. Missing genes include mfdA and mdaA, encoding a multi-drug translocase and a major nitroreductase, respectively, both absent in TA98; dps, encoding a DNA-binding protein absent in TA1537 and TA98; and dinG, encoding a lexA-regulated repair enzyme, absent in three DeltauvrB lineages. Genes involved in molybdenum cofactor biosynthesis and a number of ORFs of unknown functions are missing in all DeltauvrB strains investigated. Studies in DeltauvrB strains of Escherichia coli have found that the enhanced mutagenesis of some base analogues was due to the deletion of genes involved in molybdenum cofactor biosynthesis rather than to deletion of uvrB. These discoveries do not diminish the value of the data generated in the Ames strains. However, absence of genes other than uvrB may account for the enhanced mutagenicity of some compounds in DeltauvrB Ames strains. In general, microarrays will be useful for characterizing the extent and nature of deletion and amplification mutations.

Postier, B. L., H. L. Wang, et al. (2003). "The construction and use of bacterial DNA microarrays based on an optimized two-stage PCR strategy." BMC Genomics **4**(1): 23.

BACKGROUND: DNA microarrays are a powerful tool with important applications such as global gene expression profiling. Construction of bacterial DNA microarrays from genomic sequence data using a two-stage PCR amplification approach for the production of arrayed DNA is attractive because it allows, in principal, the continued re-amplification of DNA fragments and facilitates further utilization of the DNA fragments for additional uses (e.g. over-expression of protein). We describe the successful construction and use of DNA microarrays by the two-stage amplification approach and discuss the technical challenges that were met and resolved during the project. RESULTS: Chimeric primers that contained both gene-specific and shared, universal sequence allowed the two-stage amplification of the 3,168 genes identified on the genome of Synechocystis sp. PCC6803, an important prokaryotic model organism for the study of oxygenic photosynthesis. The gene-specific component of the primer was of variable length to maintain uniform annealing temperatures during the 1st round of PCR synthesis, and situated to preserve full-length ORFs. Genes were truncated at 2 kb for efficient amplification, so that

about 92% of the PCR fragments were full-length genes. The two-stage amplification had the additional advantage of normalizing the yield of PCR products and this improved the uniformity of DNA features robotically deposited onto the microarray surface. We also describe the techniques utilized to optimize hybridization conditions and signal-to-noise ratio of the transcription profile. The inter-lab transportability was demonstrated by the virtual error-free amplification of the entire genome complement of 3,168 genes using the universal primers in partner labs. The printed slides have been successfully used to identify differentially expressed genes in response to a number of environmental conditions, including salt stress. CONCLUSIONS: The technique detailed here minimizes the cost and effort to replicate a PCR-generated DNA gene fragment library and facilitates several downstream processes (e.g. directional cloning of fragments and gene expression as affinity-tagged fusion proteins) beyond the primary objective of producing DNA microarrays for global gene expression profiling.

Presti, R. M., D. L. Popkin, et al. (2001). "Novel cell type-specific antiviral mechanism of interferon gamma action in macrophages." J Exp Med **193**(4): 483-96.

Interferon (IFN)-gamma and macrophages (Mphi) play key roles in acute, persistent, and latent murine cytomegalovirus (MCMV) infection. IFN-gamma mechanisms were compared in embryonic fibroblasts (MEFs) and bone marrow Mphi (BMMphi). IFN-gamma inhibited MCMV replication in a signal transducer and activator of transcription (STAT)-1alpha-dependent manner much more effectively in BMMphi (approximately 100-fold) than MEF (5-10-fold). Although initial STAT-1alpha activation by IFN-gamma was equivalent in MEF and BMMphi, microarray analysis demonstrated that IFN-gamma regulates different sets of genes in BMMphi compared with MEFs. IFN-gamma inhibition of MCMV growth was independent of known mechanisms involving IFN-alpha/beta, tumor necrosis factor alpha, inducible nitric oxide synthase, protein kinase RNA activated (PKR), RNaseL, and Mx1, and did not involve IFN-gamma-induced soluble mediators. To characterize this novel mechanism, we identified the viral targets of IFN-gamma action, which differed in MEF and BMMphi. In BMMphi, IFN-gamma reduced immediate early 1 (IE1) mRNA during the first 3 h of infection, and significantly reduced IE1 protein expression for 96 h. Effects of IFN-gamma on IE1 protein expression were independent of RNaseL and PKR. In contrast, IFN-gamma had no significant effects on IE1 protein or mRNA expression in MEFs, but did decrease late gene mRNA expression. These studies in primary cells define a novel mechanism of IFN-gamma action restricted to Mphi, a cell type key for MCMV pathogenesis and latency.

Prouty, A. M., I. E. Brodsky, et al. (2004). "Bile-salt-mediated induction of antimicrobial and bile resistance in Salmonella typhimurium." Microbiology **150**(Pt 4): 775-83.

By DNA microarray, the Salmonella typhimurium marRAB operon was identified as being bile-activated. Transcriptional assays confirm that marRAB is activated in the presence of bile and that this response is concentration-dependent. The bile salt deoxycholate is alone able to activate transcription, while there was no response in the presence of other bile salts tested or a non-ionic detergent. Deoxycholate is able to interact with MarR and interfere with its ability to bind to the mar operator. In addition, incubation of salmonellae in the presence of sublethal concentrations of bile is able to enhance resistance to chloramphenicol and bile, by means of both mar-dependent and mar-independent pathways. To further characterize putative marRAB-regulated genes that may be important for the resistance phenotype, acrAB, which encodes an efflux pump, was analysed. In S. typhimurium, acrAB is required for bile resistance, but while transcription of acrAB is activated by bile, this activation is independent of marRAB, as well as Rob, RpoS or PhoP-PhoQ. These data suggest that bile interacts with salmonellae to increase resistance to bile and other antimicrobials and that this can occur by marRAB- and acrAB-dependent pathways that function independently with respect to bile activation.

Prouty, A. M., I. E. Brodsky, et al. (2004). "Transcriptional regulation of Salmonella enterica serovar Typhimurium genes by bile." FEMS Immunol Med Microbiol **41**(2): 177-85.

DNA microarrays and two-dimensional (2-D) gel electrophoresis were utilized to analyze the

global effect of bile on transcription and protein synthesis in Salmonella enterica serovar Typhimurium. Two bile-regulated proteins, YciF and PagC, were identified by 2-D gel electrophoresis and mass spectrometry fingerprinting. The operon yciGFE-katN demonstrated increased transcriptional activity in the presence of bile. While this operon has previously been shown to be RpoS-regulated, data from this study suggested that yciGFE-katN is regulated by bile independent of RpoS. The PhoP-PhoQ-regulated PagC is decreased in the presence of bile. Characterization of the untranslated leader of pagC demonstrated that a 97-bp region is necessary for the bile-mediated repression of this promoter. Analysis of data from the DNA microarray revealed an effect of bile on important global mechanistic pathways in S. enterica serovar Typhimurium. Genes involved in type III secretion-mediated invasion of epithelial cells demonstrated an overall repression of transcription in the presence of bile, corroborating previously reported data from this laboratory [Infect. Immun. 68 (2000) 6763]. In addition, bile-mediated transcriptional repression of genes involved in flagellar biosynthesis and motility was observed. These data further demonstrate that bile is an important environmental signal sensed by Salmonella spp. and that bile plays a role in regulating bacterial gene expression in multiple virulence-associated pathways.

Puthoff, D. P., D. Nettleton, et al. (2003). "Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles." Plant J **33**(5): 911-21.

With the availability of microarray technology, the expression profiles of thousands of genes can be monitored simultaneously to help determine the mechanisms of these biological processes. We conducted Affymetrix GeneChip microarray analyses of the Arabidopsis-cyst nematode interaction and employed a statistical procedure to analyze the resultant data, which allowed us to identify significant gene expression changes. Quantitative real-time RT-PCR assays were used to confirm the microarray analyses. The results of the expression profiling revealed 128 genes with altered steady-state mRNA levels following infection by the sugar beet cyst nematode (Heterodera schachtii; BCN), in contrast to only 12 genes that had altered expression following infection by the soybean cyst nematode (H. glycines; SCN). The expression of these 12 genes also changed following infection by BCN, i.e. we did not identify any genes regulated exclusively by SCN. The identification of 116 genes whose expression changes during successful cyst nematode parasitism by BCN suggests a potential involvement of these genes in the infection events starting with successful syncytium induction. Further characterization of these genes will permit the formulation of testable hypotheses to explain successful cyst nematode parasitism.

Pysz, M. A., D. E. Ward, et al. (2004). "Transcriptional analysis of dynamic heat-shock response by the hyperthermophilic bacterium Thermotoga maritima." Extremophiles **8**(3): 209-17.

The thermal stress response of the hyperthermophilic bacterium Thermotoga maritima was characterized using a 407-open reading frame-targeted cDNA microarray. Transient gene expression was followed for 90 min, following a shift from 80 degrees C to 90 degrees C. While some aspects of mesophilic heat-shock response were conserved in T. maritima, genome content suggested differentiating features that were borne out by transcriptional analysis. Early induction of predicted heat-shock operons hrcA-grpE-dnaJ (TM0851-TM0850-TM0849), groES-groEL (TM0505-TM0506), and dnaK-sHSP (TM0373-TM0374) was consistent with conserved CIRCE elements upstream of hrcA and groES. Induction of the T. maritima rpoE/ sigW and rpoD/ sigA homologs suggests a mechanism for global heat-shock response in the absence of an identifiable ortholog to a major heat-shock sigma factor. In contrast to heat-shock response in Escherichia coli, the majority of genes encoding ATP-dependent proteases were downregulated, including clpP (TM0695), clpQ (TM0521), clpY (TM0522), lonA (TM1633), and lonB (TM1869). Notably, T. maritima showed indications of a late heat-shock response with the induction of a marR homolog (TM0816), several other putative transcriptional regulators (TM1023, TM1069), and two alpha-glucosidases (TM0434 and TM1068). Taken together, the results reported here indicate that, while T. maritima shares core elements of the bacterial heat-shock response with mesophiles, the thermal stress regulatory strategies of this organism differ significantly. However, it remains to be elucidated whether

these differences are related to thermophilicity or phylogenetic placement.

Microarray Technology

Q-Z

Qin, Z. S., L. A. McCue, et al. (2003). "Identification of co-regulated genes through Bayesian clustering of predicted regulatory binding sites." Nat Biotechnol **21**(4): 435-9.

The identification of co-regulated genes and their transcription-factor binding sites (TFBS) are key steps toward understanding transcription regulation. In addition to effective laboratory assays, various computational approaches for the detection of TFBS in promoter regions of coexpressed genes have been developed. The availability of complete genome sequences combined with the likelihood that transcription factors and their cognate sites are often conserved during evolution has led to the development of phylogenetic footprinting. The modus operandi of this technique is to search for conserved motifs upstream of orthologous genes from closely related species. The method can identify hundreds of TFBS without prior knowledge of co-regulation or coexpression. Because many of these predicted sites are likely to be bound by the same transcription factor, motifs with similar patterns can be put into clusters so as to infer the sets of co-regulated genes, that is, the regulons. This strategy utilizes only genome sequence information and is complementary to and confirmative of gene expression data generated by microarray experiments. However, the limited data available to characterize individual binding patterns, the variation in motif alignment, motif width, and base conservation, and the lack of knowledge of the number and sizes of regulons make this inference problem difficult. We have developed a Gibbs sampling-based Bayesian motif clustering (BMC) algorithm to address these challenges. Tests on simulated data sets show that BMC produces many fewer errors than hierarchical and K-means clustering methods. The application of BMC to hundreds of predicted gamma-proteobacterial motifs correctly identified many experimentally reported regulons, inferred the existence of previously unreported members of these regulons, and suggested novel regulons.

Qu, N., U. Schittko, et al. (2004). "Consistency of Nicotiana attenuata's herbivore- and jasmonate-induced transcriptional responses in the allotetraploid species Nicotiana quadrivalvis and Nicotiana clevelandii." Plant Physiol **135**(1): 539-48.

We examined the consistency of the native diploid Nicotiana attenuata (Na)'s herbivore-induced transcriptional changes in the two allotetraploid natives, Nicotiana clevelandii (Nc) and Nicotiana quadrivalvis (Nq), which are thought to be derived from hybridizations with an ancestral Na. An analysis of nuclear-encoded chloroplast-expressed Gln synthetase gene (ncpGS) sequences found strong similarity between Nc and Na and between N. trigonophylla and the two allopolyploids. All species were elicited with methyl jasmonate (MeJA), or were wounded and treated with either water, Manduca sexta oral secretions and regurgitant (R), or the two most abundant fatty acid amino acid conjugates (F) in R to simulate herbivory. The induced transcriptional responses in all three species were compared with a cDNA microarray enriched in Na genes. Na had the fastest transcriptional responses followed by Nc and then Nq. Na's R- and F-elicited responses were more similar to those from Nq, while the MeJA- or wound-elicited responses were more consistent in Nc. Treatment of wounds with the full cocktail of elicitors found in R elicits more complex responses than does treatment with F. The species differ in their elicited JA responses, and these differences are mirrored in the expression of oxylipin genes (LOX, HPL, AOS, and alpha-DOX) and downstream JA-elicited genes (TD). Elicitation decreases the expression of growth-related genes in all three species. We propose that this is a valuable system to examine the modification of complex, polygenic, adaptive responses during allopolyploid speciation.

Raddatz, G., M. Dehio, et al. (2001). "PrimeArray: genome-scale primer design for DNA-microarray construction." Bioinformatics **17**(1): 98-9.

PrimeArray is a Windows program that computes oligonuceotide primer pairs for genome-scale gene amplification by the Polymerase Chain Reaction (PCR). The program supports the automated extraction of coding sequences (CDS) from various input-file formats and allows highly automated primer pair-optimization.

Radhakrishnan, S., J. Otte, et al. (2003). "JC virus-induced changes in cellular gene expression in primary human astrocytes." J Virol **77**(19): 10638-44.

Cell-type-specific transcription of the JC virus (JCV) promoter in glial cells initiates a series of events leading to viral replication in the brain and the development of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML) in patients with neurologic complications due to infection with human immunodeficiency virus type 1. Here we employed an in vitro infection of primary cultures of human astrocytes to compare the transcriptional profile of cellular genes after JCV infection by using an oligonucleotide-based microarray of 12600 genes. Transcription of nearly 355 genes was enhanced and expression of 130 genes was decreased to various degrees. Many transcripts that were increased upon JCV infection were found to encode proteins with properties that suggest their involvement in cell proliferation, including cyclin A and cyclin B1; signaling pathways, such as transforming growth factor beta receptor 1, platelet-derived growth factor receptor and fibroblast growth factor family receptor; and other regulatory events, such as inflammatory responses, including cyclo-oxygenase-2 (Cox-2). Microarray-based data for several cell cycle-regulatory genes were further examined by using Western blot analysis of in vitro infected astrocytes harvested early and late during the infection. Results demonstrate that protein levels of all upregulated genes were found to increase at some point during the infection time course. In parallel, immunohistochemical assessment of cell cycle proteins, including cyclins A, B1, E, and Cdk2, showed positive staining of astrocytes within PML lesions of brain tissue from patients with neuro-AIDS. Microarray analysis was found to be a useful predictor of gene expression in infected cells; however, it may not directly correlate with protein levels during infection with JCV.

Rajashekara, G., J. D. Glasner, et al. (2004). "Comparative whole-genome hybridization reveals genomic islands in Brucella species." J Bacteriol **186**(15): 5040-51.

Brucella species are responsible for brucellosis, a worldwide zoonotic disease causing abortion in domestic animals and Malta fever in humans. Based on host preference, the genus is divided into six species. Brucella abortus, B. melitensis, and B. suis are pathogenic to humans, whereas B. ovis and B. neotomae are nonpathogenic to humans and B. canis human infections are rare. Limited genome diversity exists among Brucella species. Comparison of Brucella species whole genomes is, therefore, likely to identify factors responsible for differences in host preference and virulence restriction. To facilitate such studies, we used the complete genome sequence of B. melitensis 16M, the species highly pathogenic to humans, to construct a genomic microarray. Hybridization of labeled genomic DNA from Brucella species to this microarray revealed a total of 217 open reading frames (ORFs) altered in five Brucella species analyzed. These ORFs are often found in clusters (islands) in the 16M genome. Examination of the genomic context of these islands suggests that many are horizontally acquired. Deletions of genetic content identified in Brucella species are conserved in multiple strains of the same species, and genomic islands missing in a given species are often restricted to that particular species. These findings suggest that, whereas the loss or gain of genetic material may be related to the host range and virulence restriction of certain Brucella species for humans, independent mechanisms involving gene inactivation or altered expression of virulence determinants may also contribute to these differences.

Rajpal, A., Y. A. Cho, et al. (2003). "Transcriptional activation of known and novel apoptotic pathways by Nur77 orphan steroid receptor." Embo J **22**(24): 6526-36.

Nur77 is a nuclear orphan steroid receptor that has been implicated in negative selection. Expression of Nur77 in thymocytes and cell lines leads to apoptosis through a mechanism that remains

unclear. In some cell lines, Nur77 was reported to act through a transcription-independent mechanism involving translocation to mitochondria, leading to cytochrome c release. However, we show here that Nur77-mediated apoptosis in thymocytes does not involve cytoplasmic cytochrome c release and cannot be rescued by Bcl-2. Microarray analysis shows that Nur77 induces many genes, including two novel genes (NDG1, NDG2) and known apoptotic genes FasL and TRAIL. Characterization of NDG1 and NDG2 indicates that NDG1 initiates a novel apoptotic pathway in a Bcl-2-independent manner. Thus Nur77-mediated apoptosis in T cells involves Bcl-2 independent transcriptional activation of several known and novel apoptotic pathways.

Raman, S., R. Hazra, et al. (2004). "Transcription regulation by the Mycobacterium tuberculosis alternative sigma factor SigD and its role in virulence." J Bacteriol **186**(19): 6605-16.

Mycobacterium tuberculosis, an obligate mammalian pathogen, adapts to its host during the course of infection via the regulation of gene expression. Of the regulators of transcription that play a role in this response, several alternative sigma factors of M. tuberculosis have been shown to control gene expression in response to stresses, and some of these are required for virulence or persistence in vivo. For this study, we examined the role of the alternative sigma factor SigD in M. tuberculosis gene expression and virulence. Using microarray analysis, we identified several genes whose expression was altered in a strain with a sigD deletion. A small number of these genes, including sigD itself, the gene encoding the autocrine growth factor RpfC, and a gene of unknown function, Rv1815, appear to be directly regulated by this sigma factor. By identifying the in vivo promoters of these genes, we have determined a consensus promoter sequence that is putatively recognized by SigD. The expression of several genes encoding PE-PGRS proteins, part of a large family of related genes of unknown function, was significantly increased in the sigD mutant. We found that the expression of sigD is stable throughout log phase and stationary phase but that it declines rapidly with oxygen depletion. In a mouse infection model, the sigD mutant strain was attenuated, with differences in survival and the inflammatory response in the lung between mice infected with the mutant and those infected with the wild type.

Ramarathnam, R. and S. Subramaniam (2000). "A novel microarray strategy for detecting genes and pathways in microbes with unsequenced genomes." Microb Comp Genomics **5**(3): 153-61.

Expression profile analysis of genes provides valuable information concerning the genetic response of cells to stimuli. We describe an adaptation of this technology that can be used to probe for the expression of specific families of genes in microbial species. In our method a combination of sets of oligonucleotide probes representing fingerprint sequences specific to protein families is used to identify the presence and expression levels of family homologs in a microbial cell. We demonstrate computationally, using exemplars, that when the cDNA complement from an organism is sequentially screened against a set of specific motif oligonucleotides, statistically significant information can be obtained concerning the expression of the corresponding genes. This method can be used to identify specific genes and pathways simultaneously in several organisms of interest even in the absence of sequence information from the organisms.

Ramaswamy, S. V., R. Reich, et al. (2003). "Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis." Antimicrob Agents Chemother **47**(4): 1241-50.

Isoniazid (INH) is a central component of drug regimens used worldwide to treat tuberculosis. Previous studies have identified resistance-associated mutations in katG, inhA, kasA, ndh, and the oxyR-ahpC intergenic region. DNA microarray-based experiments have shown that INH induces several genes in Mycobacterium tuberculosis that encode proteins physiologically relevant to the drug's mode of action. To gain further insight into the molecular genetic basis of INH resistance, 20 genes implicated in INH resistance were sequenced for INH resistance-associated mutations. Thirty-eight INH-monoresistant clinical isolates and 86 INH-susceptible isolates of M. tuberculosis were obtained from the Texas Department of Health and the Houston Tuberculosis Initiative. Epidemiologic independence was

established for all isolates by IS6110 restriction fragment length polymorphism analysis. Susceptible isolates were matched with resistant isolates by molecular genetic group and IS6110 profiles. Spoligotyping was done with isolates with five or fewer IS6110 copies. A major genetic group was established on the basis of the polymorphisms in katG codon 463 and gyrA codon 95. MICs were determined by the E-test. Semiquantitative catalase assays were performed with isolates with mutations in the katG gene. When the 20 genes were sequenced, it was found that 17 (44.7%) INH-resistant isolates had a single-locus, resistance-associated mutation in the katG, mabA, or Rv1772 gene. Seventeen (44.7%) INH-resistant isolates had resistance-associated mutations in two or more genes, and 76% of all INH-resistant isolates had a mutation in the katG gene. Mutations were also identified in the fadE24, Rv1592c, Rv1772, Rv0340, and iniBAC genes, recently shown by DNA-based microarray experiments to be upregulated in response to INH. In general, the MICs were higher for isolates with mutations in katG and the isolates had reduced catalase activities. The results show that a variety of single nucleotide polymorphisms in multiple genes are found exclusively in INH-resistant clinical isolates. These genes either are involved in mycolic acid biosynthesis or are overexpressed as a response to the buildup or cellular toxicity of INH.

Ramsay, G. (1998). "DNA chips: state-of-the art." Nat Biotechnol **16**(1): 40-4.

The technology and applications of microarrays of immobilized DNA or oligonucleotides are reviewed. DNA arrays are fabricated by high-speed robotics on glass or nylon substrates, for which labeled probes are used to determine complementary binding allowing massively parallel gene expression and gene discovery studies. Oligonucleotide microarrays are fabricated either by in situ light-directed combinational synthesis or by conventional synthesis followed by immobilization on glass substrates. Sample DNA is amplified by the polymerase chain reaction (PCR), and a fluorescent label is inserted and hybridized to the microarray. This technology has been successfully applied to the simultaneous expression of many thousands of genes and to large-scale gene discovery, as well as polymorphism screening and mapping of genomic DNA clones.

Rao, Z. M., H. T. Dong, et al. (2002). "[Analysis of gene expression profiles during host-Magnaporthe grisea interactions in a pair of near isogenic lines of rice]." Yi Chuan Xue Bao **29**(10): 887-93.

A pair of near isogenic lines G205 and G71 were selected from recombinant inbred lines (RIL) of Zhong156 x Gumei2. On the resistance locus Pi-25(t), G205 had the resistant allele that was from Gumei 2 while G71 had the susceptible allele that was from Zhong156. For the genetic background, different alleles were detected on only 24 loci out of the 672 RFLP or SSLP loci surveyed. The expression profiles of G205 and G71 in response to Magnaporthe grisea were investigated using cDNA microarray containing 2200 Expression Sequence Tags (ESTs). The leaves were inoculated with the pathogen for 12 hours at 4-leaf stage and 998 genes were identified in total. Three genes were up-regulated significantly by the fungus in G205 only. The functions of two genes were known but that of the third gene were unknown. The two genes encoded casein kinase II alpha subunit and retrotransponson TOS17 insertion element respectively. Other thirty-five genes had similar expression patterns between NILs. Among them, 17 genes were up-regulated while 18 genes were down-regulated by the inoculation. The functions of 33 out of the 35 genes were known. BLAST analysis showed that all thirty-five. BLAST analysis showed that all thirty-five genes with known functions were relative to defense reactions, signal transduction, stress response, photosynthesis and sugar metabolism. Northern blot confirmed that four of five differentially displayed genes randomly selected had the same expression patterns as those detected in cDNA microarray. Two of them were up-regulated genes encoding casein kinase II alpha subunit and glycine-rich protein (Grp), and the other two down-regulated genes encoding nitrilase-associated protein and 18S small subnit ribosomal RNA gene respectively. Northern blot also revealed that the expression of Grp was consistently up-regulated from 0 to 36 h after the inoculation of the fungus. These results showed that cDNA microarray was a useful tool to study the molecular mechanisms of disease resistance in plants.

Rasti, N., M. Wahlgren, et al. (2004). "Molecular aspects of malaria pathogenesis." FEMS Immunol Med Microbiol **41**(1): 9-26.

Plasmodium falciparum being the most lethal plasmodiae is still a major cause of the disease burden and mortality in malaria endemic areas. Due to the wide spread drug resistance in combination with poor socio-economic situation in the vast majority of the endemic countries, malaria is today a great global challenge. The scientific community is, however, progressing. The 23 Mb genome of P. falciparum has been decoded and publicly available. Data of transcriptional profiling at certain developmental stages have already been generated. More than 50% of P. falciparum genes are transcribed constitutively in all the developmental stages of parasite life cycle. Functional disruption of these genes might have implications for parasite growth and development. Available microarray data indicate that P. falciparum preferentially expresses rif and stevor gene families at gametocyte and sporozoite stages while var genes are predominantly expressed at the erythrocytic stage. Gene regulation mechanisms of the variant gene families in P. falciparum are still not understood though some regulatory elements have been proposed. The occurrence of severe malaria is determined by both parasite and human host factors. Sequestration and antigenic variation are two of the evasion mechanisms utilized by P. falciparum in order to escape the human host defences. Understanding the molecular mechanisms underlying these phenomena is of a major importance and interest in malaria research. Here, we summarize and highlight the recent progress in molecular aspects of severe malaria.

Rawls, J. F., B. S. Samuel, et al. (2004). "Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota." Proc Natl Acad Sci U S A **101**(13): 4596-601.

Animals have developed the means for supporting complex and dynamic consortia of microorganisms during their life cycle. A transcendent view of vertebrate biology therefore requires an understanding of the contributions of these indigenous microbial communities to host development and adult physiology. These contributions are most obvious in the gut, where studies of gnotobiotic mice have disclosed that the microbiota affects a wide range of biological processes, including nutrient processing and absorption, development of the mucosal immune system, angiogenesis, and epithelial renewal. The zebrafish (Danio rerio) provides an opportunity to investigate the molecular mechanisms underlying these interactions through genetic and chemical screens that take advantage of its transparency during larval and juvenile stages. Therefore, we developed methods for producing and rearing germ-free zebrafish through late juvenile stages. DNA microarray comparisons of gene expression in the digestive tracts of 6 days post fertilization germ-free, conventionalized, and conventionally raised zebrafish revealed 212 genes regulated by the microbiota, and 59 responses that are conserved in the mouse intestine, including those involved in stimulation of epithelial proliferation, promotion of nutrient metabolism, and innate immune responses. The microbial ecology of the digestive tracts of conventionally raised and conventionalized zebrafish was characterized by sequencing libraries of bacterial 16S rDNA amplicons. Colonization of germ-free zebrafish with individual members of its microbiota revealed the bacterial species specificity of selected host responses. Together, these studies establish gnotobiotic zebrafish as a useful model for dissecting the molecular foundations of host-microbial interactions in the vertebrate digestive tract.

Ray, N. and L. W. Enquist (2004). "Transcriptional response of a common permissive cell type to infection by two diverse alphaherpesviruses." J Virol **78**(7): 3489-501.

Pseudorabies virus (PRV) and herpes simplex virus type 1 (HSV-1) are distantly related alphaherpesviruses whose natural hosts are pigs and humans, respectively. Adult infections of natural hosts are mild and rarely lethal. However, both viruses are also able to infect other hosts, often with lethal effects. In this report, we use the paradigm of infection of a common permissive cell type and microarray analysis to determine if these two diverse alphaherpesviruses engage similar or different cellular pathways to obtain a common outcome: productive infection. We compared cellular gene expression in growth-arrested, primary rat embryonic fibroblasts that were mock infected or infected with either

purified PRV-Becker or HSV-1(F). Infections by either virus affect the transcription of more than 1,500 cellular genes by threefold or more. Few differences are detected early, and the majority of changes occur during the late stages of infection. Remarkably, the transcripts of about 500 genes are regulated in common, while the rest are regulated in a virus-specific manner. Genes whose expression is affected by infection fall into a diverse group of functional classes and cellular pathways. Furthermore, a comparison of the cellular response to HSV-1 infection of primary human and rat fibroblasts revealed unexpected diversity in the transcript profiles.

Raychaudhuri, S., J. M. Stuart, et al. (2000). "Pattern recognition of genomic features with microarrays: site typing of Mycobacterium tuberculosis strains." Proc Int Conf Intell Syst Mol Biol **8**: 286-95.

Mycobacterium tuberculosis (M. tb.) strains differ in the number and locations of a transposon-like insertion sequence known as IS6110. Accurate detection of this sequence can be used as a fingerprint for individual strains, but can be difficult because of noisy data. In this paper, we propose a non-parametric discriminant analysis method for predicting the locations of the IS6110 sequence from microarray data. Polymerase chain reaction extension products generated from primers specific for the insertion sequence are hybridized to a microarray containing targets corresponding to each open reading frame in M. tb. To test for insertion sites, we use microarray intensity values extracted from small windows of contiguous open reading frames. Rank-transformation of spot intensities and first-order differences in local windows provide enough information to reliably determine the presence of an insertion sequence. The nonparametric approach outperforms all other methods tested in this study.

Read, T. D., S. N. Peterson, et al. (2003). "The genome sequence of Bacillus anthracis Ames and comparison to closely related bacteria." Nature **423**(6935): 81-6.

Bacillus anthracis is an endospore-forming bacterium that causes inhalational anthrax. Key virulence genes are found on plasmids (extra-chromosomal, circular, double-stranded DNA molecules) pXO1 (ref. 2) and pXO2 (ref. 3). To identify additional genes that might contribute to virulence, we analysed the complete sequence of the chromosome of B. anthracis Ames (about 5.23 megabases). We found several chromosomally encoded proteins that may contribute to pathogenicity--including haemolysins, phospholipases and iron acquisition functions--and identified numerous surface proteins that might be important targets for vaccines and drugs. Almost all these putative chromosomal virulence and surface proteins have homologues in Bacillus cereus, highlighting the similarity of B. anthracis to near-neighbours that are not associated with anthrax. By performing a comparative genome hybridization of 19 B. cereus and Bacillus thuringiensis strains against a B. anthracis DNA microarray, we confirmed the general similarity of chromosomal genes among this group of close relatives. However, we found that the gene sequences of pXO1 and pXO2 were more variable between strains, suggesting plasmid mobility in the group. The complete sequence of B. anthracis is a step towards a better understanding of anthrax pathogenesis.

Reitzer, L. and B. L. Schneider (2001). "Metabolic context and possible physiological themes of sigma(54)-dependent genes in Escherichia coli." Microbiol Mol Biol Rev **65**(3): 422-44, table of contents.

Sigma(54) has several features that distinguish it from other sigma factors in Escherichia coli: it is not homologous to other sigma subunits, sigma(54)-dependent expression absolutely requires an activator, and the activator binding sites can be far from the transcription start site. A rationale for these properties has not been readily apparent, in part because of an inability to assign a common physiological function for sigma(54)-dependent genes. Surveys of sigma(54)-dependent genes from a variety of organisms suggest that the products of these genes are often involved in nitrogen assimilation; however, many are not. Such broad surveys inevitably remove the sigma(54)-dependent genes from a potentially coherent metabolic context. To address this concern, we consider the function and metabolic context of sigma(54)-dependent genes primarily from a single organism, Escherichia coli, in which a reasonably complete list of sigma(54)-dependent genes has been identified by computer analysis combined with a

DNA microarray analysis of nitrogen limitation-induced genes. E. coli appears to have approximately 30 sigma(54)-dependent operons, and about half are involved in nitrogen assimilation and metabolism. A possible physiological relationship between sigma(54)-dependent genes may be based on the fact that nitrogen assimilation consumes energy and intermediates of central metabolism. The products of the sigma(54)-dependent genes that are not involved in nitrogen metabolism may prevent depletion of metabolites and energy resources in certain environments or partially neutralize adverse conditions. Such a relationship may limit the number of physiological themes of sigma(54)-dependent genes within a single organism and may partially account for the unique features of sigma(54) and sigma(54)-dependent gene expression.

Ren, D., L. A. Bedzyk, et al. (2004). "Differential gene expression to investigate the effect of (5Z)-4-bromo- 5-(bromomethylene)-3-butyl-2(5H)-furanone on Bacillus subtilis." Appl Environ Microbiol **70**(8): 4941-9.

(5Z)-4-Bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone (furanone) from the red marine alga Delisea pulchra was found previously to inhibit the growth, swarming, and biofilm formation of gram-positive bacteria. Using the gram-positive bacterium Bacillus subtilis as a test organism, we observed cell killing by 20 microg of furanone per ml, while 5 microg of furanone per ml inhibited growth approximately twofold without killing the cells. To discover the mechanism of this inhibition on a genetic level and to investigate furanone as a novel antibiotic, full-genome DNA microarrays were used to analyze the gene expression profiles of B. subtilis grown with and without 5 microg of furanone per ml. This agent induced 92 genes more than fivefold ($P < 0.05$) and repressed 15 genes more than fivefold ($P < 0.05$). The induced genes include genes involved in stress responses (such as the class III heat shock genes clpC, clpE, and ctsR and the class I heat shock genes groES, but no class II or IV heat shock genes), fatty acid biosynthesis, lichenan degradation, transport, and metabolism, as well as 59 genes with unknown functions. The microarray results for four genes were confirmed by RNA dot blotting. Mutation of a stress response gene, clpC, caused B. subtilis to be much more sensitive to 5 microg of furanone per ml (there was no growth in 8 h, while the wild-type strain grew to the stationary phase in 8 h) and confirmed the importance of the induction of this gene as identified by the microarray analysis.

Ren, J. and J. F. Prescott (2003). "Analysis of virulence plasmid gene expression of intra-macrophage and in vitro grown Rhodococcus equi ATCC 33701." Vet Microbiol **94**(2): 167-82.

Rhodococcus equi is a soil organism that infects macrophages of foals and immunocompromised humans. Virulence in foal isolates is tightly associated with an 80kb plasmid, which includes a pathogenicity island (PI) with a virulence-associated gene family, vap. A DNA microarray containing 66 of 69 putative open reading frames (ORFs) of the virulence plasmid was developed. Virulence plasmid gene expression of R. equi grown in macrophages or under different conditions in vitro was compared against in vitro growth at 30 degrees C, pH=7. When grown in macrophages, all seven vap family genes as well as six ORFs within, but not outside, the PI were induced. Cluster analysis of the gene expression matrix assembled from different growth conditions suggested that those genes that actively responded to environmental changes divided broadly into two groups. One group, orf1, 2, 5, 6-8, 12-15, 19, and 20 (which includes all the vap genes), was induced at 37 degrees C, mostly by low iron, and to a lesser extent by the synergy of low calcium and pH=5. The second group, orf3, 9, and 10, was induced at 37 degrees C by magnesium depletion (produced by EDTA treatment of growth medium). Temperature (37 degrees C) was the most important factor inducing gene expression for the both groups. Iron restriction led to down-regulation of Group II genes and magnesium restriction led to down-regulation of Group I genes. A putative consensus IdeR binding site was identified upstream of vapA, suggesting that vapA is a member of an IdeR regulon in R. equi. Expression of genes inside macrophages was most closely but not completely mimicked by growth of bacteria at 37 degrees C, pH=5, under conditions of restricted iron, calcium and magnesium; that is, similar to environmental factors found inside macrophages.

Ren, Q., S. J. Robertson, et al. (2003). "Comparative DNA microarray analysis of host cell transcriptional responses to infection by Coxiella burnetii or Chlamydia trachomatis." Ann N Y Acad Sci **990**: 701-13.

DNA microarray analysis was conducted to investigate the transcriptional responses of the human monocytic leukemia cell line THP-1 to infection by Chlamydia trachomatis or Coxiella burnetii. RNA was isolated from mock infected cells and cells infected for 36 hours using TRIzol reagent. Biotinylated probes synthesized from RNA samples were hybridized to an Affymetrix U133A human genome chip consisting of 18,462 human gene probe sets. A total of 335 and 548 THP-1 genes were up- or downregulated at least twofold in cells infected with C. burnetii or C. trachomatis, respectively, when compared to uninfected cells. There was a high degree of overlap in transcriptional responses to infection with shared responses observed for 39 downregulated and 189 upregulated genes. Numerous pathogen-specific transcriptional responses were also observed. Quantitative RT-PCR and immunoblotting confirmed up- or down-regulation of a subset of THP-1 genes in response to infection by C. burnetii. This study provides insight into the host transcriptional responses to infection by Chlamydia and Coxiella that may affect the infectious cycle of each organism.

Revel, A. T., A. M. Talaat, et al. (2002). "DNA microarray analysis of differential gene expression in Borrelia burgdorferi, the Lyme disease spirochete." Proc Natl Acad Sci U S A **99**(3): 1562-7.

DNA microarrays were used to survey the adaptive genetic responses of Borrelia burgdorferi (Bb) B31, the Lyme disease spirochete, when grown under conditions analogous to those found in unfed ticks (UTs), fed ticks (FTs), or during mammalian host adaptation (Bb in dialysis membrane chambers implanted in rats). Microarrays contained 95.4% of the predicted B31 genes, 150 (8.6%) of which were differentially regulated (changes of > or = 1.8-fold) among the three growth conditions. A substantial proportion (46%) of the differentially regulated genes encoded proteins with predicted export signals (29% from predicted lipoproteins), emphasizing the importance to Bb of modulating its extracellular proteome. For B31 cultivated at the more restrictive UT condition, microarray data provided evidence of a bacterial stringent response and factors that restrict cell division. A large proportion of genes were responsive to the FT growth condition, wherein increased temperature and reduced pH were prominent environmental parameters. A surprising theme, supported by cluster analysis, was that many of the gene expression changes induced during the FT growth condition were transient and largely tempered as B31 adapted to the mammalian host, suggesting that once Bb gains entry and adapts to mammalian tissues, fewer differentially regulated genes are exploited. It therefore would seem that although widely dissimilar, the UT and dialysis membrane chamber growth conditions promote more static patterns of gene expression in Bb. The microarray data thus provide a basis for formulating new testable hypotheses regarding the life cycle of Bb and attaining a more complete understanding of many aspects of Bb's complex parasitic strategies.

Reyes-Lopez, M. A., A. Mendez-Tenorio, et al. (2003). "Fingerprinting of prokaryotic 16S rRNA genes using oligodeoxyribonucleotide microarrays and virtual hybridization." Nucleic Acids Res **31**(2): 779-89.

An oligonucleotide microarray hybridization system to differentiate microbial species was designed and tested. Seven microbial species were studied, including one Bacillus and six Pseudomonas strains. DNA sequences near the 5' end of 16S rRNA genes were aligned and two contiguous regions of high variability, flanked by highly conserved sequences, were found. The conserved sequences were used to design PCR primers which efficiently amplified these polymorphic regions from all seven species. The amplicon sequences were used to design 88 9mer hybridization probes which were arrayed onto glass slides. Single-stranded, fluorescence-tagged PCR products were hybridized to the microarrays at 15 degrees C. The experimental results were compared with the DeltaG(0) values for all matched and mismatched duplexes possible between the synthetic probes and the 16S target sequences of the seven test species, calculated using a 'virtual hybridization' software program. Although the observed hybridization patterns differed significantly from patterns predicted solely on the basis of perfect sequence matches, a unique hybridization fingerprint was obtained for each of the species, including closely related

Pseudomonas species, and there was a reasonable correlation between the intensity of observed hybridization signals and the calculated DeltaG(0) values. The results suggest that both perfect and mismatched pairings can contribute to microbial identification by hybridization fingerprinting.

Reymond, P., H. Weber, et al. (2000). "Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis." Plant Cell **12**(5): 707-20.

Wounding in multicellular eukaryotes results in marked changes in gene expression that contribute to tissue defense and repair. Using a cDNA microarray technique, we analyzed the timing, dynamics, and regulation of the expression of 150 genes in mechanically wounded leaves of Arabidopsis. Temporal accumulation of a group of transcripts was correlated with the appearance of oxylipin signals of the jasmonate family. Analysis of the coronatine-insensitive coi1-1 Arabidopsis mutant that is also insensitive to jasmonate allowed us to identify a large number of COI1-dependent and COI1-independent wound-inducible genes. Water stress was found to contribute to the regulation of an unexpectedly large fraction of these genes. Comparing the results of mechanical wounding with damage by feeding larvae of the cabbage butterfly (Pieris rapae) resulted in very different transcript profiles. One gene was specifically induced by insect feeding but not by wounding; moreover, there was a relative lack of water stress-induced gene expression during insect feeding. These results help reveal a feeding strategy of P. rapae that may minimize the activation of a subset of water stress-inducible, defense-related genes.

Roberts, E. S., M. A. Zandonatti, et al. (2003). "Induction of pathogenic sets of genes in macrophages and neurons in NeuroAIDS." Am J Pathol **162**(6): 2041-57.

The etiology of the central nervous system (CNS) alterations after human immunodeficiency virus (HIV) infection, such as dementia and encephalitis, remains unknown. We have used microarray analysis in a monkey model of neuroAIDS to identify 98 genes, many previously unrecognized in lentiviral CNS pathogenesis, whose expression is significantly up-regulated in the frontal lobe of simian immunodeficiency virus-infected brains. Further, through immunohistochemical illumination, distinct classes of genes were found whose protein products localized to infiltrating macrophages, endothelial cells and resident glia, such as CD163, Glut5, and ISG15. In addition we found proteins induced in cortical neurons (ie, cyclin D3, tissue transglutaminase, alpha1-antichymotrypsin, and STAT1), which have not previously been described as participating in simian immunodeficiency virus or HIV-related CNS pathology. This molecular phenotyping in the infected brains revealed pathways promoting entry of macrophages into the brain and their subsequent detrimental effects on neurons. These data support the hypothesis that in HIV-induced CNS disease products of activated macrophages and astrocytes lead to CNS dysfunction by directly damaging neurons, as well as by induction of altered gene and protein expression profiles in neurons themselves which are deleterious to their function.

Robertson, G. T., W. L. Ng, et al. (2002). "Global transcriptional analysis of clpP mutations of type 2 Streptococcus pneumoniae and their effects on physiology and virulence." J Bacteriol **184**(13): 3508-20.

Streptococcus pneumoniae is an important human pathogen that contains single copies of genes encoding the ClpP and FtsH ATP-dependent proteases but lacks the Lon and HslV proteases. We constructed and characterized the phenotypes of clpP, clpC, and clpX deletion replacement mutants, which lack the ClpP protease subunit or the putative ClpC or ClpX ATPase specificity factor. A DeltaclpP mutant, but not a DeltaclpC or DeltaclpX mutant, of the virulent D39 type 2 strain of S. pneumoniae grew poorly at 30 degrees C and failed to grow at 40 degrees C. Despite this temperature sensitivity, transcription of the heat shock regulon determined by microarray analysis was induced in a DeltaclpP mutant, which was also more sensitive to oxidative stress by H2O2 and to puromycin than its clpP+ parent strain. A DeltaclpP mutant, but not a DeltaclpC mutant, was strongly attenuated for virulence in the murine lung and sepsis infection models. All of these phenotypes were complemented in a DeltaclpP/clpP+ merodiploid strain. Consistent with these complementation patterns, clpP was found to be in a monocistronic operon, whose transcription was induced about fivefold by heat shock in S.

pneumoniae as determined by Northern and real-time reverse transcription-PCR analyses. Besides clpP, transcription of clpC, clpE, and clpL, but not clpX or ftsH, was induced by heat shock or entry into late exponential growth phase. Microarray analysis of DeltaclpP mutants showed a limited change in transcription pattern (approximately 80 genes) consistent with these phenotypes, including repression of genes involved in oxidative stress, metal ion transport, and virulence. In addition, transcription of the early and late competence regulon was induced in the DeltaclpP mutant, and competence gene expression and DNA uptake seemed to be constitutively induced throughout growth. Together, these results indicate that ClpP-mediated proteolysis plays a complex and central role in numerous pneumococcal stress responses, development of competence, and virulence.

Roep, B. O. (2003). "Molecular mimicry in autoimmune neurological disease after viral infection." Curr Med Chem **10**(19): 1939-43.

Viral infections have been associated with the development of several neurological and neuroendocrine autoimmune diseases. Structural similarities between environmental proteins and self-proteins have long been proposed to be targets for immune cross reactivity associated with initiation of autoimmune diseases. This mechanism called molecular mimicry has also been put forward for immune mediated neurological diseases associated with viral infection. Although many potential candidates for cross reactivity have been put forward, only few have been substantiated on the molecular level. For the definition of cellular immune cross-reactivity, it proved critical to appreciate that recognition patterns of T-cells are not linear. Subsequent microarray studies unequivocally demonstrated functional mimicry of seemingly disparate amino acid sequences. This review summarises the present evidence for molecular mimicry in neurological autoimmune diseases and virus

Roep, B. O., H. S. Hiemstra, et al. (2002). "Molecular mimicry in type 1 diabetes: immune cross-reactivity between islet autoantigen and human cytomegalovirus but not Coxsackie virus." Ann N Y Acad Sci **958**: 163-5.

Type 1 diabetes is caused by a T cell-mediated autoimmune destruction of the pancreatic beta cells. Molecular mimicry between viral pathogens and beta cell protein has been a popular theory to explain loss of tolerance in type 1 diabetes. However, functional data in support of this hypothesis have been lacking, presumably because the homologies were defined on the basis of linear similarities in peptide sequences, which ignores the criteria of HLA versus T cell receptor contact residues in peptide epitopes required for T cell recognition. We applied a HLA-binding dedicated peptide microarray analysis using autoreactive T cell clones specific for the autoantigen GAD65 to determine the algorithm of T cell recognition by this given T cell clone. The subsequent database search identified a 100% fit with cytomegalovirus peptide, which was subsequently shown to be recognized by these clonal T cells. However, T cell clones reactive with linear homologies previously described as putative candidates for T cell cross-reactivity between GAD65 and Coxsackie virus peptide were unable to recognize the homologous counterparts.

Rogers, P. D., J. Thornton, et al. (2003). "Pneumolysin-dependent and -independent gene expression identified by cDNA microarray analysis of THP-1 human mononuclear cells stimulated by Streptococcus pneumoniae." Infect Immun **71**(4): 2087-94.

Pneumolysin is an important virulence factor of Streptococcus pneumoniae, interacting with the membranes of host cells to elicit a multitude of inflammatory responses. We used cDNA microarrays to identify genes which are responsive to S. pneumoniae in a pneumolysin-dependent and -independent fashion. The THP-1 human monocytic cell line was coincubated for 3 h with medium alone, with the virulent type 2 S. pneumoniae strain D39, or with the isogenic strain PLN, which does not express pneumolysin. RNA was isolated from the monocytes and hybridized on cDNA microarrays. Of 4,133 genes evaluated, 142 were found to be responsive in a pneumolysin-dependent fashion, whereas 40 were found to be responsive independent of pneumolysin. Genes that were up-regulated in cells exposed to

D39 relative to those exposed to PLN included genes encoding proteins such as mannose binding lectin 1, lysozyme, alpha-1 catenin, cadherin 17, caspases 4 and 6, macrophage inflammatory protein 1beta (MIP-1beta), interleukin 8 (IL-8), monocyte chemotactic protein 3 (MCP-3), IL-2 receptor beta (IL-2Rbeta), IL-15 receptor alpha (IL-15Ralpha), interferon receptor 2, and prostaglandin E synthase. Down-regulated genes included those encoding complement component receptor 2/CD21, platelet-activating factor acetylhydrolase, and oxidized low-density lipoprotein receptor 1 (OLR1). Pneumolysin-independent responses included down-regulation of the genes encoding CD68, CD53, CD24, transforming growth factor beta2, and signal transducers and activators of transcription 1. These results demonstrate the striking effects of pneumolysin on the host cell upon exposure to S. pneumoniae.

Rozen, Y., R. A. Larossa, et al. (2002). "Gene expression analysis of the response by Escherichia coli to seawater." Antonie Van Leeuwenhoek **81**(1-4): 15-25.

Gene expression of Escherichia coli cells exposed to seawater for 20 h was compared to that of exponentially growing cells (mops-glucose 0.2%) using DNA microarray technology. The expression of most (ca. 3,000) of the 4,228 open reading frames on the microarray remained unchanged; the relative expression of about 320 genes decreased in seawater, whereas that of ca. one fourth (937) increased. Clearly coherent expression patterns were observed for several functional gene groups. Induced genes were numerous in groups specifying the degradation of small molecules (carbon compounds, amino acids and fatty acids), energy metabolism (aerobic and anaerobic respiration, pyruvate dehydrogenase and TCA cycle), chemotaxis and mobility, flagella biosynthesis, surface structures and phage related functions. Repressed genes were clustered in two groups, cell division and nucleotides biosynthesis, indicating a cessation of growth.

Ruberg, S., Z. X. Tian, et al. (2003). "Construction and validation of a Sinorhizobium meliloti whole genome DNA microarray: genome-wide profiling of osmoadaptive gene expression." J Biotechnol **106**(2-3): 255-68.

Based on the complete Sinorhizobium meliloti genome sequence we established DNA microarrays as a comprehensive tool for systematic genome-wide gene expression analysis in S. meliloti 1021. For these PCR fragment-based microarrays, called Sm6kPCR, a collection of probes for the 6207 predicted protein-coding genes consisting of 6046 gene-specific PCR fragments and 161 70 mer oligonucleotides was arrayed in high density on glass slides. To obtain these PCR fragments primer pairs were designed to amplify internal gene-specific DNA fragments of 80-350 bp. Additionally, these primers were characterized by a 5' extension that allowed for reamplification using standard primers after the first amplification employing the specific primers. In order to ascertain the quality of the Sm6kPCR microarrays and to validate gene expression studies in S. meliloti parallel hybridizations based on RNA samples obtained from cells cultured under identical conditions were performed. In addition, gene expression in S. meliloti in response to an osmotic upshift imposed by the addition of 0.38 M NaCl was monitored. 137 genes were identified showing significant changes in gene expression resulting from the osmotic upshift. From these genes 52 were induced and 85 genes were repressed. Among the genes displaying different RNA levels some functional groups could be identified that are particularly remarkable. Repression was observed for 8 genes related to motility and chemotaxis, 7 genes encoding amino acid biosynthesis enzymes and 15 genes involved in iron uptake whereas 14 genes involved in transport of small molecules and 4 genes related to polysaccharide biosynthesis were induced.

Rushton, J. J., L. M. Davis, et al. (2003). "Distinct changes in gene expression induced by A-Myb, B-Myb and c-Myb proteins." Oncogene **22**(2): 308-13.

The c-Myb, A-Myb and B-Myb transcription factors have nearly identical DNA-binding domains, activate the same reporter gene constructs in animal cells, but have different biological roles. The Myb proteins are often coexpressed in the same cells, raising questions about whether they activate similar or distinct gene expression profiles, and whether they cooperate or compete in regulating the same

promoters. Here, recombinant adenoviruses were used to express each protein in human mammary cells, and then microarray assays were used to assess global changes in gene expression. Each Myb protein induced a unique and specific set of changes, displaying activities far more complex than revealed by standard reporter gene assays. These results have important implications for the roles of various Myb proteins in normal and transformed human cells, for regulatory pathways that might modify their activities and for the importance of acquired mutations that may qualitatively alter their functions in tumors.

Ruutu, M., P. Peitsaro, et al. (2002). "Transcriptional profiling of a human papillomavirus 33-positive squamous epithelial cell line which acquired a selective growth advantage after viral integration." Int J Cancer **100**(3): 318-26.

Alterations in gene expression represent key events in carcinogenesis. We have studied HPV-induced cervical carcinogenesis, using an HPV-33-positive cell line (UT-DEC-1) established from a low-grade vaginal dysplasia (VAIN-I). Early-passage cells contained HPV-33 in episomal form, but these were superseded at later passages by cells carrying only integrated virus. To gain insight into the biologic significance of HPV integration, we compared the level of gene expression in normal vaginal keratinocytes, early-passage and late-passage UT-DEC-1 cells, using cDNA microarrays. Total RNA was isolated from cells by CsCl-gradient centrifugation, reverse-transcribed with MMLV reverse transcriptase and labeled with alpha-(32)P ATP. A cDNA microarray expression profile analysis was performed with Clontech's Human Cancer 1.2 cDNA expression array kit. The 16 upregulated genes (cut-off 2-fold), identified by comparing both cell types to control keratinocytes, appeared to support cell-cycle progression or to be functional in mitosis. These included, e.g., MCM4 DNA replication licensing factor, cdc2p34 and chromatin assembly factor 1 p48 subunit. Downregulated genes (44 altogether) interfered with apoptosis and cell adhesion, including the apoptosis-inducing genes FRAP, Bik and caspase-9 precursor. The most significant differences between the late and early passages (29 and 46 constantly up- and downregulated genes without any fluctuation) were overexpression of the transcription factors E2F5 with its dimerization partner DP1, NF-kappa B and serine/threonine kinases and underexpression of enzymes of the MAPK pathway. Acquisition of a selective growth advantage after viral integration might be explained by a major shift from a MAPK pathway to cell-cycle dysregulation (G(2)/M).

Ryu, M., J. Dae Kim, et al. (2004). "Nonlinear matching measure for the analysis of on-off type DNA microarray images." J Biomed Opt **9**(3): 432-8.

We propose a new nonlinear matching measure for automatic analysis of the on-off type DNA microarray images in which the hybridized spots are detected by a template-matching method. The proposed measure is obtained by binary thresholding over the entire template region and taking the number of white pixels inside the spotted area. This measure is compared with the normalized covariance method in terms of classifying the ability to successfully locate markers. The proposed measure was evaluated for scanned images of human papillomavirus (HPV) DNA microarrays where locating markers is a critical issue because of the small number of spots. The targeting spots of HPV DNA chips are designed for genotyping twenty-two types of the human papillomavirus. The proposed measure is proven to give a more discriminative response, reducing the missed cases of successful marker location. The locating accuracy of the proposed method is also shown to have the same performance as that of the normalized covariance.

Sabatti, C., L. Rohlin, et al. (2002). "Co-expression pattern from DNA microarray experiments as a tool for operon prediction." Nucleic Acids Res **30**(13): 2886-93.

The prediction of operons, the smallest unit of transcription in prokaryotes, is the first step towards reconstruction of a regulatory network at the whole genome level. Sequence information, in particular the distance between open reading frames, has been used to predict if adjacent Escherichia coli genes are in an operon. While appreciably successful, these predictions need to be validated and refined

experimentally. As a growing number of gene expression array experiments on E.coli became available, we investigated to what extent they could be used to improve and validate these predictions. To this end, we examined a large collection of published microarry data. The correlation between expression ratios of adjacent genes was used in a Bayesian classification scheme to predict whether the genes are in an operon or not. We found that for the genes whose expression levels change significantly across the experiments in the data set, the currently available gene expression data allowed a significant refinement of the sequenced-based predictions. We report these co-expression correlations in an E.coli genomic map. For a significant portion of gene pairs, however, the set of array experiments considered did not contain sufficient information to determine whether they are in the same transcriptional unit. This is not due to unreliability of the array data per se, but to the design of the experiments analyzed. In general, experiments that perturb a large number of genes offer more information for operon prediction than confined perturbations. These results provide a rationale for conducting expression studies comparing conditions that cause global changes in gene expression.

Sabina, J., N. Dover, et al. (2003). "Interfering with different steps of protein synthesis explored by transcriptional profiling of Escherichia coli K-12." J Bacteriol **185**(20): 6158-70.

Escherichia coli responses to four inhibitors that interfere with translation were monitored at the transcriptional level. A DNA microarray method provided a comprehensive view of changes in mRNA levels after exposure to these agents. Real-time reverse transcriptase PCRanalysis served to verify observations made with microarrays, and a chromosomal grpE::lux operon fusion was employed to specifically monitor the heat shock response. 4-Azaleucine, a competitive inhibitor of leucyl-tRNA synthetase, surprisingly triggered the heat shock response. Administration of mupirocin, an inhibitor of isoleucyl-tRNA synthetase activity, resulted in changes reminiscent of the stringent response. Treatment with kasugamycin and puromycin (targeting ribosomal subunit association as well as its peptidyl-transferase activity) caused accumulation of mRNAs from ribosomal protein operons. Abundant biosynthetic transcripts were often significantly diminished after treatment with any of these agents. Exposure of a relA strain to mupirocin resulted in accumulation of ribosomal protein operon transcripts. However, the relA strain's response to the other inhibitors was quite similar to that of the wild-type strain.

Sahu, S. N., S. Acharya, et al. (2003). "The bacterial adaptive response gene, barA, encodes a novel conserved histidine kinase regulatory switch for adaptation and modulation of metabolism in Escherichia coli." Mol Cell Biochem **253**(1-2): 167-77.

Histidine kinases are important prokaryotic determinants of cellular adaptation to environmental conditions, particularly stress. The highly conserved histidine kinase, BarA, encoded by the bacterial adaptive response gene, barA, is a member of the family of tripartite histidine kinases, and is involved in stress adaptation. BarA has been implicated to play a role during infection of epithelial cells. Homologues and orthologues of BarA have been found in pathogenic yeast, fungi, mould and in plants. The primary aim of this review is to assimilate evidence present in the current literature linking the role of BarA in stress response, and to support it with preliminary experimental evidence indicating that, it is indeed a global response regulator. In particular, the review focuses on the unusual domain structure of the BarA protein, its role in oxidative, weak acid, and osmotic stress responses and its role in biofilm formation. A preliminary genomic approach to identify downstream genes regulated by the BarA signaling pathway, using DNA microarray, is reported. The results demonstrate that BarA plays a global response regulatory role in cell division, carbon metabolism, iron metabolism and pili formation. The evolutionary significance of these types of histidine kinase sensors is reviewed in light of their roles in pathogenesis.

Salama, N., K. Guillemin, et al. (2000). "A whole-genome microarray reveals genetic diversity among Helicobacter pylori strains." Proc Natl Acad Sci U S A **97**(26): 14668-73.

Helicobacter pylori colonizes the stomach of half of the world's population, causing a wide spectrum of disease ranging from asymptomatic gastritis to ulcers to gastric cancer. Although the basis for

these diverse clinical outcomes is not understood, more severe disease is associated with strains harboring a pathogenicity island. To characterize the genetic diversity of more and less virulent strains, we examined the genomic content of 15 H. pylori clinical isolates by using a whole genome H. pylori DNA microarray. We found that a full 22% of H. pylori genes are dispensable in one or more strains, thus defining a minimal functional core of 1281 H. pylori genes. While the core genes encode most metabolic and cellular processes, the strain-specific genes include genes unique to H. pylori, restriction modification genes, transposases, and genes encoding cell surface proteins, which may aid the bacteria under specific circumstances during their long-term infection of genetically diverse hosts. We observed distinct patterns of the strain-specific gene distribution along the chromosome, which may result from different mechanisms of gene acquisition and loss. Among the strain-specific genes, we have found a class of candidate virulence genes identified by their coinheritance with the pathogenicity island.

Salgado, H., S. Gama-Castro, et al. (2004). "RegulonDB (version 4.0): transcriptional regulation, operon organization and growth conditions in Escherichia coli K-12." Nucleic Acids Res **32 Database issue**: D303-6.

RegulonDB is the primary database of the major international maintained curation of original literature with experimental knowledge about the elements and interactions of the network of transcriptional regulation in Escherichia coli K-12. This includes mechanistic information about operon organization and their decomposition into transcription units (TUs), promoters and their sigma type, binding sites of specific transcriptional regulators (TRs), their organization into 'regulatory phrases', active and inactive conformations of TRs, as well as terminators and ribosome binding sites. The database is complemented with clearly marked computational predictions of TUs, promoters and binding sites of TRs. The current version has been expanded to include information beyond specific mechanisms aimed at gathering different growth conditions and the associated induced and/or repressed genes. RegulonDB is now linked with Swiss-Prot, with microarray databases, and with a suite of programs to analyze and visualize microarray experiments. We provide a summary of the biological knowledge contained in RegulonDB and describe the major changes in the design of the database. RegulonDB can be accessed on the web at the URL: http://www.cifn.unam.mx/Computational_Biology/regulondb/.

Salmon, K., S. P. Hung, et al. (2003). "Global gene expression profiling in Escherichia coli K12. The effects of oxygen availability and FNR." J Biol Chem **278**(32): 29837-55.

The work presented here is a first step toward a long term goal of systems biology, the complete elucidation of the gene regulatory networks of a living organism. To this end, we have employed DNA microarray technology to identify genes involved in the regulatory networks that facilitate the transition of Escherichia coli cells from an aerobic to an anaerobic growth state. We also report the identification of a subset of these genes that are regulated by a global regulatory protein for anaerobic metabolism, FNR. Analysis of these data demonstrated that the expression of over one-third of the genes expressed during growth under aerobic conditions are altered when E. coli cells transition to an anaerobic growth state, and that the expression of 712 (49%) of these genes are either directly or indirectly modulated by FNR. The results presented here also suggest interactions between the FNR and the leucine-responsive regulatory protein (Lrp) regulatory networks. Because computational methods to analyze and interpret high dimensional DNA microarray data are still at an early stage, and because basic issues of data analysis are still being sorted out, much of the emphasis of this work is directed toward the development of methods to identify differentially expressed genes with a high level of confidence. In particular, we describe an approach for identifying gene expression patterns (clusters) obtained from multiple perturbation experiments based on a subset of genes that exhibit high probability for differential expression values.

Sandler, N. G., M. M. Mentink-Kane, et al. (2003). "Global gene expression profiles during acute pathogen-induced pulmonary inflammation reveal divergent roles for Th1 and Th2 responses in tissue repair." J Immunol **171**(7): 3655-67.

T helper 1 responses are typically proinflammatory, while Th2 responses have been considered regulatory. Interestingly, Th2 responses characterize a number of pulmonary diseases, many of which terminate in tissue remodeling and fibrosis. We developed a mouse model using Schistosoma mansoni eggs and cytokine-deficient mice to induce highly polarized Th1- or Th2-type inflammation in the lung. In this study, we examined the pathology and cytokine profiles in Th1- and Th2-polarized environments and used oligonucleotide microarray analysis to decipher the genes responsible for these effects. We further elaborated on the results using IL-10- and IL-13-deficient mice because these cytokines are believed to be the central regulators of Th2-associated pathology. We found that the Th1-polarized mice developed small granulomas with less fibrosis while expressing genes characteristic of tissue damage. Th2-polarized mice, in contrast, formed large granulomas with massive collagen deposition and up-regulated genes associated with wound healing, specifically, arginase, collagens, matrix metalloproteinases (MMPs), and tissue inhibitors of MMP. In addition, several members of the chitinase-like family were up-regulated in the lung following egg challenge. We also developed a method of defining the net collagen deposition using the expression profiles of several collagen, MMP, and tissue inhibitors of MMP genes. We found that Th1-polarized mice did not elaborate collagens or MMPs and therefore did not have a significant capacity for repair in this model. Thus, Th1-mediated inflammation is characterized by tissue damage, while Th2 directs wound healing and fibrosis.

Sasik, R., N. Iranfar, et al. (2002). "Extracting transcriptional events from temporal gene expression patterns during Dictyostelium development." Bioinformatics **18**(1): 61-6.

MOTIVATION: The DNA microarray technology can generate a large amount of data describing the time-course of gene expression. These data, when properly interpreted, can yield a great deal of information concerning differential gene expression during development. Much current effort in bioinformatics has been devoted to the analysis of gene expression data, usually via some 'clustering analysis' on the raw data in some abstract high dimensional space. Here, we describe a method where we first 'process' the raw time-course data using a simple biologically based kinetic model of gene expression. This allows us to reduce the vast data to a few vital attributes characterizing each expression profile, e.g. the times of the onset and cessation of the expression of the developmentally regulated genes. These vital attributes can then be trivially clustered by visual inspection to reveal biologically significant effects. RESULTS: We have applied this approach to microarray expression data from samples isolated every 2 h throughout the 24 h developmental program of Dictyostelium discoideum. mRNA accumulation patterns for 50 developmental genes were found to fit the kinetic model with a p-value of 0.05 or better. Transcription of these genes appears to be initiated in bursts at well-defined periods during development, in a manner suggestive of a dependent sequence. This approach can be applied to analyses of other temporal gene expression patterns, including those of the cell cycle.

Sassetti, C. M., D. H. Boyd, et al. (2001). "Comprehensive identification of conditionally essential genes in mycobacteria." Proc Natl Acad Sci U S A **98**(22): 12712-7.

An increasing number of microbial genomes have been completely sequenced, and the identified genes are categorized based on their homology to genes of known function. However, the function of a large number of genes cannot be determined on this basis alone. Here, we describe a technique, transposon site hybridization (TraSH), which allows rapid functional characterization by identifying the complete set of genes required for growth under different conditions. TraSH combines high-density insertional mutagenesis with microarray mapping of pools of mutants. We have made large pools of independent transposon mutants in mycobacteria by using a mariner-based transposon and efficient phage transduction. By using TraSH, we have defined the set of genes required for growth of Mycobacterium bovis bacillus Calmette-Guerin on minimal but not rich medium. Genes of both known and unknown functions were identified. Of the genes with known functions, nearly all were involved in amino acid biosynthesis. TraSH is a powerful method for categorizing gene function that should be applicable to a variety of microorganisms.

Sauvonnet, N., B. Pradet-Balade, et al. (2002). "Regulation of mRNA expression in macrophages after Yersinia enterocolitica infection. Role of different Yop effectors." J Biol Chem **277**(28): 25133-42.

The Yop virulon, which comprises a complete type III secretion system and secreted proteins, allows bacteria from the genus Yersinia to resist the nonspecific immune response of the host. This virulon, which is encoded by a plasmid called pYV in Yersinia enterocolitica, enables extracellular bacteria to inject six Yop effectors (YopE, -H, -T, -O, -P, -M) into the host cell. To investigate the role of YopP, YopM, and the other pYV-encoded factors on the expression of the host cell genes, we characterized the transcriptome alterations in infected mouse macrophages using the microarray technique. PU5-1.8 macrophages were infected either with an avirulent (pYV(-)), a wild type (pYV(+)), or two knockout (yopP(-) and yopM(-)) mutants of Y. enterocolitica. Expression alterations in response to Y. enterocolitica infection were monitored for 6657 genes. Among those, 857 genes were affected, 339 of which were specifically regulated by the action of the Yop virulon. Further analysis of those 339 genes allowed identification of specific targets of YopP, YopM, or the other pYV-encoded factors. According to these results, the main action of the Yop virulon is to counteract the host cell pro-inflammatory response to the infection. YopP participates to this inhibition, whereas another pYV-encoded factor appears to also be involved in this down-regulation. Besides, YopM was found to induce the regulation of genes involved in cell cycle and cell growth, revealing for the first time an in vitro effect for YopM. In addition to YopM, other pYV factors distinct from YopP affected the expression of genes involved in cycling. In conclusion, these results provide new insight into the mechanisms of Yersinia pathogenicity by identifying the changes in host genes expression after infection and highlight the concerted actions of the different Yop effectors.

Sax, J. K. and W. S. El-Deiry (2003). "Identification and characterization of the cytoplasmic protein TRAF4 as a p53-regulated proapoptotic gene." J Biol Chem **278**(38): 36435-44.

The role of p53 in tumor suppression partly relies on its ability to transcriptionally regulate target genes involved in the initiation of cell cycle arrest or the activation of programmed cell death. In recent years many genes have been identified as p53-regulated genes; however, no single target gene has been shown to be required for the full apoptotic effect. We have identified TRAF4 as a p53-regulated gene in a microarray screen using a Murine 11K Affymetrix GeneChip hybridized with cRNA from the p53 temperature-sensitive cell line, Vm10. TRAF4 is a member the TRAF family of adaptor proteins that mediate cellular signaling by binding to various members of the tumor necrosis family receptor superfamily and interleukin-1/Toll-like receptor super-family. In contrast to its other family members, TRAF4 has not been shown to bind to a member of the tumor necrosis factor receptor superfamily in vivo, nor has it been shown to regulate signaling pathways common to its other family members. Therefore the role of TRAF4 in a signaling pathway has not yet been established and requires further study. TRAF4 is specifically regulated by p53 in response to temperature sensitive p53, overexpression of p53 by use of an adenovirus, and stabilization of p53 in response to DNA damage. The murine TRAF4 promoter contains a functional p53 DNA-binding site approximately 1 kilobase upstream of the initiating methionine. TRAF4 localizes to the cytoplasm and appears to remain in the cytoplasm following DNA damage. Interestingly, the overexpression of TRAF4 induces apoptosis and suppresses colony formation. These data suggest a correlation that the orphan adaptor protein TRAF4 may play a role in p53-mediated proapoptotic signaling in the response to cellular stress.

Saxena, A., E. A. Worthey, et al. (2003). "Evaluation of differential gene expression in Leishmania major Friedlin procyclics and metacyclics using DNA microarray analysis." Mol Biochem Parasitol **129**(1): 103-14.

The various Leishmania species are flagellated protozoans, responsible for a wide spectrum of human diseases. The sequence of the L. major genome is nearing completion and a large proportion of the identified genes have yet to be ascribed functions. DNA microarrays containing PCR-amplified DNA from a random amplified genomic library of L. major Friedlin (LmjF) [Mol. Biochem. Parasitol. 113

(2001) 337] were hybridized with fluorescent probes made from L. major Friedlin RNA from five time-points during differentiation from procyclics to metacyclics. The data were normalized for background and probe intensity and the relative abundance of RNA for each spot was calculated. Almost 15% (1387/9282) of the DNAs showed statistically significant (P<0.01) changes in expression (1.1-5-fold) during the transition, with 1.16% (108) showing up-regulation at two or more time-points and 0.14% (13) showing down-regulation. Northern blot analyses of selected genes confirmed these results. These studies confirmed the stage-specific expression of several known genes, as well as identifying a number of novel genes that are up-regulated in either procyclics or metacyclics.

Scheible, W. R., B. Fry, et al. (2003). "An Arabidopsis mutant resistant to thaxtomin A, a cellulose synthesis inhibitor from Streptomyces species." Plant Cell **15**(8): 1781-94.

Thaxtomin A is a phytotoxin produced by Streptomyces scabies and other Streptomyces species, the causative agents of common scab disease in potato and other taproot crops. At nanomolar concentrations, thaxtomin causes dramatic cell swelling, reduced seedling growth, and inhibition of cellulose synthesis in Arabidopsis. We identified a mutant of Arabidopsis, designated txr1, that exhibits increased resistance to thaxtomin as a result of a decrease in the rate of toxin uptake. The TXR1 gene was identified by map-based cloning and found to encode a novel, small protein with no apparent motifs or organelle-targeting signals. The protein, which has homologs in all fully sequenced eukaryotic genomes, is expressed in all tissues and during all developmental stages analyzed. Microarray transcript profiling of some 14,300 genes revealed two stomatin-like genes that were expressed differentially in the txr1 mutant and the wild type. We propose that TXR1 is a regulator of a transport mechanism.

Schembri, M. A., D. W. Ussery, et al. (2002). "DNA microarray analysis of fim mutations in Escherichia coli." Mol Genet Genomics **267**(6): 721-9.

Bacterial adhesion is often mediated by complex polymeric surface structures referred to as fimbriae. Type 1 fimbriae of Escherichia coli represent the archetypical and best characterised fimbrial system. These adhesive organelles mediate binding to D-mannose and are directly associated with virulence in the urinary tract. A typical type 1 fimbriated bacterium has up to 500 fimbriae on its surface, with each fimbria consisting of approximately 1000 individual subunits. This equates to approximately 8% of the total cellular protein and is potentially a significant resource drain for the cell. Here we have used DNA microarray analysis to examine the molecular events involved in response to fimbrial gene expression in E. coli K-12. Observed differential expression levels of the fim genes were in good agreement with our current knowledge of the stoichiometry of type 1 fimbriae. Changes in fim expression correlated directly with alterations in colony morphology. Deletion of the entire fim gene cluster resulted in the converse expression of another surface protein Antigen 43 (Ag43). Specific deletion of the fimH gene did not affect expression of other fim genes or Ag43, but did dramatically reduce the number of fimbriae expressed on the cell surface. The use of high-resolution oligonucleotide arrays for defining points of transcription initiation and termination is also demonstrated.

Schilling, O., S. Ruggeberg, et al. (2004). "Characterization of an Escherichia coli elaC deletion mutant." Biochem Biophys Res Commun **320**(4): 1365-73.

The elaC gene of Escherichia coli encodes a binuclear zinc phosphodiesterase (ZiPD). ZiPD homologs from various species act as3' tRNA processing endoribonucleases, and although the homologous gene in Bacillus subtilis is essential for viability [EMBO J. 22(2003) 4534], the physiological function of E. coli ZiPD has remained enigmatic. In order to investigate the function of E. coli ZiPDwe generated and characterized an E. coli elaC deletion mutant. Surprisingly, the E. coli elaC deletion mutant was viable and had wild-type like growth properties. Microarray-based transcriptional analysis indicated expression of the E. coli elaC gene at basal levels during aerobic growth. The elaC gene deletion had no effect on the expression of genes coding for RNases or amino-acyl tRNA synthetases or any other gene among a total of > 1300 genes probed. 2D-PAGE analysis showed that the

elaC mutation, like-wise, had no effect on the proteome. These results strengthen doubts about the involvement of E. coli ZiPD in tRNA maturation and suggest functional diversity within the ZiPD/ElaC1 protein family. In addition to these unexpected features of the E. coli elaC deletion mutant, a sequence comparison of ZiPD (ElaC1) proteins revealed specific regions for either enterobacterial or mammalian ZiPD (ElaC1) proteins.

Schmitt, W. A., Jr. and G. Stephanopoulos (2003). "Prediction of transcriptional profiles of Synechocystis PCC6803 by dynamic autoregressive modeling of DNA microarray data." Biotechnol Bioeng **84**(7): 855-63.

Time-series profiles of gene expression generated by DNA microarrays possess sufficient information for building dynamic models of transcriptional behavior. This, however, requires properly designed experiments and sufficient independent data to validate such models. Here we report the use of AutoRegressive with eXogenous input (ARX) models to fit dynamic gene expression data obtained by subjecting cultures of the photosynthetic bacterium Synechocystis PCC6803 to consecutive light-to-dark transitions. Autoregressive with exogenous input models of appropriate complexity were selected by applying Akaike's information criterion (AIC) such as to maximize agreement between model predictions with experimental data without overfitting. These models were subsequently used to design the experimental profile of an optimal validating data set. Predictions from these models were tested in a second experiment and were found to match well with the validation data. Additionally, the models with the least error in predicting the expression profiles of the validation data set exactly match the model complexity predicted by AIC. Such models offer insights into cellular responses to environmental conditions and form the basis for hypothesizing and quantifying relationships that are presently poorly understood at the level of fundamental mechanisms.

Scholle, F., K. Li, et al. (2004). "Virus-host cell interactions during hepatitis C virus RNA replication: impact of polyprotein expression on the cellular transcriptome and cell cycle association with viral RNA synthesis." J Virol **78**(3): 1513-24.

Considerable controversy surrounds the impact of hepatitis C virus (HCV) protein expression on viability of host cells and regulation of the cell cycle. Both promotion of cellular proliferation and apoptosis have been observed in different experimental systems. To determine whether expression of the entire complement of HCV proteins in the context of ongoing viral RNA replication significantly alters the host cell transcriptome and cell cycle regulatory processes, we carried out high-density oligonucleotide microarray studies and analyzed cell cycle distributions and S-phase entry in Huh7 cell clones harboring selectable, full-length, replicating HCV RNAs that express the entire genotype 1b, HCV-N polyprotein, and clonally related cells in which all viral RNA was eliminated by prior treatment with alpha interferon. Oligonucleotide microarray analyses revealed only subtle, coordinated differences in the mRNA profiles of cells containing replicating viral RNA and their interferon-cured progeny, with variation between different cell clones having a greater influence on the cellular transcriptome than the presence or absence of replicating HCV RNA. Flow cytometric analysis demonstrated no significant differences in cell cycle distribution among populations of asynchronously growing cells of both types. Cell lines containing replicating viral RNA and their interferon-cured progeny were able to reenter the cell cycle similarly after transient G(1) arrest. In contrast, although viral protein expression and genome replication did not alter cell cycle control in these cells, HCV genome replication was highly dependent on cellular proliferation, with viral RNA synthesis strongly decreased in poorly proliferating, confluent, or serum-starved cells and substantially enhanced in the S phase of the cell cycle.

Schoolnik, G. K. (2002). "Microarray analysis of bacterial pathogenicity." Adv Microb Physiol **46**: 1-45.

The DNA microarray, a surface that contains an ordered arrangement of each identified open reading frame of a sequenced genome, is the engine of functional genomics. Its output, the expression profile, provides a genome wide snap-shot of the transcriptome. Refined by array-specific statistical

instruments and data-mined by clustering algorithms and metabolic pathway databases, the expression profile discloses, at the transcriptional level, how the microbe adapts to new conditions of growth--the regulatory networks that govern the adaptive response and the metabolic and biosynthetic pathways that effect the new phenotype. Adaptation to host microenvironments underlies the capacity of infectious agents to persist in and damage host tissues. While monitoring the whole genome transcriptional response of bacterial pathogens within infected tissues has not been achieved, it is likely that the complex, tissue-specific response is but the sum of individual responses of the bacteria to specific physicochemical features that characterize the host milieu. These are amenable to experimentation in vitro and whole-genome expression studies of this kind have defined the transcriptional response to iron starvation, low oxygen, acid pH, quorum-sensing pheromones and reactive oxygen intermediates. These have disclosed new information about even well-studied processes and provide a portrait of the adapting bacterium as a 'system', rather than the product of a few genes or even a few regulons. Amongst the regulated genes that compose this adaptive system are transcription factors. Expression profiling experiments of transcription factor mutants delineate the corresponding regulatory cascade. The genetic basis for pathogenicity can also be studied by using microarray-based comparative genomics to characterize and quantify the extent of genetic variability within natural populations at the gene level of resolution. Also identified are differences between pathogen and commensal that point to possible virulence determinants or disclose evolutionary history. The host vigorously engages the pathogen; expression studies using host genome microarrays and bacterially infected cell cultures show that the initial host reaction is dominated by the innate immune response. However, within the complex expression profile of the host cell are components mediated by pathogen-specific determinants. In the future, the combined use of bacterial and host microarrays to study the same infected tissue will reveal the dialogue between pathogen and host in a gene-by-gene and site- and time-specific manner. Translating this conversation will not be easy and will probably require a combination of powerful bioinformatic tools and traditional experimental approaches--and considerable effort and time.

Schoolnik, G. K. (2002). "Functional and comparative genomics of pathogenic bacteria." Curr Opin Microbiol **5**(1): 20-6.

Microarray expression profiling and the development of data-mining tools and new statistical instruments affords an unprecedented opportunity for the genome-scale study of bacterial pathogenicity. Expression profiles obtained from bacteria grown in media simulating host microenvironments yield a portrait of interacting metabolic pathways and multistage developmental programs and disclose regulatory networks. The analysis of closely related strains and species by microarray-based comparative genomics provides a measure of genetic variability within natural populations and identifies crucial differences between pathogen and commensal. In the near future, the combined use of bacterial and host microarrays to study the same infected tissue will reveal the host-pathogen dialogue in a gene-by-gene and site- and time-specific manner. This review discusses the use of microarray-based expression profiling to identify genes of pathogenic bacteria that are differentially regulated in response to host-specific signals. Additionally, the review describes the application of microarray methods to disclose differences in gene content between taxonomically related strains that vary with respect to pathogenic phenotype.

Schroeder, B. G., L. M. Peterson, et al. (2002). "Improved quantitation and reproducibility in Mycobacterium tuberculosis DNA microarrays." J Mol Microbiol Biotechnol **4**(2): 123-6.

We show here that the amount of labeled cDNA and its specific activity can play a significant role in the quantitation of microarray experiments. Standard reverse transcription of 2 microg total bacterial RNA with concomitant incorporation of cyanine dye-conjugated nucleotides did not produce enough label for optimal hybridization results in our Mycobacterium tuberculosis DNA microarray. Therefore we turned to an alternative labeling method using the incorporation of aminoallyl nucleotides followed by conjugation to Cy-dye. The method allows up to 10 fold more label to be produced, and at

higher specific activity. In particular, more transcripts can be detected and variability between replicate features can be reduced by using more labeled cDNA. We show that optimizing the labeling protocol is a critical element in conducting microarray experiments and obtaining reproducible and interpretable data.

Schwarze, S. R., S. E. DePrimo, et al. (2002). "Novel pathways associated with bypassing cellular senescence in human prostate epithelial cells." J Biol Chem **277**(17): 14877-83.

Cellular senescence forms a barrier that inhibits the acquisition of an immortal phenotype, a critical feature in tumorigenesis. The inactivation of multiple pathways that positively regulate senescence are required for immortalization. To identify these pathways in an unbiased manner, we performed DNA microarray analyses to assess the expression of 20,000 genes in human prostate epithelial cells (HPECs) passaged to senescence. These gene expression patterns were then compared with those of HPECs immortalized with the human Papillomavirus 16 E7 oncoprotein. Senescent cells display gene expression patterns that reflect their nonproliferative, differentiated phenotype and express secretory proteases and extracellular matrix components. A comparison of genes transcriptionally up-regulated in senescence to those in which expression is significantly down-regulated in immortalized HPECs identified three genes: the chemokine BRAK, DOC1, and a member of the insulin-like growth factor axis, IGFBP-3. Expression of these genes is found to be uniformly lost in human prostate cancer cell lines and xenografts, and previously, their inactivation was documented in tumor samples. Thus, these genes may function in novel pathways that regulate senescence and are inactivated during immortalization. These changes may be critical not only in allowing cells to bypass senescence in vitro but in the progression of prostate cancer in vivo.

Sebat, J. L., F. S. Colwell, et al. (2003). "Metagenomic profiling: microarray analysis of an environmental genomic library." Appl Environ Microbiol **69**(8): 4927-34.

Genomic libraries derived from environmental DNA (metagenomic libraries) are useful for characterizing uncultured microorganisms. However, conventional library-screening techniques permit characterization of relatively few environmental clones. Here we describe a novel approach for characterization of a metagenomic library by hybridizing the library with DNA from a set of groundwater isolates, reference strains, and communities. A cosmid library derived from a microcosm of groundwater microorganisms was used to construct a microarray (COSMO) containing approximately 1-kb PCR products amplified from the inserts of 672 cosmids plus a set of 16S ribosomal DNA controls. COSMO was hybridized with Cy5-labeled genomic DNA from each bacterial strain, and the results were compared with the results for a common Cy3-labeled reference DNA sample consisting of a composite of genomic DNA from multiple species. The accuracy of the results was confirmed by the preferential hybridization of each strain to its corresponding rDNA probe. Cosmid clones were identified that hybridized specifically to each of 10 microcosm isolates, and other clones produced positive results with multiple related species, which is indicative of conserved genes. Many clones did not hybridize to any microcosm isolate; however, some of these clones hybridized to community genomic DNA, suggesting that they were derived from microbes that we failed to isolate in pure culture. Based on identification of genes by end sequencing of 17 such clones, DNA could be assigned to functions that have potential ecological importance, including hydrogen oxidation, nitrate reduction, and transposition. Metagenomic profiling offers an effective approach for rapidly characterizing many clones and identifying the clones corresponding to unidentified species of microorganisms.

Sebert, M. E., L. M. Palmer, et al. (2002). "Microarray-based identification of htrA, a Streptococcus pneumoniae gene that is regulated by the CiaRH two-component system and contributes to nasopharyngeal colonization." Infect Immun **70**(8): 4059-67.

Nasopharyngeal carriage is the reservoir from which most disease with Streptococcus pneumoniae arises. Survival as a commensal in this environment is likely to require a set of adaptations distinct from those needed to cause disease, some of which may be mediated by two-component signal

transduction systems (TCSTS). We examined the contributions of nine pneumococcal TCSTS to the process of nasopharyngeal colonization by using an infant rat model. Whereas deletions in all but one of these systems have been associated previously with a high degree of attenuation in a murine model of pneumonia, only the CiaRH system was necessary for efficient carriage. Transcriptional analysis by using microarray hybridization identified a locus consisting of two adjacent genes, htrA and spoJ, that was specifically and strongly downregulated in a DeltaciaRH-null mutant. A S. pneumoniae strain lacking the htrA gene encoding a putative serine protease, but not one lacking spoJ, showed decreased fitness in a competitive model of colonization, a finding consistent with this gene mediating a portion of the carriage deficit observed with the DeltaciaRH strain.

Selinger, D. W., R. M. Saxena, et al. (2003). "Global RNA half-life analysis in Escherichia coli reveals positional patterns of transcript degradation." Genome Res **13**(2): 216-23.

Subgenic-resolution oligonucleotide microarrays were used to study global RNA degradation in wild-type Escherichia coli MG1655. RNA chemical half-lives were measured for 1036 open reading frames (ORFs) and for 329 known and predicted operons. The half-life of total mRNA was 6.8 min under the conditions tested. We also observed significant relationships between gene functional assignments and transcript stability. Unexpectedly, transcription of a single operon (tdcABCDEFG) was relatively rifampicin-insensitive and showed significant increases 2.5 min after rifampicin addition. This supports a novel mechanism of transcription for the tdc operon, whose promoter lacks any recognizable sigma binding sites. Probe by probe analysis of all known and predicted operons showed that the 5' ends of operons degrade, on average, more quickly than the rest of the transcript, with stability increasing in a 3' direction, supporting and further generalizing the current model of a net 5' to 3' directionality of degradation. Hierarchical clustering analysis of operon degradation patterns revealed that this pattern predominates but is not exclusive. We found a weak but highly significant correlation between the degradation of adjacent operon regions, suggesting that stability is determined by a combination of local and operon-wide stability determinants. The 16 ORF dcw gene cluster, which has a complex promoter structure and a partially characterized degradation pattern, was studied at high resolution, allowing a detailed and integrated description of its abundance and degradation. We discuss the application of subgenic resolution DNA microarray analysis to study global mechanisms of RNA transcription and processing.

Semnani, R. T., M. Law, et al. (2004). "Filaria-induced immune evasion: suppression by the infective stage of Brugia malayi at the earliest host-parasite interface." J Immunol **172**(10): 6229-38.

To assess the physiologic interactions between the infective stage of Brugia malayi--one of the extracellular parasites responsible for lymphatic filariasis in humans--and the APC with which they come in contact during their development and routes of travel, we have investigated the interaction between the infective stage (L3) of B. malayi and human Langerhans cells (LC) in the skin. Our data indicate that live L3 result in increased migration of LC from the epidermis without affecting the viability of these cells and up-regulation of the IL-18 cytokine involved in LC migration. Live L3 also result in down-regulation of MHC class I and II on the LC cell surface. Additionally, microarray data indicate that live L3 significantly down-regulated expression of IL-8 as well as of multiple genes involved in Ag presentation, reducing the capacity of LC to induce CD4(+) T cells in allogeneic MLR, and thus resulting in a decreased ability of LC to promote CD4(+) T cell proliferation and production of IFN-gamma and IL-10. These data suggest that L3 exert a down-regulatory response in epidermal LC that leads to a diminished capacity of these cells to activate CD4(+) T cells.

Semnani, R. T., A. Y. Liu, et al. (2003). "Brugia malayi microfilariae induce cell death in human dendritic cells, inhibit their ability to make IL-12 and IL-10, and reduce their capacity to activate CD4+ T cells." J Immunol **171**(4): 1950-60.

Parasite Ag-specific T cell unresponsiveness and diminished IFN-gamma production are

immunologic hallmarks of patent infection with lymph-dwelling filarial nematodes. Although this diminished responsiveness is directed primarily against the intravascular microfilarial (MF) parasite stage and mediated in part by reduced APC function, the mechanisms involved are not fully understood. In this report, we demonstrate that human dendritic cells (DC) exposed to live MF up-regulate both the cell surface and gene expression of CD54 (ICAM-1). Moreover, live MF result in a 3-fold increase in DC death compared with MF-unexposed DC, primarily due to apoptosis. Notably, microarray and real-time RT-PCR data indicate that live MF concurrently up-regulate mRNA expression of proinflammatory molecules such as IL-8, RANTES, IL-1alpha, TNF-alpha, and IL-beta in DC, the presence of which is also detected at the protein level, while inhibiting the production of IL-12 (p40 and p70) and IL-10. Soluble excretory-secretory products from live MF diminished IL-12 and IL-10 production and induced DC death, although to a lesser degree. Moreover, exposure of DC to live MF resulted in a decrease in the ability of DC to promote CD4(+) T cell production of IFN-gamma and IL-5. Our findings clearly suggest that the interaction between live MF and DC is complex but contributes to the hyporesponsiveness and parasite persistence associated with the MF(+) state in the infected human. These data further suggest that MF induce an orchestrated response in APC that leads to a diminished capacity to function appropriately, which in turn has significant consequences for CD4(+) T cells.

Semret, M., G. Zhai, et al. (2004). "Extensive genomic polymorphism within Mycobacterium avium." J Bacteriol **186**(18): 6332-4.

We have initiated comparative genomic analysis of Mycobacterium avium subspecies by DNA microarray, uncovering 14 large sequence polymorphisms (LSPs) comprising over 700 kb that distinguish M. avium subsp. avium from M. avium subsp. paratuberculosis. Genes predicted to encode metabolic pathways were overrepresented in the LSPs, and analysis revealed a polymorphism within the mycobactin biosynthesis operon that potentially explains the in vitro mycobactin dependence of M. avium subsp. paratuberculosis.

Sepulveda, A. R., H. Tao, et al. (2002). "Screening of gene expression profiles in gastric epithelial cells induced by Helicobacter pylori using microarray analysis." Aliment Pharmacol Ther **16 Suppl 2**: 145-57.

BACKGROUND: H. pylori infection is a major risk factor in gastric cancer development. The availability of cDNA microarrays creates the unprecedented opportunity to examine simultaneously dynamic changes of multiple pathways affected by H. pylori infection. AIM: In this study we examined broad patterns of gene expression induced by H. pylori in the gastric cancer cell line 1739-CRL AGS cells in culture using the U95A microarray. METHODS: H. pylori were cocultured with AGS cells for 4, 12, 24 and 48 h. Total RNA was extracted and after labelling was used for detection of genes represented in the human U95A microarray set. Data analyses were performed using GeneChip and CLUSFAVOR software. RESULTS: Nearly 6000 genes present in the array were expressed by AGS cells. We report approximately 200 genes that showed the most marked changes. Our studies confirm the up-regulation of c-jun, jun-B, c-fos and cyclin D1 by H. pylori. We report for the first time the induction of the serine threonine kinase pim-1 and ATF3 by H. pylori infection of AGS cells. CONCLUSIONS: In this microarray analysis of gene expression induced by H. pylori in gastric epithelial cells, we identified a large number of unsuspected genes affected by H. pylori. Further, we show that unsupervised hierarchical cluster analysis can provide useful insight into the possible contribution of genes in specific pathways, based on their profile of expression.

Serizawa, M., H. Yamamoto, et al. (2004). "Systematic analysis of SigD-regulated genes in Bacillus subtilis by DNA microarray and Northern blotting analyses." Gene **329**: 125-36.

The SigD-regulated genes in Bacillus subtilis were systematically analyzed by comparing the pattern of transcripts derived from wild-type cells with those from sigD mutant cells using DNA microarray technology. One hundred and fifty-eight genes were found to be SigD-dependent candidates, 46 of which being known SigD-regulated genes. Northern blot analysis revealed that 18 of the remaining

genes were SigD-dependent. The SigD consensus sequence was newly identified in the upstream regions of nine operons (11 genes): ybdO, yfmT-yfmS, hemAT, yjcP-yjcQ, yjfB, ylqB, yoaH, yscB and yxkC, and the other seven genes were assumed to be indirectly affected by a SigD mutation. Furthermore, yviE-yviF are likely to be SigD-dependent genes, because three independent sets of array data for yviE and yviF indicated they are SigD-dependent, and these genes are neighbors of flgL and hag transcribed by SigD RNA polymerase.

Sexton, A. C., R. T. Good, et al. (2004). "Transcriptional profiling reveals suppressed erythropoiesis, up-regulated glycolysis, and interferon-associated responses in murine malaria." J Infect Dis **189**(7): 1245-56.

The primary pathophysiological events contributing to fatal malaria are the cerebral syndrome, anemia, and lactic acidosis. The molecular basis of each event has been unclear. In the present study, microarray analysis of murine transcriptional responses during the development of severe disease revealed temporal, organ-specific, and pathway-specific patterns. More than 400 genes in the brain and 600 genes in the spleen displayed transcriptional changes. Dominant patterns revealed strongly suppressed erythropoiesis, starting early during infection, and highly up-regulated transcription of genes that control host glycolysis, including lactate dehydrogenase. The latter presents a mechanism that may contribute to metabolic acidosis. No evidence for hypoxia-mediated regulation of these events was observed. Interferon-regulated gene transcripts dominated the inflammatory response to cytokines. These results demonstrate previously unknown transcriptional changes in the host that may underlie the development of malarial syndromes, such as anemia and metabolic dysregulation, and increase the utility of murine models in investigation of basic malarial pathogenesis.

Shaheduzzaman, S., V. Krishnan, et al. (2002). "Effects of HIV-1 Nef on cellular gene expression profiles." J Biomed Sci **9**(1): 82-96.

The early human immunodeficiency virus (HIV) accessory protein Nef makes an important contribution to virulence, but the mechanisms by which Nef influences pathogenesis remain unclear. Many well-studied effects of Nef, like CD4 and class I MHC downregulation, occur posttranslationally. However, Nef has the potential to affect gene expression by interfering with cell signaling pathways and by virtue of structural features such as the Pro-X-X-Pro motif, which may interact with src homology region-3 domains of src-like kinases. We used a cDNA microarray screening strategy to identify cellular genes whose steady state transcriptional levels may be affected by Nef. We generated HeLa cell lines expressing wild-type or mutant HIV-1 nef protein sequences. Using cDNA microarray technology, we compared the patterns of cellular gene expression in the various cell lines to the pattern in non-Nef-expressing HeLa cells. By matching the patterns of cellular gene expression in HeLa cell lines expressing various Nefs with that of parental HeLa cells, we identified several cellular genes whose expression was modulated differentially by Nef and its mutants. We confirmed the differential expression of selected genes by RNA filter blotting. Genes expressed at higher levels included proteases, transcription factors, protein kinases, nuclear import/export proteins, adaptor molecules and cyclins, some of which have previously been implicated as being important for HIV replication and pathogenesis. The results indicate that Nef expression can alter the expression of cellular genes and suggest that this alteration in cellular gene expression may serve to optimize the cell to support the subsequent stages of viral replication.

Shaw, G., S. Morse, et al. (2002). "Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells." Faseb J **16**(8): 869-71.

The 293 cell line was derived by transformation of primary cultures of human embryonic kidney (HEK) cells with sheared adenovirus (Ad)5 DNA. A combination of immunostaining, immunoblot, and microarray analysis showed that 293 cells express the neurofilament (NF) subunits NF-L, NF-M, NF-H, and a-internexin as well as many other proteins typically found in neurons. Three other independently derived HEK lines, two transformed by Ad5 and one by Ad12, also expressed NFs, as did one human

embryonic retinal cell line transformed with Ad5. Two rodent kidney lines transformed with Ad12 were also found to express NF proteins, although several rodent kidney cell lines transformed by Ad5 DNA and three HEK cell lines transformed by the SV40 early region did not express NFs. These results suggest that human Ads preferentially transform human neuronal lineage cells. We also demonstrate that the widely used HEK293 cells have an unexpected relationship to neurons, a finding that may require reinterpretation of many previous studies in which it was assumed that HEK293 cells resembled more typical kidney epithelial cells.

Sheeter, D., P. Du, et al. (2003). "Surface CD4 expression modulated by a cellular factor induced by HIV type 1 infection." AIDS Res Hum Retroviruses **19**(2): 117-23.

Human immunodeficiency virus type 1 (HIV-1) alters gene expression in infected cells, leading to cellular dysfunction. We uncovered a number of host cell genes that are modulated in both CD4(+) T cell lines and primary CD4(+) T lymphocytes infected with HIV-1, using high-density oligonucleotide probe microarray technology. We focused on one gene in particular, nuclear factor I-B2 (NFI-B2), because of its high level of expression. NFI-B2 is a member of the nuclear factor I family of nuclear proteins, which are known to be involved in viral and cellular transcription. To better understand the role of NFI-B2 during HIV-1 infection, we generated a Jurkat T cell line that constitutively expressed NFI-B2. After infection with HIV-1, these cells produced fewer viruses because of a downregulation of surface CD4 expression. The surface expression of the coreceptor, CXCR4, remained unchanged. Furthermore, levels of CD4 mRNA were reduced in NFI-B2-producing cells, suggesting that expression of NFI-B2 impairs CD4 transcription. Modulation of NFI-B2 by HIV-1 may represent yet another mechanism by which HIV infection reduces cell surface expression of CD4.

Sherman, D. R., M. Voskuil, et al. (2001). "Regulation of the Mycobacterium tuberculosis hypoxic response gene encoding alpha -crystallin." Proc Natl Acad Sci U S A **98**(13): 7534-9.

Unlike many pathogens that are overtly toxic to their hosts, the primary virulence determinant of Mycobacterium tuberculosis appears to be its ability to persist for years or decades within humans in a clinically latent state. Since early in the 20th century latency has been linked to hypoxic conditions within the host, but the response of M. tuberculosis to a hypoxic signal remains poorly characterized. The M. tuberculosis alpha-crystallin (acr) gene is powerfully and rapidly induced at reduced oxygen tensions, providing us with a means to identify regulators of the hypoxic response. Using a whole genome microarray, we identified >100 genes whose expression is rapidly altered by defined hypoxic conditions. Numerous genes involved in biosynthesis and aerobic metabolism are repressed, whereas a high proportion of the induced genes have no known function. Among the induced genes is an apparent operon that includes the putative two-component response regulator pair Rv3133c/Rv3132c. When we interrupted expression of this operon by targeted disruption of the upstream gene Rv3134c, the hypoxic regulation of acr was eliminated. These results suggest a possible role for Rv3132c/3133c/3134c in mycobacterial latency.

Shih, A. Y., D. A. Johnson, et al. (2003). "Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress." J Neurosci **23**(8): 3394-406.

Astrocytes have a higher antioxidant potential in comparison to neurons. Pathways associated with this selective advantage include the transcriptional regulation of antioxidant enzymes via the action of the Cap'n'Collar transcription factor Nrf2 at the antioxidant response element (ARE). Here we show that Nrf2 overexpression can reengineer neurons to express this glial pathway and enhance antioxidant gene expression. However, Nrf2-mediated protection from oxidative stress is conferred primarily by glia in mixed cultures. The antioxidant properties of Nrf2-overexpressing glia are more pronounced than those of neurons, and a relatively small number of these glia (< 1% of total cell number added) could protect fully cocultured naive neurons from oxidative glutamate toxicity associated with glutathione (GSH) depletion. Microarray and biochemical analyses indicate a coordinated upregulation of enzymes involved

in GSH biosynthesis (xCT cystine antiporter, gamma-glutamylcysteine synthetase, and GSH synthase), use (glutathione S-transferase and glutathione reductase), and export (multidrug resistance protein 1) with Nrf2 overexpression, leading to an increase in both media and intracellular GSH. Selective inhibition of glial GSH synthesis and the supplementation of media GSH indicated that an Nrf2-dependent increase in glial GSH synthesis was both necessary and sufficient for the protection of neurons, respectively. Neuroprotection was not limited to overexpression of Nrf2, because activation of endogenous glial Nrf2 by the small molecule ARE inducer, tert-butylhydroquinone, also protected against oxidative glutamate toxicity.

Shim, K. S., S. K. Cho, et al. (2004). "Identification of fungal (Magnaporthe grisea) stress-induced genes in wild rice (Oryza minuta)." Plant Cell Rep **22**(8): 599-607.

To identify fungal stress-related genes in wild rice, Oryza minuta, we constructed a subtracted library using suppression subtractive hybridization in combination with mirror orientation selection. DNA chips containing 960 randomly selected cDNA clones were applied by reverse Northern analysis to eliminate false positive clones from the library and to prescreen differentially expressed genes. In total, 377 cDNA clones were selected on the basis of their signal intensities and expression ratios. Sequence analyses of these 377 cDNA fragments revealed that 180 of them (47.7%) represented unique genes. Of these 180 cDNAs, 89 clones (49.6%) showed significant homologies to previously known genes, while the remaining 91 did not match any known sequences. The putative functions of the 180 unique ESTs were categorized by aligning them with MIPS data. They were classified into seven different groups using microarray data-derived expression patterns and verified by Northern blotting.

Shrager, J., C. Hauser, et al. (2003). "Chlamydomonas reinhardtii genome project. A guide to the generation and use of the cDNA information." Plant Physiol **131**(2): 401-8.

The National Science Foundation-funded Chlamydomonas reinhardtii genome project involves (a) construction and sequencing of cDNAs isolated from cells exposed to various environmental conditions, (b) construction of a high-density cDNA microarray, (c) generation of genomic contigs that are nucleated around specific physical and genetic markers, (d) generation of a complete chloroplast genome sequence and analyses of chloroplast gene expression, and (e) the creation of a Web-based resource that allows for easy access of the information in a format that can be readily queried. Phases of the project performed by the groups at the Carnegie Institution and Duke University involve the generation of normalized cDNA libraries, sequencing of cDNAs, analysis and assembly of these sequences to generate contigs and a set of predicted unique genes, and the use of this information to construct a high-density DNA microarray. In this paper, we discuss techniques involved in obtaining cDNA end-sequence information and the ways in which this information is assembled and analyzed. Descriptions of protocols for preparing cDNA libraries, assembling cDNA sequences and annotating the sequence information are provided (the reader is directed to Web sites for more detailed descriptions of these methods). We also discuss preliminary results in which the different cDNA libraries are used to identify genes that are potentially differentially expressed.

Singh, A. K., L. M. McIntyre, et al. (2003). "Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium Synechocystis sp. PCC 6803." Plant Physiol **132**(4): 1825-39.

A full-genome microarray of the (oxy)photosynthetic cyanobacterium Synechocystis sp. PCC 6803 was used to identify genes that were transcriptionally regulated by growth in iron (Fe)-deficient versus Fe-sufficient media. Transcript accumulation for 3,165 genes in the genome was analyzed using an analysis of variance model that accounted for slide and replicate (random) effects and dye (a fixed) effect in testing for differences in the four time periods. We determined that 85 genes showed statistically significant changes in the level of transcription (P </= 0.05/3,165 = 0.0000158) across the four time points examined, whereas 781 genes were characterized as interesting (P </= 0.05 but greater than

0.0000158; 731 of these had a fold change >1.25 x). The genes identified included those known previously to be Fe regulated, such as isiA that encodes a novel chlorophyll-binding protein responsible for the pigment characteristics of low-Fe (LoFe) cells. ATP synthetase and phycobilisome genes were down-regulated in LoFe, and there were interesting changes in the transcription of genes involved in chlorophyll biosynthesis, in photosystem I and II assembly, and in energy metabolism. Hierarchical clustering demonstrated that photosynthesis genes, as a class, were repressed in LoFe and induced upon the re-addition of Fe. Specific regulatory genes were transcriptionally active in LoFe, including two genes that show homology to plant phytochromes (cph1 and cph2). These observations established the existence of a complex network of regulatory interactions and coordination in response to Fe availability.

Singh, U., J. L. Brewer, et al. (2002). "Genetic analysis of tachyzoite to bradyzoite differentiation mutants in Toxoplasma gondii reveals a hierarchy of gene induction." Mol Microbiol **44**(3): 721-33.

Developmental switching in Toxoplasma gondii, from the virulent tachyzoite to the relatively quiescent bradyzoite stage, is responsible for disease propagation and reactivation. We have generated tachyzoite to bradyzoite differentiation (Tbd-) mutants in T. gondii and used these in combination with a cDNA microarray to identify developmental pathways in bradyzoite formation. Four independently generated Tbd- mutants were analysed and had defects in bradyzoite development in response to multiple bradyzoite-inducing conditions, a stable phenotype after in vivo passages and a markedly reduced brain cyst burden in a murine model of chronic infection. Transcriptional profiles of mutant and wild-type parasites, growing under bradyzoite conditions, revealed a hierarchy of developmentally regulated genes, including many bradyzoite-induced genes whose transcripts were reduced in all mutants. A set of non-developmentally regulated genes whose transcripts were less abundant in Tbd- mutants were also identified. These may represent genes that mediate downstream effects and/or whose expression is dependent on the same transcription factors as the bradyzoite-induced set. Using these data, we have generated a model of transcription regulation during bradyzoite development in T. gondii. Our approach shows the utility of this system as a model to study developmental biology in single-celled eukaryotes including protozoa and fungi.

Siwkowski, A. M., L. A. Madge, et al. (2004). "Effects of antisense oligonucleotide-mediated depletion of tumor necrosis factor (TNF) receptor 1-associated death domain protein on TNF-induced gene expression." Mol Pharmacol **66**(3): 572-9.

Tumor necrosis factor (TNF) receptor 1-associated death domain protein (TRADD) is an adaptor protein known to be involved in the TNF signaling pathway as well as signaling of other members of the TNF receptor superfamily, including DR3, DR6, p75(NTR), and the Epstein-Barr virus latent membrane protein 1. Current knowledge of the function of the adaptor protein has been derived from studies examining its over-expression in either wild-type or mutated forms. In this study, we analyzed the consequences of antisense oligonucleotide (ASO)-mediated depletion of endogenous TRADD on TNF induction of inflammation-related gene products, such as intercellular adhesion molecule-1, and associated kinase signaling pathways in human umbilical vein endothelial cells. A broader perspective of TRADD's role in TNF signaling was indicated by microarray gene expression analysis, where 20 of 24 genes that showed a 5-fold or greater increase in TNF-induced mRNA expression levels displayed a reduction in TNF-induced expression as a consequence of ASO-mediated knockdown of TRADD. Reduced activation of the nuclear factor-kappaB and c-Jun NH(2)-terminal kinase pathways, as measured by IkappaB-alpha protein levels and the extent of c-Jun phosphorylation, was also observed. These results indicate usage of antisense inhibitors of TRADD expression for modulating diseases associated with TRADD-dependent signal transduction pathways.

Smith, D., B. D. Collins, et al. (2003). "Sensitivity and specificity of photoaptamer probes." Mol Cell Proteomics **2**(1): 11-8.

The potential of photoaptamers as proteomic probes was investigated. Photoaptamers are defined

as aptamers that bear photocross-linking functionality, in this report, 5-bromo-2'-deoxyuridine. A key question regarding the use of photoaptamer probes is the specificity of the cross-linking reaction. The specificity of three photoaptamers was explored by comparing their reactions with target proteins and non-target proteins. The range of target/non-target specificity varies from 100- to >10(6)-fold with most values >10(4)-fold. The contributions of the initial binding step and the photocross-linking step were evaluated for each reaction. Photocross-linking never degraded specificity and significantly increased aptamer specificity in some cases. The application of photoaptamer technology to proteomics was investigated in microarray format. Immobilized anti-human immunodeficiency virus-gp120 aptamer was able to detect subnanomolar concentrations of target protein in 5% human serum. The levels of sensitivity and specificity displayed by photoaptamers, combined with other advantageous properties of aptamers, should facilitate development of protein chip technology.

Smith, M. W., Z. N. Yue, et al. (2003). "Hepatitis C virus and liver disease: global transcriptional profiling and identification of potential markers." Hepatology **38**(6): 1458-67.

Microarray analysis of RNA from hepatitis C virus (HCV)-infected cirrhotic livers was performed to identify a gene expression signature of liver disease. The expression levels of approximately 13600 genes were analyzed using surgical material and core biopsy specimens from HCV-infected cirrhotic liver explants in comparison with reference samples of normal nondiseased liver. In addition, normal liver samples were compared with each other to determine normal physiologic variation in gene expression. A set of genes, including some associated with stress, acute-phase immune response, and hepatic stellate cell activation, had variable expression levels in normal livers. These genes were subtracted from the sets of genes differentially expressed in cirrhotic livers. To exclude cancer-related genes from our marker sets, we subtracted genes that also were expressed differentially in hepatocellular carcinomas. The resultant HCV- and liver disease-associated gene set provided a molecular portrait of several processes occurring in the HCV-infected liver. It included (1). genes expressed in activated lymphocytes infiltrating the cirrhotic liver, and activated liver macrophages; (2). genes involved in remodeling of extracellular matrix-cell and cell-cell interactions associated with cytoskeleton rearrangements; (3). genes related to the anti-apoptotic pathway of Bcl-2 signaling; and (4). genes involved with the interferon response and virus-host interactions. In conclusion, our microarray analysis identified several potential gene markers of HCV-associated liver disease and contributed to our rapidly expanding database of experiments describing HCV pathogenesis.

Smoot, J. C., K. D. Barbian, et al. (2002). "Genome sequence and comparative microarray analysis of serotype M18 group A Streptococcus strains associated with acute rheumatic fever outbreaks." Proc Natl Acad Sci U S A **99**(7): 4668-73.

Acute rheumatic fever (ARF), a sequelae of group A Streptococcus (GAS) infection, is the most common cause of preventable childhood heart disease worldwide. The molecular basis of ARF and the subsequent rheumatic heart disease are poorly understood. Serotype M18 GAS strains have been associated for decades with ARF outbreaks in the U.S. As a first step toward gaining new insight into ARF pathogenesis, we sequenced the genome of strain MGAS8232, a serotype M18 organism isolated from a patient with ARF. The genome is a circular chromosome of 1,895,017 bp, and it shares 1.7 Mb of closely related genetic material with strain SF370 (a sequenced serotype M1 strain). Strain MGAS8232 has 178 ORFs absent in SF370. Phages, phage-like elements, and insertion sequences are the major sources of variation between the genomes. The genomes of strain MGAS8232 and SF370 encode many of the same proven or putative virulence factors. Importantly, strain MGAS8232 has genes encoding many additional secreted proteins involved in human-GAS interactions, including streptococcal pyrogenic exotoxin A (scarlet fever toxin) and two uncharacterized pyrogenic exotoxin homologues, all phage-associated. DNA microarray analysis of 36 serotype M18 strains from diverse localities showed that most regions of variation were phages or phage-like elements. Two epidemics of ARF occurring 12 years apart in Salt Lake City, UT, were caused by serotype M18 strains that were genetically identical, or nearly so.

Our analysis provides a critical foundation for accelerated research into ARF pathogenesis and a molecular framework to study the plasticity of GAS genomes.

Smoot, L. M., J. C. Smoot, et al. (2001). "Global differential gene expression in response to growth temperature alteration in group A Streptococcus." Proc Natl Acad Sci U S A **98**(18): 10416-21.

Pathogens are exposed to different temperatures during an infection cycle and must regulate gene expression accordingly. However, the extent to which virulent bacteria alter gene expression in response to temperatures encountered in the host is unknown. Group A Streptococcus (GAS) is a human-specific pathogen that is responsible for illnesses ranging from superficial skin infections and pharyngitis to severe invasive infections such as necrotizing fasciitis and streptococcal toxic shock syndrome. GAS survives and multiplies at different temperatures during human infection. DNA microarray analysis was used to investigate the influence of temperature on global gene expression in a serotype M1 strain grown to exponential phase at 29 degrees C and 37 degrees C. Approximately 9% of genes were differentially expressed by at least 1.5-fold at 29 degrees C relative to 37 degrees C, including genes encoding transporter proteins, proteins involved in iron homeostasis, transcriptional regulators, phage-associated proteins, and proteins with no known homologue. Relatively few known virulence genes were differentially expressed at this threshold. However, transcription of 28 genes encoding proteins with predicted secretion signal sequences was altered, indicating that growth temperature substantially influences the extracellular proteome. TaqMan real-time reverse transcription-PCR assays confirmed the microarray data. We also discovered that transcription of genes encoding hemolysins, and proteins with inferred roles in iron regulation, transport, and homeostasis, was influenced by growth at 40 degrees C. Thus, GAS profoundly alters gene expression in response to temperature. The data delineate the spectrum of temperature-regulated gene expression in an important human pathogen and provide many unforeseen lines of pathogenesis investigation.

Smothers, J. F. and S. Henikoff (2001). "Predicting in vivo protein peptide interactions with random phage display." Comb Chem High Throughput Screen **4**(7): 585-91.

Binding sites in protein complexes occasionally map to small peptides within one or more proteins. Random peptide display methods simulate binding interactions by providing all possible peptide combinations with an equal opportunity to bind a protein of interest. The natural substrates for the protein are typically known in advance. However, it is often the case that such substrates are identified as putative partner proteins by using in vivo methods such as yeast two hybrid screening. Unfortunately, such methods often produce lengthy datasets of protein sequences and offer little mechanistic insight into how such interactions might take place in vivo. Here, we review an approach that addresses this problem. First, sequence alignment tools identify and characterize blocks of conserved sequences among peptides recovered during random peptide display. Next, searching programs detect similar blocks of conserved sequences within naturally occurring proteins to predict partner proteins. Finally, the significance of an interaction is tested using site specific mutagenesis, binding competition or co-immunoprecipitation experiments. This strategy should become increasingly powerful with the growing popularity of interaction studies, sequencing projects and microarray analyses in modern biology.

Snyder, L. A., J. K. Davies, et al. (2004). "Microarray genomotyping of key experimental strains of Neisseria gonorrhoeae reveals gene complement diversity and five new neisserial genes associated with Minimal Mobile Elements." BMC Genomics **5**(1): 23.

BACKGROUND: There are four widely used experimental strains of N. gonorrhoeae, one of which has been sequenced and used as the basis for the construction of a multi-strain, mutli-species pan-neisserial microarray. Although the N. gonorrhoeae population structure is thought to be less diverse than N. meningitidis, there are some recognized gene-complement differences between strains, including the 59 genes of the Gonococcal Genetic Island. In this study we have investigated the three experimental strains that have not been sequenced to determine the extent and nature of their similarities and

differences. RESULTS: Using the Pan-Neisseria microarray, three commonly used gonococcal laboratory experimental strains were investigated (F62, MS11, & FA19). Genes absent from these strains, but present in strain FA1090, were assessed as is possible with typical microarrays. Due to the design of this microarray, additional genes were also identified. Differences were associated with Minimal Mobile Elements (MMEs) or known divergences. Genomotyping indicates the presence of genes previously only described in meningococci and shows the presence of the complete Gonococcal Genetic Island in N. gonorrhoeae strain FA19. Five new neisserial genes were identified through microarray genomotyping and subsequent sequencing of two divergent MMEs in N. gonorrhoeae strain MS11 and four MMEs in N. gonorrhoeae strain FA19. No differences were identified between N. gonorrhoeae strains FA1090 and F62, indicating that these strains are very similar. CONCLUSION: This study shows extensive similarity between the experimental strains, associated with a varying number of strain-specific genes. This provides a framework for those working with these strains to refer to the available gonococcal genome sequence, and is the first detailed comparison of gene complements between gonococcal strains.

Song, Y. J. and M. F. Stinski (2002). "Effect of the human cytomegalovirus IE86 protein on expression of E2F-responsive genes: a DNA microarray analysis." Proc Natl Acad Sci U S A **99**(5): 2836-41.

We have previously reported that the immediate early (IE)-86 protein of human cytomegalovirus (HCMV) pushes the cell cycle toward S phase but inhibits cell division [Murphy, E. A., Streblow, D. N., Nelson, J. A. & Stinski, M. F. (2000) J. Virol. 74, 7108-7118]. We determined the cellular genes activated by the IE86 protein in permissive human fibroblast cells. A 4-fold or greater increase in the steady-state RNA from many cellular genes that regulate the cell cycle, the enzymes for DNA precursor synthesis, and the initiation of cellular DNA replication was detected by high-density DNA microarray analysis. Northern blot analysis confirmed the DNA microarray data. The viral IE86 protein induced a significant increase in the cellular steady-state RNA level from the B-myb, cyclin E, cdk-2, E2F-1, ribonucleotide reductase 1, ribonucleotide reductase 2, thymidylate synthetase, MCM3, and MCM7 genes, but actin RNA was not affected. Cellular genes regulated by the E2F transcription factors were strongly activated by the IE86 protein. In most cases, the cellular genes induced by the IE86 protein were also induced by HCMV infection. This study demonstrates the global array of cellular genes activated by the IE86 protein that pushes progression of the cell cycle from G0/G1 toward the G1/S transition point.

Spender, L. C., G. H. Cornish, et al. (2002). "Expression of transcription factor AML-2 (RUNX3, CBF(alpha)-3) is induced by Epstein-Barr virus EBNA-2 and correlates with the B-cell activation phenotype." J Virol **76**(10): 4919-27.

To identify cell proteins regulated by the Epstein-Barr virus (EBV) transcription factor EBNA-2, we analyzed a cell line with conditional EBNA-2 activity by using microarray expression profiling. This led to the identification of two novel target genes induced by EBNA-2. The first of these, interleukin-16, is an immunomodulatory cytokine involved in the regulation of CD4 T cells. The second, AML-2, is a member of the Runt domain family of transcription factors. Quiescent B cells initially expressed AML-1 but, 48 h after virus infection, the levels of AML-1 decreased dramatically, whereas the amount of AML-2 protein increased. Analysis of a panel of B-cell lines indicated that AML-2 expression is normally predominant in EBV latency III, whereas AML-1 is associated with EBV latency I or EBV-negative cells. The AML genes are the first example of cell transcription factors whose expression correlates with the latency I/III phenotype.

Springer, A. L., L. R. Booth, et al. (2003). "A rapid method for manual or automated purification of fluorescently labeled nucleic acids for sequencing, genotyping, and microarrays." J Biomol Tech **14**(1): 17-32.

Fluorescent dyes provide specific, sensitive, and multiplexed detection of nucleic acids. To maximize sensitivity, fluorescently labeled reaction products (e.g., cycle sequencing or primer extension products) must be purified away from residual dye-labeled precursors. Successful high-throughput

analyses require that this purification be reliable, rapid, and amenable to automation. Common methods for purifying reaction products involve several steps and require processes that are not easily automated. Prolinx, Inc. has devel oped RapXtract superparamagnetic separation technology affording rapid and easy-to-perform methods that yield high-quality product and are easily automated. The technology uses superparamagnetic particles that specifically remove unincorporated dye-labeled precursors. These particles are efficiently pelleted in the presence of a magnetic field, making them ideal for purification because of the rapid separations that they allow. RapXtract-purified sequencing reactions yield data with good signal and high Phred quality scores, and they work with various sequencing dye chemistries, including BigDye and near-infrared fluorescence IRDyes. RapXtract technology can also be used to purify dye primer sequencing reactions, primer extension reactions for genotyping analysis, and nucleic acid labeling reactions for microarray hybridization. The ease of use and versatility of RapXtract technology makes it a good choice for manual or automated purification of fluorescently labeled nucleic acids.

Sreekumar, R., B. Rosado, et al. (2003). "Hepatic gene expression in histologically progressive nonalcoholic steatohepatitis." Hepatology **38**(1): 244-51.

Although the molecular basis for the pathophysiology of nonalcoholic steatohepatitis (NASH) is poorly understood, insulin resistance and mitochondrial dysfunction are physiologic hallmarks of this condition. We sought evidence of a transcriptional or pretranscriptional basis for insulin resistance and mitochondrial dysfunction through measurement of hepatic gene expression (messenger RNA [mRNA]) using high-density synthetic oligonucleotide microarray analysis (Hu6800 GeneChip, Affymetrix, CA). Global hepatic gene expression was determined in snap-frozen liver biopsy specimens from 4 groups: (1) patients with cirrhotic-stage NASH (n = 6), (2) patients with cirrhosis caused by hepatitis C virus (HCV) (n = 6), (3) patients with cirrhosis secondary to primary biliary cirrhosis (PBC) (n = 6), and (4) healthy controls (n = 6). Genes were considered to be expressed differentially in NASH only if there was a greater than 2-fold difference in abundance of mRNA when compared with each of the control groups. Sixteen genes were uniquely differentially expressed (4 overexpressed and 12 underexpressed) in patients with cirrhotic-stage NASH. Genes that were significantly underexpressed included genes important for maintaining mitochondrial function (copper/zinc superoxide dismutase, aldehyde oxidase, and catalase). Glucose 6-phospatase, alcohol dehydrogenase, elongation factor-TU, methylglutaryl coenzyme A (CoA), acyl CoA synthetase, oxoacyl CoA thiolase, and ubiquitin also were underexpressed in NASH. Genes that were overexpressed in NASH included complement component C3 and hepatocyte-derived fibrinogen-related protein, potentially contributing to impaired insulin sensitivity. In conclusion, these studies provide evidence for a transcriptional or pretranscriptional basis for impaired mitochondrial function (attenuated capacity for the dismutation of reactive oxygen species) and diminished insulin sensitivity (increased acute phase reactants) in patients with histologically progressive NASH. Further studies are required to determine the mechanism and the physiologic significance of these findings.

Stingley, S. W., J. J. Ramirez, et al. (2000). "Global analysis of herpes simplex virus type 1 transcription using an oligonucleotide-based DNA microarray." J Virol **74**(21): 9916-27.

More than 100 transcripts of various abundances and kinetic classes are expressed during phases of productive and latent infections by herpes simplex virus (HSV) type 1. To carry out rapid global analysis of variations in such patterns as a function of perturbation of viral regulatory genes and cell differentiation, we have made DNA microchips containing sets of 75-mer oligonucleotides specific for individual viral transcripts. About half of these are unique for single transcripts, while others function for overlapping ones. We have also included probes for 57 human genes known to be involved in some aspect of stress response. The chips efficiently detect all viral transcripts, and analysis of those abundant under various conditions of infection demonstrates excellent correlation with known kinetics of mRNA accumulation. Further, quantitative sensitivity is high. We have further applied global analysis of transcription to an investigation of mRNA populations in cells infected with a mutant virus in which the

essential immediate-early alpha27 (U(L)54) gene has been functionally deleted. Transcripts expressed at 6 h following infection with this mutant can be classified into three groups: those whose abundance is augmented (mainly immediate-early transcripts) or unaltered, those whose abundance is somewhat reduced, and those where there is a significant reduction in transcript levels. These do not conform to any particular kinetic class. Interestingly, levels of many cellular transcripts surveyed are increased. The high proportion of such transcripts suggests that the alpha27 gene plays a major role in the early decline in cellular gene expression so characteristic of HSV infection.

Stintzi, A. (2003). "Gene expression profile of Campylobacter jejuni in response to growth temperature variation." J Bacteriol **185**(6): 2009-16.

The foodborne pathogen Campylobacter jejuni is the primary causative agent of gastroenteritis in humans. In the present study a whole genome microarray of C. jejuni was constructed and validated. These DNA microarrays were used to measure changes in transcription levels over time, as C. jejuni cells responded to a temperature increase from 37 to 42 degrees C. Approximately 20% of the C. jejuni genes were significantly up- or downregulated over a 50-min period after the temperature increase. The global change in C. jejuni transcriptome was found to be essentially transient, with only a small subset of genes still differentially expressed after 50 min. A substantial number of genes with a downregulated coexpression pattern were found to encode for ribosomal proteins. This suggests a short growth arrest upon temperature stress, allowing the bacteria to reshuffle their energy toward survival and adaptation to the new growth temperature. Genes encoding chaperones, chaperonins, and heat shock proteins displayed the most dramatic and rapid upregulation immediately after the temperature change. Interestingly, genes encoding proteins involved in membrane structure modification were differentially expressed, either up- or downregulated, suggesting a different protein membrane makeup at the two different growth temperatures. Overall, these data provide new insights into the primary response of C. jejuni to surmount a sudden temperature upshift, allowing the bacterium to survive and adapt its transcriptome to a new steady state.

Stjepandic, D., C. Weinel, et al. (2002). "The genome structure of Pseudomonas putida: high-resolution mapping and microarray analysis." Environ Microbiol **4**(12): 819-23.

As part of a collaborative project aimed at sequencing and functionally analysing the entire genome of Pseudomonas putida strain KT2440, a physical clone map was produced as an initial resource. To this end, a high-coverage cosmid library was arrayed and ordered by clone hybridizations. Restriction fragments generated by rare-cutting enzymes and plasmids containing the rrn operon and 23S rDNA of Pseudomonas aeruginosa were used as probes and, parts of the cosmids were end-sequenced. This provided the information necessary for merging and comparing the macro-restriction map, cosmid clone order and sequence information, thereby assuring co-linearity of the eventual sequence assembly with the actual genome. A tiling path of clones was selected, from the shotgun clones used for sequencing, for the production of DNA microarrays that represent the entire genome including its non-coding portions.

Stolarov, J., K. Chang, et al. (2001). "Design of a retroviral-mediated ecdysone-inducible system and its application to the expression profiling of the PTEN tumor suppressor." Proc Natl Acad Sci U S A **98**(23): 13043-8.

We have engineered the ecdysone-inducible mammalian expression system for general retroviral delivery to cultured mammalian cells. We inducibly expressed PTEN in the glioblastoma cell line, U87MG, lacking this gene. Because nearly all cells are recruited on induction, we find both up- and down-regulated genes by cDNA microarray analysis. The changes we see are similar to those observed after treatment with LY294002, an inhibitor of phosphatidylinositol 3-OH kinase, fully consistent with the model that PTEN antagonizes phosphatidylinositol 3-OH kinase. Both treatments result in suppressed expression of the transforming growth factor (TGF)-beta gene and the genes of the cholesterol biosynthesis pathway. Our results illustrate the power of using a fully inducible expression system in

conjunction with cDNA microarray analysis for exploring gene function.

Stowe-Evans, E. L., J. Ford, et al. (2004). "Genomic DNA microarray analysis: identification of new genes regulated by light color in the cyanobacterium Fremyella diplosiphon." J Bacteriol **186**(13): 4338-49.

Many cyanobacteria use complementary chromatic adaptation to efficiently utilize energy from both green and red regions of the light spectrum during photosynthesis. Although previous studies have shown that acclimation to changing light wavelengths involves many physiological responses, research to date has focused primarily on the expression and regulation of genes that encode proteins of the major photosynthetic light-harvesting antennae, the phycobilisomes. We have used two-dimensional gel electrophoresis and genomic DNA microarrays to expand our understanding of the physiology of acclimation to light color in the cyanobacterium Fremyella diplosiphon. We found that the levels of nearly 80 proteins are altered in cells growing in green versus red light and have cloned and positively identified 17 genes not previously known to be regulated by light color in any species. Among these are homologs of genes present in many bacteria that encode well-studied proteins lacking clearly defined functions, such as tspO, which encodes a tryptophan-rich sensory protein, and homologs of genes encoding proteins of clearly defined function in many species, such as nblA and chlL, encoding phycobilisome degradation and chlorophyll biosynthesis proteins, respectively. Our results suggest novel roles for several of these gene products and highly specialized, unique uses for others.

Strzelczyk, B., M. Slominska-Wojewodzka, et al. (2003). "Non-random distribution of GATC sequences in regions of promoters stimulated by the SeqA protein of Escherichia coli." Acta Biochim Pol **50**(4): 941-5.

The SeqA protein of Escherichia coli is not only the main negative regulator of DNA replication initiation but also a specific transcription factor. It binds to hemimethylated GATC sequences and, with somewhat different specificity, to fully methylated GATC regions. Recently, a microarray analysis was reported, in which transcriptomes of wild-type and DeltaseqA strains were compared. Although in the seqA mutant the levels of some transcripts were significantly decreased while certain transcripts were evidently more abundant relative to wild-type bacteria, no correlation between the presence of GATC motifs in promoter sequences and transcription activity was found. However, here we show that when larger DNA fragments, encompassing positions from -250 to +250 relative to the transcription start site, are analyzed, some common features of GATC distribution near the promoters activated by SeqA can be demonstrated. Nevertheless, it seems that the GATC pattern is not the only determinant of SeqA-dependence of promoter activity.

Suerbaum, S., C. Josenhans, et al. (2003). "The complete genome sequence of the carcinogenic bacterium Helicobacter hepaticus." Proc Natl Acad Sci U S A **100**(13): 7901-6.

Helicobacter hepaticus causes chronic hepatitis and liver cancer in mice. It is the prototype enterohepatic Helicobacter species and a close relative of Helicobacter pylori, also a recognized carcinogen. Here we report the complete genome sequence of H. hepaticus ATCC51449. H. hepaticus has a circular chromosome of 1,799,146 base pairs, predicted to encode 1,875 proteins. A total of 938, 953, and 821 proteins have orthologs in H. pylori, Campylobacter jejuni, and both pathogens, respectively. H. hepaticus lacks orthologs of most known H. pylori virulence factors, including adhesins, the VacA cytotoxin, and almost all cag pathogenicity island proteins, but has orthologs of the C. jejuni adhesin PEB1 and the cytolethal distending toxin (CDT). The genome contains a 71-kb genomic island (HHGI1) and several genomic islets whose G+C content differs from the rest of the genome. HHGI1 encodes three basic components of a type IV secretion system and other virulence protein homologs, suggesting a role of HHGI1 in pathogenicity. The genomic variability of H. hepaticus was assessed by comparing the genomes of 12 H. hepaticus strains with the sequenced genome by microarray hybridization. Although five strains, including all those known to have caused liver disease, were indistinguishable from

ATCC51449, other strains lacked between 85 and 229 genes, including large parts of HHGI1, demonstrating extensive variation of genome content within the species.

Sugiura, M., H. Aiba, et al. (2003). "Identification and classification of two-component systems that affect rpoS expression in Escherichia coli." Biosci Biotechnol Biochem **67**(7): 1612-5.

The rpoS-encoded sigmaS subunit of RNA polymerase regulates the expression of stationary phase and stress response genes in Escherichia coli. Recent study of our DNA microarray analysis suggested that the rpoS expression is affected by multiple two-component systems. In this study, we identified two-component-system mutants in which the rpoS expression increased. The regulatory manner of the systems on rpoS expression is suggested.

Sun, A., G. V. Devi-Rao, et al. (2004). "Immediate-early expression of the herpes simplex virus type 1 ICP27 transcript is not critical for efficient replication in vitro or in vivo." J Virol **78**(19): 10470-8.

We constructed a promoter mutation altering the immediate-early expression of the herpes simplex virus type 1 (HSV-1) ICP27 transcript and its cognate wild-type rescue viruses in order to assess the role of the ICP27 protein in the earliest stages of viral infection by global transcriptional analysis with a DNA microarray. This mutant, ICP27/VP16, replaces the whole ICP27 promoter/enhancer with the VP16 promoter. It demonstrates loss of immediate-early expression of ICP27 according to the criteria expression in the absence of de novo protein synthesis and earliest expression in the kinetic cascade. Significant differences in relative transcript abundances between the mutant and wild-type rescue viruses were limited at the earliest times measured and not evident at all by 4 h after infection. Consistent with this observation, levels of some critical proteins were reduced in the mutant as compared to rescue virus infections at the earliest times tested, but were equivalent by 8 h postinfection. Further, both single and multistep levels of virus replication were equivalent with both mutant and rescue viruses. Thus, altering the immediate-early kinetics of ICP27 leads to a suboptimal quantitative lag phase in gene expression but without consequence for replication fitness in vitro. Infections in vivo also revealed equivalent ability of mutant and rescue viruses to invade the central nervous system of mice following footpad injections. Limitations to an immediate-early role of ICP27 in the biology of HSV are discussed in light of these observations.

Sun, R., P. J. Converse, et al. (2004). "Mycobacterium tuberculosis ECF sigma factor sigC is required for lethality in mice and for the conditional expression of a defined gene set." Mol Microbiol **52**(1): 25-38.

Bacterial alternative RNA polymerase sigma factors are key global adaptive response regulators with a likely role in Mycobacterium tuberculosis pathogenesis. We constructed a mutant lacking the sigma factor gene, sigC, by allelic exchange, in the virulent CDC1551 strain of M. tuberculosis and compared the resulting mutant with the isogenic wild-type strain and complemented mutant strain. In vitro, compared to the wild-type and complemented strains, the mutant was found to have similar ability to survive in both murine bone marrow-derived macrophages and activated J774 macrophages. In time-to-death experiments in the mouse model, the DeltasigC mutant was significantly attenuated, causing no death in infected mice whereas the wild-type and complemented strains caused 100% mortality within 235 days after aerosol infection with a median time to death of 170 days. Mouse organ bacterial burdens indicated that the mutant proliferated and persisted at the same level as the wild-type and complemented strains in lung tissue and was able to persist in mice without causing death for > 300 days. A complete genomic microarray study demonstrated that SigC modulates the expression of several key virulence-associated genes including hspX, senX3 and mtrA, encoding the alpha-crystallin homologue, a two-component sensor kinase and a two-component response regulator respectively. Altered expression of a subset of these genes was confirmed by quantitative RT-PCR analysis. Analysis of genes modulated by SigC also revealed a putative consensus DNA recognition sequence for SigC of SSSAAT-N(16-20)-CGTSSS (S = C or G). Promoter recognition for one of these genes was confirmed by in vitro transcription analysis after purification of recombinant SigC and reconstitution of an Esigma(C) RNA

polymerase holoenzyme. These data indicate that the M. tuberculosis transcription factor SigC governs expression of an important M. tuberculosis regulon and is essential for lethality in mice, but is not required for bacterial survival in this species. These observations place the DeltasigC mutant in a class of M. tuberculosis mutants which persist in tissues but are attenuated in their ability to elicit lethal immunopathology.

Suscovich, T. J., M. Paulose-Murphy, et al. (2004). "Defective immune function of primary effusion lymphoma cells is associated with distinct KSHV gene expression profiles." Leuk Lymphoma **45**(6): 1223-38.

Primary effusion lymphomas (PEL) are uniformly infected with Kaposi's sarcoma-associated herpesvirus (KSHV), and thus likely present both tumor and viral antigens to the immune system. In order to grow unrestricted and cause disease, multiple immune evasion strategies may be utilized by PEL to evade immune surveillance. Using six well-established PEL cell lines and comparing these to Epstein-Barr virus-transformed B cell lines and peripheral blood B cells, significant differences were found in the surface expression of molecules involved in antigen presentation, T cell activation and cell-cell adhesion. Significantly reduced stimulation of cytotoxic T lymphocytes, lowered sensitivity to natural killer cell-mediated lysis and impaired function as antigen presenting cells in mixed leukocyte reactions were found for three PEL cell lines with particularly low CD54, CD58 and CD81 expression. Comparative microarray analysis demonstrated specific patterns of KSHV-encoded gene expression that were associated with the different immune functions of these cell lines. Thus, the present data suggest that distinct patterns of KSHV gene expression may be associated with particular phenotypic and functional characteristics of PEL cells, which may influence PEL pathogenesis.

Suwa, T., M. Chen, et al. (2003). "Zonal expression of dickkopf-3 and components of the Wnt signalling pathways in the human adrenal cortex." J Endocrinol **178**(1): 149-58.

The mechanisms underlying the differentiation of the adrenal cortex into zones are unclear. Microarray studies on RNA from microdissected zona reticularis (ZR) and zona fasciculata/zona glomerulosa (ZF/ZG) derived from adult human adrenal glands showed that a gene of the dickkopf family (DKK), DKK3, is differentially expressed in the zones. The Dickkopf proteins are morphogens involved in Wnt signalling. Northern blotting showed higher DKK3 transcript levels in ZF/ZG than ZR samples. In situ hybridization on adult human adrenal gland sections showed that DKK3 expression was much higher in the ZG than in the ZF or ZR. DKK3 expression was also higher in the medulla. We screened for expression of other members of the DKK family and the related Wingless-type mouse mammary tumor virus integration site gene family (WNT), frizzled (FZD), and dishevelled (DVL) gene families. Among dickkopf family members, only DKK3 was expressed at a detectable level in both human and mouse adrenocortical RNA samples. Consistent with previously published data on the effects of Wnt4 gene disruption in the mouse, we found only WNT4 expression within the WNT family in both human and mouse RNA. Northern blotting showed that WNT4 was expressed at a higher level in ZF/ZG cells than in ZR. The higher level of DKK3 and WNT4 expression in ZF/ZG cells was confirmed by real-time PCR. In the frizzled and dishevelled families we found FZD1, FZD2 and DVL3 transcripts in human adrenocortical RNA, and FZD2 and DVL3 in mouse adrenocortical RNA. These data show that a variety of genes of the Wnt signalling pathways are expressed in the adrenal cortex. The zonal distribution of DKK3 expression suggests that it could be involved in zonal differentiation or growth.

Suzuki, I., Y. Kanesaki, et al. (2001). "Cold-regulated genes under control of the cold sensor Hik33 in Synechocystis." Mol Microbiol **40**(1): 235-44.

A histidine kinase, Hik33, appears to sense decreases in temperature and to regulate the expression of certain cold-inducible genes in the cyanobacterium Synechocystis sp. PCC6803. To examine the role of Hik33 in the regulation of gene expression, we analysed a DeltaHik33 mutant using the DNA microarray technique. In wild-type cells, genes that were strongly induced at low temperature

encoded proteins that were predominantly subunits of the transcriptional and translational machinery. Most cold-repressible genes encoded components of the photosynthetic machinery. Mutation of the hik33 gene suppressed the expression of some of these cold-regulated genes, which could be divided into three groups according to the effect of the mutation of hik33. In the first group, regulation of gene expression by low temperature was totally abolished; in the second group, the extent of such regulation was reduced by half; and, in the third group, such regulation was totally unaffected. These results suggest that expression of the genes in the first group is regulated solely by Hik33, expression of genes in the third group is regulated by an as yet unidentified cold sensor, and expression of genes in the second group is regulated by both these cold sensors.

Swartz, J. M., J. Bystrom, et al. (2004). "Plasminogen activator inhibitor-2 (PAI-2) in eosinophilic leukocytes." J Leukoc Biol **76**(4): 812-9.

Plasminogen activator inhibitor-2 (PAI-2) as a potential eosinophil protein was inferred from our gene microarray study of mouse eosinophilopoiesis. Here, we detect 47 kDa intracellular and approximately 60 kDa secretory forms of PAI-2 in purified human eosinophil extracts. PAI-2 is present at variable concentrations in eosinophil lysates, ranging from 30 to 444 ng/10(6) cells, with a mean of 182 ng/10(6) cells from 10 normal donors, which is the highest per-cell concentration among all leukocyte subtypes evaluated. Enzymatic assay confirmed that eosinophil-derived PAI-2 is biologically active and inhibits activation of its preferred substrate, urokinase. Immunohistochemical and immunogold staining demonstrated PAI-2 localization in eosinophil-specific granules. Immunoreactive PAI-2 was detected in extracellular deposits in and around the eosinophil-enriched granuloma tissue encapsulating the parasitic egg in livers of wild-type mice infected with the helminthic parasite Schistosoma mansoni. Among the possibilities, we consider a role for eosinophil-derived PAI-2 in inflammation and remodeling associated with parasitic infection as well as allergic airways disease, respiratory virus infection, and host responses to tumors and metastasis in vivo.

Syder, A. J., S. M. Karam, et al. (2004). "A transgenic mouse model of metastatic carcinoma involving transdifferentiation of a gastric epithelial lineage progenitor to a neuroendocrine phenotype." Proc Natl Acad Sci U S A **101**(13): 4471-6.

Human neuroendocrine cancers (NECs) arise in various endoderm-derived epithelia, have diverse morphologic features, exhibit a wide range of growth phenotypes, and generally have obscure cellular origins and ill-defined molecular mediators of initiation and progression. We describe a transgenic mouse model of metastatic gastric cancer initiated by expressing simian virus 40 large tumor antigen (SV40 TAg), under control of regulatory elements from the mouse Atp4b gene, in the progenitors of acid-producing parietal cells. Parietal cells normally do not express endocrine or neural features, and Atp4b-Cre bitransgenic mice with a Cre reporter confirmed that the Atp4b regulatory elements are not active in gastric enteroendocrine cells. GeneChip analyses were performed on laser capture microdissected SV40 TAg-expressing cells in preinvasive foci and invasive tumors. Genes that distinguish invasive from preinvasive cells were then hierarchically clustered with DNA microarray datasets obtained from human lung and gastric cancers. The results, combined with immunohistochemical and electron microscopy studies of Apt4b-SV40 TAg stomachs, revealed that progression to invasion was associated with transdifferentiation of parietal cell progenitors to a neuroendocrine phenotype, and that invasive cells shared molecular features with NECs arising in the human pulmonary epithelium, including transcription factors that normally regulate differentiation of various endocrine lineages and maintain neural progenitors in an undifferentiated state. The 399 mouse genes identified as regulated during acquisition of an invasive phenotype and concomitant neuroendocrine transdifferentiation, plus their human orthologs associated with lung NECs, provide a foundation for molecular classification of NECs arising in other tissues and for genetic tests of the molecular mechanisms underlying NEC pathogenesis.

Tabuchi, Y. (2004). "Development of cell model with specific functions and its application to the study of

global gene expression." Yakugaku Zasshi **124**(5): 261-8.

The use of in vitro cell culture models has been of central importance in the development of our understanding of the cellular and molecular biology of organs and tissues. Transgenic mice and rats harboring temperature-sensitive simian virus 40 large T-antigen genes are useful for establishing cell lines from organs and tissues that have proved difficult to culture in vitro. Many conditionally immortalized cell lines with normal specific functions were generated from transgenic animals. DNA microarray technology has broad applications and is directed toward the study of global gene expression. Using established cell lines and DNA microarrays, we identified many genes that were up- and down-regulated in the process of the cell differentiation or cell death. In this review, the characteristics of established cell lines and possible applications of the study of global gene expression are discussed.

Taddeo, B., T. R. Luo, et al. (2003). "Activation of NF-kappaB in cells productively infected with HSV-1 depends on activated protein kinase R and plays no apparent role in blocking apoptosis." Proc Natl Acad Sci U S A **100**(21): 12408-13.

Microarray data reported elsewhere indicated that herpes simplex virus 1 induces the up-regulation of nuclear factor kappaB (NF-kappaB)-regulated genes, including that of its inhibitor, IkappaBalpha, consistent with the reports that wild-type virus induces the activation of NF-kappaB. In this report we show that activation of NF-kappaB in infected cells is linked to the activation of protein kinase R (PKR). Specifically: (i) PKR is activated in infected cells although the effects of the activated enzyme on protein synthesis are negated by the viral gene gamma134.5, which encodes a protein phosphatase 1alpha accessory factor that enables the dephosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. NF-kappaB is activated in wild-type murine embryonic fibroblasts but not in related PKR-null cells. (ii) In cells infected with a replication-competent Deltagamma134.5 mutant (R5104), but carrying a US11 gene expressed early in infection, eukaryotic translation initiation factor 2alpha is not phosphorylated, and in in vitro assays, PKR bound to the US11 protein is not phosphorylated on subsequent addition of double-stranded RNA. Here we report that this mutant does not activate PKR, has no effect on the accumulation of IkappaBalpha, and does not cause the translocation of NF-kappaB in infected cells. (iii) One hypothesis advanced for the activation of NF-kappaB is that it blocks apoptosis induced by viral gene products. The replication-competent R5104 mutant does not induce the programmed cell's death. We conclude that in herpes simplex virus 1-infected cells, activation of NF-kappaB depends on activation of PKR and that NF-kappaB is not required to block apoptosis in productively infected cells.

Talaat, A. M., S. T. Howard, et al. (2002). "Genomic DNA standards for gene expression profiling in Mycobacterium tuberculosis." Nucleic Acids Res **30**(20): e104.

A fundamental problem in DNA microarray analysis is the lack of a common standard to compare the expression levels of different samples. Several normalization protocols have been proposed to overcome variables inherent in this technology. As yet, there are no satisfactory methods to exchange gene expression data among different research groups or to compare gene expression values under different stimulus-response profiles. We have tested a normalization procedure based on comparing gene expression levels to the signals generated from hybridizing genomic DNA (genomic normalization). This procedure was applied to DNA microarrays of Mycobacterium tuberculosis using RNA extracted from cultures growing to the logarithmic and stationary phases. The applied normalization procedure generated reproducible measurements of expression level for 98% of the putative mycobacterial ORFs, among which 5.2% were significantly changed comparing the logarithmic to stationary growth phase. Additionally, analysis of expression levels of a subset of genes by real time PCR technology revealed an agreement in expression of 90% of the examined genes when genomic DNA normalization was applied instead of 29-68% agreement when RNA normalization was used to measure the expression levels in the same set of RNA samples. Further examination of microarray expression levels displayed clusters of genes differentially expressed between the logarithmic, early stationary and late stationary growth phases.

We conclude that genomic DNA standards offer advantages over conventional RNA normalization procedures and can be adapted for the investigation of microbial genomes.

Talaat, A. M., P. Hunter, et al. (2000). "Genome-directed primers for selective labeling of bacterial transcripts for DNA microarray analysis." Nat Biotechnol **18**(6): 679-82.

DNA microarrays have the ability to analyze the expression of thousands of the same set of genes under at least two different experimental conditions. However, DNA microarrays require substantial amounts of RNA to generate the probes, especially when bacterial RNA is used for hybridization (50 microg of bacterial total RNA contains approximately 2 microg of mRNA). We have developed a computer-based algorithm for prediction of the minimal number of primers to specifically anneal to all genes in a given genome. The algorithm predicts, for example, that 37 oligonucleotides should prime all genes in the Mycobacterium tuberculosis genome. We tested the usefulness of the genome-directed primers (GDPs) in comparison to random primers for gene expression profiling using DNA microarrays. Both types of primers were used to generate fluorescent-labeled probes and to hybridize to an array of 960 mycobacterial genes. Compared to random-primer probes, the GDP probes were more sensitive and more specific, especially when mammalian RNA samples were spiked with mycobacterial RNA. The GDPs were used for gene expression profiling of mycobacterial cultures grown to early log or stationary growth phases. This approach could be useful for accurate genome-wide expression analysis, especially for in vivo gene expression profiling, as well as directed amplification of sequenced genomes.

Tani, T. H., A. Khodursky, et al. (2002). "Adaptation to famine: a family of stationary-phase genes revealed by microarray analysis." Proc Natl Acad Sci U S A **99**(21): 13471-6.

Bacterial adaptation to nutrient limitation and increased population densities is central to survival and virulence. Surprisingly, <3% of Escherichia coli genes are known to play roles specific to the stationary phase. There is evidence that the leucine-responsive regulatory protein (Lrp) may play an important role in stationary phase, so this study used microarrays representing >98% of E. coli genes to more comprehensively identify those controlled by Lrp. The primary analysis compared isogenic Lrp(+) and Lrp(-) strains in cells growing in steady state in glucose minimal medium, either in the presence or absence of leucine. More than 400 genes were significantly Lrp-responsive under the conditions used. Transcription of 147 genes was lower in Lrp(+) than in Lrp(-) cells whether or not leucine was present; most of these genes were tightly coregulated under several conditions, including a burst of synthesis on transition to stationary phase. This cluster includes 56 of 115 genes already known to play roles in stationary phase. Our results suggest that the actual number of genes induced on entrance into stationary phase is closer to 200 and that Lrp affects nearly three-quarters of them, including genes involved in response to nutrient limitation, high concentrations of organic acids, and osmotic stress.

Taylor, L. A., C. M. Carthy, et al. (2000). "Host gene regulation during coxsackievirus B3 infection in mice: assessment by microarrays." Circ Res **87**(4): 328-34.

Host genetic responses that characterize enteroviral myocarditis have not yet been determined. The injurious and inflammatory process in heart muscle may reflect host responses of benefit to the virus and ultimately result in congestive heart failure and dilated cardiomyopathy. On the other hand, host responses within the myocardium may secure the host against acute or protracted damage. To investigate the nature of modified gene expression in comparison with normal tissue, mRNA species were assessed in myocardium using cDNA microarray technology at days 3, 9, and 30 after infection. Of 7000 clones initially screened, 169 known genes had a level of expression significantly different at 1 or more postinfection time points as compared with baseline. The known regulated genes were sorted according to their functional groups and normalized expression patterns and, subsequently, interpreted in the context of viremic, inflammatory, and healing phases of the myocarditic process.

Thijs, G., K. Marchal, et al. (2002). "A Gibbs sampling method to detect overrepresented motifs in the

upstream regions of coexpressed genes." J Comput Biol **9**(2): 447-64.

Microarray experiments can reveal important information about transcriptional regulation. In our case, we look for potential promoter regulatory elements in the upstream region of coexpressed genes. Here we present two modifications of the original Gibbs sampling algorithm for motif finding (Lawrence et al., 1993). First, we introduce the use of a probability distribution to estimate the number of copies of the motif in a sequence. Second, we describe the technical aspects of the incorporation of a higher-order background model whose application we discussed in Thijs et al. (2001). Our implementation is referred to as the Motif Sampler. We successfully validate our algorithm on several data sets. First, we show results for three sets of upstream sequences containing known motifs: 1) the G-box light-response element in plants, 2) elements involved in methionine response in Saccharomyces cerevisiae, and 3) the FNR O(2)-responsive element in bacteria. We use these data sets to explain the influence of the parameters on the performance of our algorithm. Second, we show results for upstream sequences from four clusters of coexpressed genes identified in a microarray experiment on wounding in Arabidopsis thaliana. Several motifs could be matched to regulatory elements from plant defence pathways in our database of plant cis-acting regulatory elements (PlantCARE). Some other strong motifs do not have corresponding motifs in PlantCARE but are promising candidates for further analysis.

Thomas, J. T., S. T. Oh, et al. (2001). "Cellular changes induced by low-risk human papillomavirus type 11 in keratinocytes that stably maintain viral episomes." J Virol **75**(16): 7564-71.

Infections by low-risk papillomavirus types, such as human papillomavirus (HPV) type 6 (HPV-6) and HPV-11, induce benign genital warts that rarely progress to malignancy. In contrast, lesions induced by high-risk HPV types have the potential to progress to cancer. Considerable information is available concerning the pathogenesis of high-risk HPV types, but little is known about the life cycle of low-risk HPV types. Although functionally distinct, both high- and low-risk virus types infect keratinocytes and induce virion production upon differentiation. This information suggests that they may share common mechanisms for regulating their productive life cycles. Using tissue culture methods developed to study high-risk HPV types, we examined the ability of HPV-11 to be stably maintained as episomes following transfection of normal human keratinocytes with cloned viral DNA. HPV-11 genomes were found to be maintained in keratinocytes for extended passages in cultures in 14 independent experiments involving transfection of cloned HPV-11 DNA. Interestingly, the HPV-11-positive cells exhibited an extended life span that averaged approximately twofold longer than that of control neomycin-transfected cells. In organotypic cultures, HPV-11-positive cells exhibited altered differentiation patterns, but the extent of disruption was less severe than that seen with high-risk HPV types. In addition, the amplification of HPV-11 DNA, as well as the induction of several viral messages, was observed following differentiation of transfected cells in semisolid media. To determine whether global changes in cellular gene expression induced by HPV-11 were similar to those observed with high-risk HPV-31 (Y. E. Chang and L. A. Laimins, J. Virol. 74:4174-4182, 2000), microarray analysis of 7,075 expressed sequences was performed. A spectrum of cellular genes different from that previously reported for HPV-31 was found to be activated or repressed by HPV-11. The expression of only a small set of genes was similarly altered by both high- and low-risk HPV types. This result suggests that different classes of HPVs have distinct effects on global cellular transcription patterns during infection. The methods described allow for a genetic analysis of HPV-11 in the context of its differentiation-dependent life cycle.

Thompson, D. K., A. S. Beliaev, et al. (2002). "Transcriptional and proteomic analysis of a ferric uptake regulator (fur) mutant of Shewanella oneidensis: possible involvement of fur in energy metabolism, transcriptional regulation, and oxidative stress." Appl Environ Microbiol **68**(2): 881-92.

The iron-directed, coordinate regulation of genes depends on the fur (ferric uptake regulator) gene product, which acts as an iron-responsive, transcriptional repressor protein. To investigate the biological function of a fur homolog in the dissimilatory metal-reducing bacterium Shewanella oneidensis

MR-1, a fur knockout strain (FUR1) was generated by suicide plasmid integration into this gene and characterized using phenotype assays, DNA microarrays containing 691 arrayed genes, and two-dimensional polyacrylamide gel electrophoresis. Physiological studies indicated that FUR1 was similar to the wild-type strain when they were compared for anaerobic growth and reduction of various electron acceptors. Transcription profiling, however, revealed that genes with predicted functions in electron transport, energy metabolism, transcriptional regulation, and oxidative stress protection were either repressed (ccoNQ, etrA, cytochrome b and c maturation-encoding genes, qor, yiaY, sodB, rpoH, phoB, and chvI) or induced (yggW, pdhC, prpC, aceE, fdhD, and ppc) in the fur mutant. Disruption of fur also resulted in derepression of genes (hxuC, alcC, fhuA, hemR, irgA, and ompW) putatively involved in iron uptake. This agreed with the finding that the fur mutant produced threefold-higher levels of siderophore than the wild-type strain under conditions of sufficient iron. Analysis of a subset of the FUR1 proteome (i.e., primarily soluble cytoplasmic and periplasmic proteins) indicated that 11 major protein species reproducibly showed significant ($P < 0.05$) differences in abundance relative to the wild type. Protein identification using mass spectrometry indicated that the expression of two of these proteins (SodB and AlcC) correlated with the microarray data. These results suggest a possible regulatory role of S. oneidensis MR-1 Fur in energy metabolism that extends the traditional model of Fur as a negative regulator of iron acquisition systems.

Thompson, L. J., D. S. Merrell, et al. (2003). "Gene expression profiling of Helicobacter pylori reveals a growth-phase-dependent switch in virulence gene expression." Infect Immun **71**(5): 2643-55.

The global pattern of growth-phase-dependent gene expression of Helicobacter pylori during in vitro culture was analyzed by using a high-density DNA microarray. To detect consistent coordinated gene expression in this bacterium, temporal changes in transcription were assessed in two independent time courses. Cluster analysis of the expression profiles highlighted a major switch in gene expression during the late log-to-stationary phase transition that we have termed the Log-Stat switch. Statistical analysis of the genes that were significantly induced or repressed during the Log-Stat switch revealed that many of these genes were related to virulence. Among these, expression of the genes for the neutrophil activating protein (napA) and the major flagellin subunit (flaA) were significantly induced. Additionally, the expression of a number of genes involved in iron homeostasis changed dramatically at this switch; the gene for the iron-storage protein, pfr, was induced, while the genes for two putative iron uptake proteins, fecA and frpB, were significantly repressed. These data suggest that the late log phase may correspond to the most virulent phase of growth in H. pylori and may be intimately related to its pathogenesis. The use of microarrays to analyze the kinetics of the transcriptional response of a bacterial pathogen to a changing environment has enabled the discovery of previously unappreciated relationships between genes by elucidation of coordinated gene expression profiles.

Thomson, N., S. Baker, et al. (2004). "The role of prophage-like elements in the diversity of Salmonella enterica serovars." J Mol Biol **339**(2): 279-300.

The Salmonella enterica serovar Typhi CT18 (S.Typhi) chromosome harbours seven distinct prophage-like elements, some of which may encode functional bacteriophages. In silico analyses were used to investigate these regions in S.Typhi CT18, and ultimately compare these integrated bacteriophages against 40 other Salmonella isolates using DNA microarray technology. S.Typhi CT18 contains prophages that show similarity to the lambda, Mu, P2 and P4 bacteriophage families. When compared to other S.Typhi isolates, these elements were generally conserved, supporting a clonal origin of this serovar. However, distinct variation was detected within a broad range of Salmonella serovars; many of the prophage regions are predicted to be specific to S.Typhi. Some of the P2 family prophage analysed have the potential to carry non-essential "cargo" genes within the hyper-variable tail region, an observation that suggests that these bacteriophage may confer a level of specialisation on their host. Lysogenic bacteriophages therefore play a crucial role in the generation of genetic diversity within S.enterica.

Tien, E. S., J. P. Gray, et al. (2003). "Comprehensive gene expression analysis of peroxisome proliferator-treated immortalized hepatocytes: identification of peroxisome proliferator-activated receptor alpha-dependent growth regulatory genes." Cancer Res **63**(18): 5767-80.

Chemicals known as peroxisome proliferators (PPs) are the subject of intense study because of their ability to cause hepatocellular carcinoma in laboratory rodents. These chemicals act through a family of proteins termed the peroxisome proliferator-activated receptors (PPARs), in particular PPARalpha. It has become increasingly apparent that the role of the PPs in the development of cancer encompasses many different aspects of cell growth regulation. Immortalized hepatocytes from wild-type (PPARalpha(+/+)) and PPARalpha(-/-) mice were generated using a temperature-sensitive SV40 virus. Characterization of the murine SV40 hepatocytes (MuSH) generated from both genotypes (MuSHalpha(+/+), MuSHalpha(-/-)) show markers of differentiation such as albumin expression, but is devoid of Kupffer cell contamination. Hallmark PPARalpha-mediated responses such as induction of acyl-CoA oxidase mRNA by PPs are present in the MuSHalpha(+/+) but are absent in MuSHalpha(-/-) cells. In contrast to most cell culture systems, the wild-type MuSH hepatocytes retain the mitogenic activity of PPs, whereas the MuSHalpha(-/-) does not respond in this manner, thus making this cell culture system an ideal tool to examine growth regulatory gene expression affected by PPs. Microarray experiments performed on both cell types identified many genes in which regulation is dependent on the presence of PPARalpha, and these changes were verified with reverse transcriptase-PCR. Genes involved in carcinogenesis and control of the cell cycle that are regulated by PPs in a PPARalpha-dependent manner include ubiquitin COOH-terminal hydrolase 37 (also known as UCT-L5) and cyclin T1. These results show that MuSH cells reflect the biological properties of both the wild-type and PPARalpha-null animals and can be used to identify novel PPARalpha-regulated genes that could be involved in regulation of the cell cycle and carcinogenesis.

Tong, H. H., J. P. Long, et al. (2004). "Alteration of gene expression in human middle ear epithelial cells induced by influenza A virus and its implication for the pathogenesis of otitis media." Microb Pathog **37**(4): 193-204.

Influenza A virus infection plays a significant role in the pathogenesis of Streptococcus pneumoniae-induced acute otitis media in children. An understanding of how influenza A virus modulates host cellular responses is critically important in efforts to explore the molecular mechanisms of this synergism. We used microarray technology to characterize the mRNA expression profile in human middle ear epithelial cells induced by influenza A virus. Alterations of mRNA expression in 142 out of approximately 12,600 genes were observed at 24h after virus infection. Of these 142 genes with altered expression, interferon inducible genes, chemokine and cytokine genes, pro- and antiapoptotic genes, signal transduction and transcription factors, cellular immune response, cell cycle and metabolism genes were the most prominent. Our results reveal several previously unknown alterations of host gene expression induced by influenza A virus which may provide new targets for further analysis of its role in this particular host-pathogen interaction.

Tong, X., J. W. Campbell, et al. (2004). "Genome-scale identification of conditionally essential genes in E. coli by DNA microarrays." Biochem Biophys Res Commun **322**(1): 347-54.

Identifying the genes required for the growth or viability of an organism under a given condition is an important step toward understanding the roles these genes play in the physiology of the organism. Currently, the combination of global transposon mutagenesis with PCR-based mapping of transposon insertion sites is the most common method for determining conditional gene essentiality. In order to accelerate the detection of essential gene products, here we test the utility and reliability of a DNA microarray technology-based method for the identification of conditionally essential genes of the bacterium, Escherichia coli, grown in rich medium under aerobic or anaerobic growth conditions using two different DNA microarray platforms. Identification and experimental verification of five hypothetical E. coli genes essential for anaerobic growth directly demonstrated the utility of the method. However, the

two different DNA microarray platforms yielded largely non-overlapping results after a two standard deviations cutoff and were subjected to high false positive background levels. Thus, further methodological improvements are needed prior to the use of DNA microarrays to reliably identify conditionally essential genes on genome-scale.

Torpey, N., S. E. Maher, et al. (2004). "Interferon alpha but not interleukin 12 activates STAT4 signaling in human vascular endothelial cells." J Biol Chem **279**(25): 26789-96.

STAT4 signaling, activated by either interleukin 12 (IL12) or interferon alpha (IFNalpha), promotes T(H)1 responses in CD4(+) T cells. Vascular endothelial cells (EC) may also become polarized in response to various cytokines, favoring recruitment and activation of T(H)1 or T(H)2 effector cells. Here we have investigated the role of the STAT4 pathway in EC. Cultured human umbilical vein EC (HUVEC) express low levels of STAT4, which may be tyrosine-phosphorylated by treatment with IFNalpha but not IL12. This is because HUVEC lack both subunits of the IL12 receptor (IL12Rbeta1 and IL12Rbeta2), even following treatment with various cytokines. IL12 phosphorylation of STAT4 can be observed in HUVEC that have been transduced to express the IL12R. To identify STAT4-induced genes we pursued three approaches: analysis by DNA microarray and quantitative RT-PCR (Q-PCR) of the IL12 responses in IL12R-transduced EC; analysis by Q-PCR of IFNalpha responses in STAT4-overexpressing EC; and analysis of IFNalpha responses in U3A neuroblastoma cell lines that express either STAT1 or STAT4, but not both. In all three instances we observe STAT4-mediated induction of the chemokine monocyte chemoattractant protein 1 (MCP1) and suppressor of cytokine signaling 3 (SOCS3) mRNA, and we confirm the production of each protein in both IL12R-transduced EC and STAT4-transduced U3A cells. These observations reveal that there is a STAT4 response of EC, activated by IFNalpha but not IL12, and that it may modulate the pro-inflammatory behavior of EC.

Tracy, B. S., K. K. Edwards, et al. (2002). "Carbon and nitrogen substrate utilization by archival Salmonella typhimurium LT2 cells." BMC Evol Biol **2**(1): 14.

BACKGROUND: A collection of over 20,000 Salmonella typhimurium LT2 mutants, sealed for four decades in agar stabs, is a unique resource for study of genetic and evolutionary changes. Previously, we reported extensive diversity among descendants including diversity in RpoS and catalase synthesis, diversity in genome size, protein content, and reversion from auxotrophy to prototrophy. RESULTS: Extensive and variable losses and a few gains of catabolic functions were observed by this standardized method. Thus, 95 catabolic reactions were scored in each of three plates in wells containing specific carbon and nitrogen substrates. CONCLUSION: While the phenotype microarray did not reveal a distinct pattern of mutation among the archival isolates, the data did confirm that various isolates have used multiple strategies to survive in the archival environment. Data from the MacConkey plates verified the changes in carbohydrate metabolism observed in the Biolog system.

Tsai, J. M., H. C. Wang, et al. (2004). "Genomic and proteomic analysis of thirty-nine structural proteins of shrimp white spot syndrome virus." J Virol **78**(20): 11360-70.

White spot syndrome virus (WSSV) virions were purified from the hemolymph of experimentally infected crayfish Procambarus clarkii, and their proteins were separated by 8 to 18% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to give a protein profile. The visible bands were then excised from the gel, and following trypsin digestion of the reduced and alkylated WSSV proteins in the bands, the peptide sequence of each fragment was determined by liquid chromatography-nano-electrospray ionization tandem mass spectrometry (LC-nanoESI-MS/MS) using a quadrupole/time-of-flight mass spectrometer. Comparison of the resulting peptide sequence data against the nonredundant database at the National Center for Biotechnology Information identified 33 WSSV structural genes, 20 of which are reported here for the first time. Since there were six other known WSSV structural proteins that could not be identified from the SDS-PAGE bands, there must therefore be a total of at least 39 (33 + 6) WSSV structural protein genes. Only 61.5% of the WSSV structural genes have a polyadenylation

signal, and preliminary analysis by 3' rapid amplification of cDNA ends suggested that some structural protein genes produced mRNA without a poly(A) tail. Microarray analysis showed that gene expression started at 2, 6, 8, 12, 18, 24, and 36 hpi for 7, 1, 4, 12, 9, 5, and 1 of the genes, respectively. Based on similarities in their time course expression patterns, a clustering algorithm was used to group the WSSV structural genes into four clusters. Genes that putatively had common or similar roles in the viral infection cycle tended to appear in the same cluster.

Tseng, G. C., M. K. Oh, et al. (2001). "Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects." Nucleic Acids Res **29**(12): 2549-57.

We consider the problem of comparing the gene expression levels of cells grown under two different conditions using cDNA microarray data. We use a quality index, computed from duplicate spots on the same slide, to filter out outlying spots, poor quality genes and problematical slides. We also perform calibration experiments to show that normalization between fluorescent labels is needed and that the normalization is slide dependent and non-linear. A rank invariant method is suggested to select non-differentially expressed genes and to construct normalization curves in comparative experiments. After normalization the residuals from the calibration data are used to provide prior information on variance components in the analysis of comparative experiments. Based on a hierarchical model that incorporates several levels of variations, a method for assessing the significance of gene effects in comparative experiments is presented. The analysis is demonstrated via two groups of experiments with 125 and 4129 genes, respectively, in Escherichia coli grown in glucose and acetate.

Tsoi, S. C., J. M. Cale, et al. (2003). "Use of human cDNA microarrays for identification of differentially expressed genes in Atlantic salmon liver during Aeromonas salmonicida infection." Mar Biotechnol (NY) **5**(6): 545-54.

Commercially available human complementary DNA microarrays were used to compare differential expression in the livers of Atlantic salmon (Salmo salar) infected with Aeromonas salmonicida and of healthy fish. Complementary DNA probes were prepared from total RNA isolated from livers of control salmon and infected salmon by reverse transcription in the presence of (33)P-dCTP and independently hybridized to human GENE-FILTERS GF211 microarrays. Of the 4131 known genes on the microarray, 241 spots gave clearly detectable signals using labeled RNA from the control salmon liver. Of these, 4 spots were consistently found to have a greater than 2-fold increase in infected salmon compared with controls when using the same pair of filters to generate hybridization data from triplicates. These up-regulated genes were ADP/ATP translocase (AAT2), Na(+)/K(+) ATPase, acyloxyacyl hydrolase (AOAH), and platelet-derived growth factor (PDFG-A). A BlastN search revealed an AAT2 homolog from Atlantic salmon, and a reverse transcriptase polymerase chain reaction assay using primers based on this sequence confirmed its up-regulation (approx. 1.8-fold) during early infection. This work demonstrates the feasibility of using human microarrays to facilitate the discovery of differentially expressed genes in Atlantic salmon, for which no homologous microarrays are available.

Tsukasaki, K., S. Tanosaki, et al. (2004). "Identifying progression-associated genes in adult T-cell leukemia/lymphoma by using oligonucleotide microarrays." Int J Cancer **109**(6): 875-81.

Adult T-cell leukemia/lymphoma (ATL) is associated with human T-lymphotropic virus type-1 (HTLV-1). To understand the changes in expression that occur in the progression of chronic phase of ATL to acute crisis, the gene expression profiles of fresh ATL cells were compared in 4 pairs of samples (progression of chronic to acute phase in 3 patients, as well as 1 typical chronic phase sample vs. 1 typical acute phase sample) using high-density oligonucleotide DNA arrays. We identified 203 genes that were commonly upregulated in acute vs. chronic phase samples including ribosomal proteins, proteosome subunits, eukaryotic translation factors, immunophilins, heat shock proteins and genes important for DNA replication. Additionally, we identified 91 commonly downregulated genes including immune molecules related to MHC and a phosphatase. Several of the genes were previously identified to be associated with

the Tax protein of HTLV-1. Some of the upregulated genes were located in amplified regions identified by comparative genomic hybridization in the corresponding chronic/acute ATL sample. Using real-time quantitative PCR, we confirmed the array-results in those specimens analyzed by microarray. These results demonstrated that distinct sets of genes that are known to be critical in cellular transformation and/or activation are up- or down-regulated during the transition to the acute phase of ATL.

Tsutsumi, T., T. Suzuki, et al. (2003). "Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice." Hepatology **38**(4): 820-8.

In human chronic hepatitis C, alcohol intake is a synergistic factor for the acceleration of hepatocarcinogenesis. Recently, we showed a significant increase of reactive oxygen species (ROS) in hepatitis C virus (HCV) core-transgenic mice fed ethanol-containing diets. Because previous studies indicated that ROS is closely associated with mitogen-activated protein kinases (MAPK), we examined activities of c-Jun N-terminal kinase, p38 MAPK, and extracellular signal-regulated kinase (ERK) in the liver of core-transgenic and nontransgenic mice with short-term ethanol feeding. Activity of ERK and p38 MAPK was increased in core-transgenic mice compared with nontransgenic mice, whereas neither ERK nor p38 MAPK was activated in core-transgenic mice with normal diets. In addition, activity of cyclic-AMP and serum responsive element, downstream pathways of p38 MAPK and ERK, was also increased. Comparison of gene expression profiles by cDNA microarray and real-time PCR revealed that galectin-1, which is associated with cell transformation, was significantly increased in ethanol-fed core-transgenic mice. On the other hand, glutathione S-transferase (GST), which plays a key role in protecting cells from oxidative stress, was decreased. In conclusion, these results suggest that HCV core protein cooperates with ethanol for the activation of some MAPK pathways, and leads to the modulation of several genes, contributing to the pathogenesis of liver disease of HCV-infected patients with high ethanol consumption.

Tummala, S. B., S. G. Junne, et al. (2003). "Antisense RNA downregulation of coenzyme A transferase combined with alcohol-aldehyde dehydrogenase overexpression leads to predominantly alcohologenic Clostridium acetobutylicum fermentations." J Bacteriol **185**(12): 3644-53.

Plasmid pAADB1 for the overexpression of the alcohol-aldehyde dehydrogenase (aad) gene and downregulation of the coenzyme A transferase (CoAT) using antisense RNA (asRNA) against ctfB (the second CoAT gene on the polycistronic aad-ctfA-ctfB message) was used in order to increase the butanol/acetone ratio of Clostridium acetobutylicum ATCC 824 fermentations. Acetone and butanol levels were drastically reduced in 824(pCTFB1AS) (expresses only an asRNA against ctfB) compared to 824(pSOS95del) (plasmid control). Compared to strain 824(pCTFB1AS), 824(pAADB1) fermentations exhibited two profound differences. First, butanol levels were ca. 2.8-fold higher in 824(pAADB1) and restored back to plasmid control levels, thus supporting the hypothesis that asRNA downregulation of ctfB leads to degradation of the whole aad-ctfA-ctfB transcript. Second, ethanol titers in 824(pAADB1) were ca. 23-fold higher and the highest (ca. 200 mM) ever reported in C. acetobutylicum. Western blot analysis confirmed that CoAT was downregulated in 824(pAADB1) at nearly the same levels as in strain 824(pCTFB1AS). Butyrate depletion in 824(pAADB1) fermentations suggested that butyryl-CoA was limiting butanol production in 824(pAADB1). This was confirmed by exogenously adding butyric acid to 824(pAADB1) fermentations to increase the butanol/ethanol ratio. DNA microarray analysis showed that aad overexpression profoundly affects the large-scale transcriptional program of the cells. Several classes of genes were differentially expressed [strain 824(pAADB1) versus strain 824(pCTFB1AS)], including genes of the stress response, sporulation, and chemotaxis. The expression patterns of the CoAT genes (ctfA and ctfB) and aad were consistent with the overexpression of aad and asRNA downregulation of ctfB.

Tzankov, A., A. Zimpfer, et al. (2003). "High-throughput tissue microarray analysis of G1-cyclin alterations in classical Hodgkin's lymphoma indicates overexpression of cyclin E1." J Pathol **199**(2): 201-7.

Deregulation of G1-cyclins (CCN) plays a key role in the pathogenesis of many human malignancies, including non-Hodgkin's lymphomas (NHLs). In contrast to NHL, little is known about phenotypic and genotypic changes in the regulation of the cell cycle in classical Hodgkin's lymphoma (cHL). To facilitate analysis of aberrant gene expression in cHL, a lymphoma tissue microarray (TMA) containing 752 cores of 330 different cHL samples was constructed. Direct comparison of Epstein-Barr virus (EBV) latent membrane protein 1 (LMP-1) expression in Hodgkin's and Reed-Sternberg (HRS) cells on conventional full sections with the corresponding duplicate/triplicate tumour cores on the TMA showed a concordance of 100%, indicating that cHL-TMA is a reliable and representative method for evaluating gene expression profiles in situ. Using TMA technology, protein expression and gene amplification of different G1-CCNs in cHL were analysed. Among the G1-CCNs analysed, cyclin E (CCNE) was expressed in 212/253 cases (84%). In most of the individual tumours, over 75% of the HRS cells stained positive for CCNE, suggesting that CCNE is overexpressed in cHL. This overexpression was not due to CCNE gene amplification, as judged by fluorescence in situ hybridization, and did not correlate with EBV infection, as assessed by the expression of LMP-1. Thus, the overexpression of CCNE could be caused by profound changes in HRS cell-cycle regulation that could contribute to the malignant phenotype.

Ulrich, R. L., H. B. Hines, et al. (2004). "Mutational analysis and biochemical characterization of the Burkholderia thailandensis DW503 quorum-sensing network." J Bacteriol **186**(13): 4350-60.

Numerous gram-negative bacteria communicate and regulate gene expression through a cell density-responsive mechanism termed quorum sensing (QS), which involves the synthesis and perception of diffusible N-acyl-homoserine lactones (AHL). In this study we genetically and physiologically characterized the Burkholderia thailandensis DW503 QS network. In silico analysis of the B. thailandensis genome revealed the presence of at least three AHL synthases (AHS) and five transcriptional regulators belonging to the LuxIR family of proteins. Mass spectrometry demonstrated that wild-type B. thailandensis synthesizes N-hexanoyl-homoserine lactone (C6-HSL), N-octanoyl-homoserine lactone (C8-HSL), and N-decanoyl-homoserine lactone (C10-HSL). Mutation of the btaI1 (luxI) AHS gene prevented accumulation of C8-HSL in culture supernatants, enhanced beta-hemolysis of sheep erythrocytes, increased lipase production, and altered colony morphology on swarming and twitching motility plates. Disruption of the btaI3 (luxI) AHS prevented biosynthesis of C6-HSL and increased lipase production and beta-hemolysis, whereas mutagenesis of the btaI2 (luxI) allele eliminated C10-HSL accumulation and reduced lipase production. Complementation of the btaI1 and btaI3 mutants fully restored the synthesis of C8-HSL and C6-HSL to parental levels. In contrast, mutagenesis of the btaR1, btaR3, btaR4, and btaR5 (luxR) transcriptional regulators had no effect on AHL accumulation, enhanced lipase production, and resulted in extensive beta-hemolysis on sheep blood agar plates. Furthermore, interruption of the btaI1, btaR1, and btaR3 genes altered colony morphology on twitching and swarming motility plates and induced pigmentation. Additionally, phenotypic microarray analysis indicated that QS in B. thailandensis both positively and negatively affects the metabolism of numerous substrates, including citric acid, formic acid, glucose 6-phosphate, capric acid, gamma-hydroxybutyric acid, and d-arabinose. These results demonstrate that mutagenesis of the B. thailandensis QS system affects various cellular processes, including lipase production, swarming and twitching motility, beta-hemolysis of sheep erythrocytes, and carbon metabolism and/or transport.

Urakawa, H., S. El Fantroussi, et al. (2003). "Optimization of single-base-pair mismatch discrimination in oligonucleotide microarrays." Appl Environ Microbiol **69**(5): 2848-56.

The discrimination between perfect-match and single-base-pair-mismatched nucleic acid duplexes was investigated by using oligonucleotide DNA microarrays and nonequilibrium dissociation rates (melting profiles). DNA and RNA versions of two synthetic targets corresponding to the 16S rRNA sequences of Staphylococcus epidermidis (38 nucleotides) and Nitrosomonas eutropha (39 nucleotides) were hybridized to perfect-match probes (18-mer and 19-mer) and to a set of probes having all possible

single-base-pair mismatches. The melting profiles of all probe-target duplexes were determined in parallel by using an imposed temperature step gradient. We derived an optimum wash temperature for each probe and target by using a simple formula to calculate a discrimination index for each temperature of the step gradient. This optimum corresponded to the output of an independent analysis using a customized neural network program. These results together provide an experimental and analytical framework for optimizing mismatch discrimination among all probes on a DNA microarray.

Valerius, M. T., L. T. Patterson, et al. (2002). "Microarray analysis of novel cell lines representing two stages of metanephric mesenchyme differentiation." Mech Dev **112**(1-2): 219-32.

Clonal cell lines representing different developmental stages of the metanephric mesenchyme were made from transgenic mice with the Simian Virus 40 T-antigen (SV40 Tag) gene driven by the Hoxa 11 promoter. The resulting mK3 cell line represented early metanephric mesenchyme, prior to induction by the ureteric bud. These cells showed a spindle-shaped, fibroblast morphology. They expressed genes characteristic of early mesenchyme, including Hoxa 11, Hoxd 11, collagen I, and vimentin. Moreover, the mK3 cells displayed early metanephric mesenchyme biological function. In organ co-culture experiments they were able to induce growth and branching of the ureteric bud. Another cell line, mK4, represented later, induced metanephric mesenchyme undergoing epithelial conversion. These cells were more polygonal, or epithelial in shape, and expressed genes diagnostic of late mesenchyme, including Pax-2, Pax-8, Wnt-4, Cadherin-6, Collagen IV, and LFB3. To better define the gene expression patterns of kidney metanephric mesenchyme cells at these two stages of development, RNAs from the mK3 and mK4 cells were hybridized to Affymetrix GeneChip probe arrays. Over 4000 expressed genes were identified and thereby implicated in kidney formation. Comparison of the mK3 and mK4 gene expression profiles revealed 121 genes showing greater than a ten-fold difference in expression level. Several are known to be expressed during metanephric mesenchyme differentiation, but most had not been previously associated with this process. In situ hybridizations were used to confirm that selected novel genes were expressed in the developing kidney.

Valerius, M. T., L. T. Patterson, et al. (2002). "Microarray analysis of novel cell lines representing two stages of metanephric mesenchyme differentiation." Mech Dev **110**(1-2): 151-64.

Clonal cell lines representing different developmental stages of the metanephric mesenchyme were made from transgenic mice with the Simian Virus 40 T-antigen (SV40 Tag) gene driven by the Hoxa 11 promoter. The resulting mK3 cell line represented early metanephric mesenchyme, prior to induction by the ureteric bud. These cells showed a spindle-shaped, fibroblast morphology. They expressed genes characteristic of early mesenchyme, including Hoxa 11, Hoxd 11, collagen I, and vimentin. Moreover, the mK3 cells displayed early metanephric mesenchyme biological function. In organ co-culture experiments they were able to induce growth and branching of the ureteric bud. Another cell line, mK4, represented later, induced metanephric mesenchyme undergoing epithelial conversion. These cells were more polygonal, or epithelial in shape, and expressed genes diagnostic of late mesenchyme, including Pax-2, Pax-8, Wnt-4, Cadherin-6, Collagen IV, and LFB3. To better define the gene expression patterns of kidney metanephric mesenchyme cells at these two stages of development, RNAs from the mK3 and mK4 cells were hybridized to Affymetrix GeneChip probe arrays. Over 4000 expressed genes were identified and thereby implicated in kidney formation. Comparison of the mK3 and mK4 gene expression profiles revealed 121 genes showing greater than a ten-fold difference in expression level. Several are known to be expressed during metanephric mesenchyme differentiation, but most had not been previously associated with this process. In situ hybridizations were used to confirm that selected novel genes were expressed in the developing kidney.

van de Peppel, J., P. Kemmeren, et al. (2003). "Monitoring global messenger RNA changes in externally controlled microarray experiments." EMBO Rep **4**(4): 387-93.

Expression profiling is a universal tool, with a range of applications that benefit from the accurate

determination of differential gene expression. To allow normalization using endogenous transcript levels, current microarray analyses assume that relatively few transcripts vary, or that any changes that occur are balanced. When normalization using endogenous genes is carried out, changes in expression levels are calculated relative to the behaviour of most of the transcripts. This does not reflect absolute changes if global shifts in messenger RNA populations occur. Using external RNA controls, we have set up microarray experiments to monitor global changes. The levels of most mRNAs were found to change during yeast stationary phase and human heat shock when external controls were included. Even small global changes had a significant effect on the number of genes reported as being differentially expressed. This suggests that global mRNA changes occur more frequently than is assumed at present, and shows that monitoring such effects may be important for the accurate determination of changes in gene expression.

Van Dyk, T. K., Y. Wei, et al. (2001). "A genomic approach to gene fusion technology." Proc Natl Acad Sci U S A **98**(5): 2555-60.

Gene expression profiling provides powerful analyses of transcriptional responses to cellular perturbation. In contrast to DNA array-based methods, reporter gene technology has been underused for this application. Here we describe a genomewide, genome-registered collection of Escherichia coli bioluminescent reporter gene fusions. DNA sequences from plasmid-borne, random fusions of E. coli chromosomal DNA to a Photorhabdus luminescens luxCDABE reporter allowed precise mapping of each fusion. The utility of this collection covering about 30% of the transcriptional units was tested by analyzing individual fusions representative of heat shock, SOS, OxyR, SoxRS, and cya/crp stress-responsive regulons. Each fusion strain responded as anticipated to environmental conditions known to activate the corresponding regulatory circuit. Thus, the collection mirrors E. coli's transcriptional wiring diagram. This genomewide collection of gene fusions provides an independent test of results from other gene expression analyses. Accordingly, a DNA microarray-based analysis of mitomycin C-treated E. coli indicated elevated expression of expected and unanticipated genes. Selected luxCDABE fusions corresponding to these up-regulated genes were used to confirm or contradict the DNA microarray results. The power of partnering gene fusion and DNA microarray technology to discover promoters and define operons was demonstrated when data from both suggested that a cluster of 20 genes encoding production of type I extracellular polysaccharide in E. coli form a single operon.

Ventura, M., C. Canchaya, et al. (2003). "Integration and distribution of Lactobacillus johnsonii prophages." J Bacteriol **185**(15): 4603-8.

In Lactobacillus johnsonii strain NCC533, two prophages were integrated into tRNA genes and one was disrupted by integration. In a survey, the prophages were restricted to strains sharing an essentially identical restriction pattern. Microarray analysis showed that the prophage DNA represents about 50% of the NCC533 strain-specific DNA.

Vo-Dinh, T., J. P. Alarie, et al. (1999). "DNA biochip using a phototransistor integrated circuit." Anal Chem **71**(2): 358-63.

This work describes the development of an integrated biosensor based on phototransistor integrated circuits (IC) for use in medical detection, DNA diagnostics, and gene mapping. The evaluation of various system components developed for an integrated biosensor microchip is discussed. Methods to develop a microarray of DNA probes on nitrocellulose substrate are discussed. The biochip device has sensors, amplifiers, discriminators, and logic circuitry on board. Integration of light-emitting diodes into the device is also possible. To achieve improved sensitivity, we have designed an IC system having each phototransistor sensing element composed of 220 phototransistor cells connected in parallel. Measurements of fluorescent-labeled DNA probe microarrays and hybridization experiments with a sequence-specific DNA probe for the human immunodeficiency virus 1 system on nitrocellulose substrates illustrate the usefulness and potential of the DNA biochip.

Wade, M. M., D. Volokhov, et al. (2004). "Accurate mapping of mutations of pyrazinamide-resistant Mycobacterium tuberculosis strains with a scanning-frame oligonucleotide microarray." Diagn Microbiol Infect Dis **49**(2): 89-97.

The increasing emergence of drug-resistant Mycobacterium tuberculosis poses significant threat to the treatment of tuberculosis. Conventional susceptibility testing for the front-line tuberculosis drug pyrazinamide (PZA) is difficult, because of the requirement for acid pH for the drug to show activity. Resistance to PZA in M. tuberculosis is caused by mutations in the pncA gene, and detection of pncA mutations can be an indicator of PZA resistance. In this study, we examined the feasibility of a microarray-based approach exploiting short overlapping oligonucleotides (sliding-frame array) to rapidly detect pncA mutations (substitutions, deletions, and insertions) in multiple strains of PZA-resistant M. tuberculosis. The genetic mapping of these mutations is necessary to link the gene sequence to the protein function defined by mutant phenotype. Microarray analysis was performed in a blind manner using 57 isolates of M. tuberculosis for which the sequence of the pncA gene was previously determined. Our results showed that all mutations could be unambiguously detected, suggesting that microarray can be a routine and valuable tool for rapid identification of drug-resistant M. tuberculosis isolates. We expect that mutation mapping with a sliding-frame microarray will accelerate the molecular analysis of drug-resistant M. tuberculosis bacteria and the microorganism populations.

Wagner, V. E., D. Bushnell, et al. (2003). "Microarray analysis of Pseudomonas aeruginosa quorum-sensing regulons: effects of growth phase and environment." J Bacteriol **185**(7): 2080-95.

Bacterial communication via quorum sensing (QS) has been reported to be important in the production of virulence factors, antibiotic sensitivity, and biofilm development. Two QS systems, known as the las and rhl systems, have been identified previously in the opportunistic pathogen Pseudomonas aeruginosa. High-density oligonucleotide microarrays for the P. aeruginosa PAO1 genome were used to investigate global gene expression patterns modulated by QS regulons. In the initial experiments we focused on identifying las and/or rhl QS-regulated genes using a QS signal generation-deficient mutant (PAO-JP2) that was cultured with and without added exogenous autoinducers [N-(3-oxododecanoyl) homoserine lactone and N-butyryl homoserine lactone]. Conservatively, 616 genes showed statistically significant differential expression (P </= 0.05) in response to the exogenous autoinducers and were classified as QS regulated. A total of 244 genes were identified as being QS regulated at the mid-logarithmic phase, and 450 genes were identified as being QS regulated at the early stationary phase. Most of the previously reported QS-promoted genes were confirmed, and a large number of additional QS-promoted genes were identified. Importantly, 222 genes were identified as being QS repressed. Environmental factors, such as medium composition and oxygen availability, eliminated detection of transcripts of many genes that were identified as being QS regulated.

Wan, J., F. M. Dunning, et al. (2002). "Probing plant-pathogen interactions and downstream defense signaling using DNA microarrays." Funct Integr Genomics **2**(6): 259-73.

The interaction between a plant and a pathogen activates a wide variety of defense responses. The recent development of microarray-based expression profiling methods, together with the availability of genomic and/or EST (expressed sequence tag) sequence data for some plant species, has allowed significant progress in the characterization of plant pathogenesis-related responses. The small number of expression profiling studies completed to date have already identified an amazing number of genes that had not previously been implicated in plant defense. Some of these genes can be associated with defense signal transduction or antimicrobial action, but the functional contribution of many others remains uncertain. Initial expression profiling work has also revealed similarities and distinctions between different defense signaling pathways, and cross-talk (both overlap and interference) between pathogenesis-related responses and plant responses to other stresses. Potential transcriptional cis-regulatory elements upstream of co-regulated genes can also be identified. Whole-genome arrays are only now becoming available, and many interactions remain to be studied (e.g. different pathogen species,

plant genotypes, mutants, time-points after infection). Expression profiling technologies, in combination with other genomic tools, will have a substantial impact on our understanding of plant-pathogen interactions and defense signaling pathways.

Wang, D., S. Liu, et al. (2002). "Carbohydrate microarrays for the recognition of cross-reactive molecular markers of microbes and host cells." Nat Biotechnol **20**(3): 275-81.

We describe here the development of a carbohydrate-based microarray to extend the scope of biomedical research on carbohydrate-mediated molecular recognition and anti-infection responses. We have demonstrated that microbial polysaccharides can be immobilized on a surface-modified glass slide without chemical conjugation. With this procedure, a large repertoire of microbial antigens (approximately 20,000 spots) can be patterned on a single micro-glass slide, reaching the capacity to include most common pathogens. Glycoconjugates of different structural characteristics are shown here to be applicable for microarray fabrication, extending the repertoires of diversity and complexity of carbohydrate microarrays. The printed microarrays can be air-dried and stably stored at room temperature for long periods of time. In addition, the system is highly sensitive, allowing simultaneous detection of a broad spectrum of antibody specificities with as little as a few microliters of serum specimen. Finally, the potential of carbohydrate microarrays is demonstrated by the discovery of previously undescribed cellular markers, Dex-Ids.

Wang, H. W., M. W. Trotter, et al. (2004). "Kaposi sarcoma herpesvirus-induced cellular reprogramming contributes to the lymphatic endothelial gene expression in Kaposi sarcoma." Nat Genet **36**(7): 687-93.

The biology of Kaposi sarcoma is poorly understood because the dominant cell type in Kaposi sarcoma lesions is not known. We show by gene expression microarrays that neoplastic cells of Kaposi sarcoma are closely related to lymphatic endothelial cells (LECs) and that Kaposi sarcoma herpesvirus (KSHV) infects both LECs and blood vascular endothelial cells (BECs) in vitro. The gene expression microarray profiles of infected LECs and BECs show that KSHV induces transcriptional reprogramming of both cell types. The lymphangiogenic molecules VEGF-D and angiopoietin-2 were elevated in the plasma of individuals with acquired immune deficiency syndrome and Kaposi sarcoma. These data show that the gene expression profile of Kaposi sarcoma resembles that of LECs, that KSHV induces a transcriptional drift in both LECs and BECs and that lymphangiogenic molecules are involved in the pathogenesis of Kaposi sarcoma.

Wang, J. and C. T. Zhang (2001). "Identification of protein-coding genes in the genome of Vibrio cholerae with more than 98% accuracy using occurrence frequencies of single nucleotides." Eur J Biochem **268**(15): 4261-8.

The published sequence of the Vibrio cholerae genome indicates that, in addition to the genes that encode proteins of known and unknown function, there are 1577 ORFs identified as conserved hypothetical or hypothetical gene candidates. Because the annotation is not 100% accurate, it is not known which of the 1577 ORFs are true protein-coding genes. In this paper, an algorithm based on the Z curve method, with sensitivity, specificity and accuracy greater than 98%, is used to solve this problem. Twenty-fold cross-validation tests show that the accuracy of the algorithm is 98.8%. A detailed discussion of the mechanism of the algorithm is also presented. It was found that 172 of the 1577 ORFs are unlikely to be protein-coding genes. The number of protein-coding genes in the V. cholerae genome was re-estimated and found to be approximately 3716. This result should be of use in microarray analysis of gene expression in the genome, because the cost of preparing chips may be somewhat decreased. A computer program was written to calculate a coding score called VCZ for gene identification in the genome. Coding/noncoding is simply determined by VCZ > 0/VCZ < 0. The program is freely available on request for academic use.

Wang, J. P., S. E. Rought, et al. (2003). "Gene expression profiling detects patterns of human macrophage

responses following Mycobacterium tuberculosis infection." FEMS Immunol Med Microbiol **39**(2): 163-72.

High-density oligonucleotide microarrays allow simultaneous monitoring of the expression of a large number of cellular genes. Microarrays were used to screen the global human monocyte-derived macrophage transcriptional response to infection with the intracellular pathogen Mycobacterium tuberculosis. The microarray detected reproducible patterns of regulated gene expression. Analysis of the expression data showed induction of cytokines and chemokines, ribosomal proteins, and the interferon-response gene Stat1. Several changes were validated by quantitative reverse transcription polymerase chain reaction and immunoblot assays. Augmentation of the respiratory burst and preservation of the response to interferon-gamma were also demonstrated. These data supplement existing knowledge on macrophage responses to tuberculosis infection.

Wang, P. L., K. Ohura, et al. (2003). "DNA microarray analysis of human gingival fibroblasts from healthy and inflammatory gingival tissues." Biochem Biophys Res Commun **305**(4): 970-3.

In the inflammatory gingival tissues of patients with periodontitis, cytokines such as interleukin (IL)-1 alpha, IL-1 beta, IL-6, IL-8, and tumor necrosis factor (TNF)-alpha have been detected. Gingival fibroblasts are the major constituents of gingival tissue. We recently demonstrated that lipopolysaccharide (LPS) from periodontopathic bacteria induces inflammatory reactions in various tissues via CD14 and/or Toll-like receptors (TLRs) in gingival tissues [Biochem. Biophys. Res. Commun. 273 (2000) 1161]. To confirm this, we examined the expression of IL-1 alpha, IL-1 beta, IL-6, IL-8, TNF-alpha, CD14, TLR2, and TLR4 in human gingival fibroblasts (HGFs) obtained from patients with healthy or inflammatory gingiva using DNA microarray analysis. We also studied the expression levels of these proteins by flow cytometric analysis (FACS). The expression levels of all eight genes in the HGFs of the Inflammatory group were significantly higher than those in the Healthy group on DNA microarray analysis. FACS revealed that the expression levels of all eight proteins on the HGFs of the Inflammatory group were higher than those on the Healthy group. Our data indicated that these eight proteins in HGFs are involved in inflammatory conditions in the gingiva, including periodontal disease. Our results suggested that these eight proteins, in turn, act directly or indirectly on the immune response by activating host cells involved in inflammatory processes.

Wang, Q., J. G. Frye, et al. (2004). "Gene expression patterns during swarming in Salmonella typhimurium: genes specific to surface growth and putative new motility and pathogenicity genes." Mol Microbiol **52**(1): 169-87.

Swarming is a specialized form of surface motility displayed by several flagellated bacterial genera, which shares features with other surface phenomenon such as biofilm formation and host invasion. Swarmer cells are generally more flagellated and longer than vegetative cells of the same species propagated in liquid media, and move within an encasement of polysaccharide 'slime'. Signals and signalling pathways controlling swarm cell differentiation are largely unknown. In order to test whether there is a genetic programme specific to swarming, we have determined global gene expression profiles of Salmonella typhimurium over an 8 h time course during swarming, and compared the microarray data with a similar time course of growth in liquid media as well as on harder agar where the bacteria do not swarm. Our data show that bacteria growing on the surface of agar have a markedly different physiology from those in broth, as judged by differential regulation of nearly one-third of the functional genome. The large number of genes showing surface-specific upregulation included those for lipopolysaccharide synthesis, iron metabolism and type III secretion. Although swarming-specific induction of flagellar gene expression was not generally apparent, genes for iron metabolism were strongly induced specifically on swarm agar. Surface-dependent regulation of many virulence genes suggests that growth on an agar surface could serve as a model for gene expression during the initial stages of host infection. Based on cluster analysis of distinctive expression patterns, we report here the identification of putative new genes involved in motility and virulence.

Wang, S. H., E. Mezosi, et al. (2004). "IFNgamma sensitization to TRAIL-induced apoptosis in human thyroid carcinoma cells by upregulating Bak expression." Oncogene **23**(4): 928-35.

TRAIL preferentially induces apoptosis in tumor cells and virus-infected cells. Unlike other tumor necrosis factor family members, TRAIL does not kill cells from most normal tissues and has thus been proposed as a promising new cancer treatment. Our study demonstrated that IFNgamma combined with TRAIL can trigger apoptosis in vitro in several resistant thyroid tumor cell lines, such as thyroid anaplastic carcinoma cells (ARO cells), while either agent alone exerts only a minimal effect. We further tested this effect on a mouse thyroid tumor model, when in vivo tumor growth was also significantly inhibited by this combination. The mechanism of how IFNgamma sensitized thyroid carcinoma cells to TRAIL-induced apoptosis was investigated by screening global gene alterations in ARO cells treated with IFNgamma. Microarray data revealed that a proapoptotic gene, Bak, is markedly upregulated by IFNgamma, and this was confirmed by RNase protection assay. Western blot analysis also showed a significant increase in Bak at the protein level. Upregulation of Bak and sensitization for apoptosis by IFNgamma was blocked by overexpression of antisense Bak in ARO cells. Furthermore, overexpression of Bak sensitized ARO cell to TRAIL-induced apoptosis without the need for IFNgamma pretreatment. This suggests that Bak is a regulatory molecule involved in IFNgamma-facilitated TRAIL-mediated apoptosis in thyroid cancer cells.

Wang, Y., M. C. Wu, et al. (2002). "Different expression of hepatitis B surface antigen between hepatocellular carcinoma and its surrounding liver tissue, studied using a tissue microarray." J Pathol **197**(5): 610-6.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and is highly associated with chronic liver disease, including hepatitis B viral infection. In order to study the association between hepatitis B virus (HBV) infection and HCC development, tissue microarrays were used to detect the expression of hepatitis B surface antigen (HBsAg) in 194 HCCs and their surrounding liver tissues, using anti-HBsAg monoclonal antibody. The results showed that the expression of HBsAg is significantly lower in tumour tissue than in non-tumour tissue. Among the 138 cases with positive serum HBsAg, expression of HBsAg was more frequently detected in non-tumour tissue (103 cases, 75%) than in tumour tissue (11 cases, 8%). RT-PCR and Southern blot analysis were performed to explore the mechanism of the decreased expression of HBsAg in tumour cells. The RT-PCR results showed that absence or decreased expression of the HBV S gene was detected in 3/15 (20%) and 6/15 (40%) HCCs, respectively. Integration of HBV in 23 pairs of HCCs and their matched non-tumour liver tissues was studied by Southern blot. The results showed that the integrated HBV S gene sequence was detected in 19/23 tumours (83%) and 1/23 non-tumour tissues (4%), whereas the free replicative virus form was observed in 3/23 tumours (13%) and 14/23 non-tumour tissues (61%). These findings suggest that HBsAg-negative results in tumour tissues were directly related to HBV DNA insertion and provide new insights into the involvement of HBsAg in hepatocarcinogenesis.

Wang, Z., G. Trillo-Pazos, et al. (2004). "Effects of human immunodeficiency virus type 1 on astrocyte gene expression and function: potential role in neuropathogenesis." J Neurovirol **10 Suppl 1**: 25-32.

Neurodegeneration and dementia caused by human immunodeficiency virus type 1 (HIV-1) infection of the brain are common complications of acquired immunodeficiency syndrome (AIDS). Introduction of highly active antiretroviral therapy (HAART) reduced the incidence of HIV-1-associated dementia, but so far had no effect on the high frequency of milder neurological disorders caused by HIV-1. This indicates that some neuropathogenic processes persist during limited HIV-1 replication in the central nervous system (CNS). The authors are evaluating the hypothesis that interaction of HIV-1 with astrocytes, which bind HIV-1 but support limited productive HIV-1 infection, may contribute to these processes by disrupting astrocyte functions that are important for neuronal activity or survival. Using laser-capture microdissection on brain tissue samples from HIV-1-infected individuals, we found that HIV-1 DNA can be detected in up to 1% of cortical and basal ganglia astrocytes, thus confirming HIV-1

infection in astrocytes from symptomatic patients. Using rapid subtraction hybridization, the authors cloned and identified 25 messenger RNAs in primary human fetal astrocytes either up-regulated or down-regulated by native HIV-1 infection or exposure to gp120 in vitro. Extending this approach to gene microarray analysis using Affymetrix U133A/B gene chips, the authors determined that HIV-1 alters globally and significantly the overall program of gene expression in astrocytes, including changes in transcripts coding for cytokines, G-coupled protein receptors, transcription factors, and others. Focusing on a specific astrocyte function relevant to neuropathogenesis, the authors showed that exposure of astrocytes to HIV-1 or gp120 in vitro impairs the ability of the cells to transport L-glutamate and the authors related this defect to transcriptional inhibition of the EAAT2 glutamate transporter gene. These findings define new pathways through which HIV-1 may contribute to neuropathogenesis under conditions of limited virus replication in the brain.

Warke, R. V., K. Xhaja, et al. (2003). "Dengue virus induces novel changes in gene expression of human umbilical vein endothelial cells." J Virol **77**(21): 11822-32.

Endothelial cells are permissive to dengue virus (DV) infection in vitro, although their importance as targets of DV infection in vivo remains a subject of debate. To analyze the virus-host interaction, we studied the effect of DV infection on gene expression in human umbilical vein endothelial cells (HUVECs) by using differential display reverse transcription-PCR (DD-RTPCR), quantitative RT-PCR, and Affymetrix oligonucleotide microarrays. DD identified eight differentially expressed cDNAs, including inhibitor of apoptosis-1, 2'-5' oligoadenylate synthetase (OAS), a 2'-5' OAS-like (OASL) gene, galectin-9, myxovirus protein A (MxA), regulator of G-protein signaling, endothelial and smooth muscle cell-derived neuropilin-like protein, and phospholipid scramblase 1. Microarray analysis of 22,000 human genes confirmed these findings and identified an additional 269 genes that were induced and 126 that were repressed more than fourfold after DV infection. Broad functional responses that were activated included the stress, defense, immune, cell adhesion, wounding, inflammatory, and antiviral pathways. These changes in gene expression were seen after infection of HUVECs with either laboratory-adapted virus or with virus isolated directly from plasma of DV-infected patients. Tumor necrosis factor alpha, OASL, and MxA and h-IAP1 genes were induced within the first 8 to 12 h after infection, suggesting a direct effect of DV infection. These global analyses of DV effects on cellular gene expression identify potentially novel mechanisms involved in dengue disease manifestations such as hemostatic disturbance.

Watanabe, S. (2004). "[Transcriptome analysis of virus-infected cells]." Uirusu **54**(1): 23-31.

Human genome project revealed that human genes are derived from 30,000-40,000 species of genetic loci, which had been estimated as approximately 100,000. The project also promoted devising novel tools that enable us to analyze biological phenomena comprehensively. Microarray technology is a representative of the novel tools in genomics and engages us to explore genome-wide expression levels of genes simultaneously (transcriptome analysis). Here we show transcriptomes obtained from 10 species of cells infected with human cytomegalovirus, as a model virus, by a synthetic DNA microarray system that we have established recently. Our system provides simultaneous and parallel description on alteration of expression of viral and host genes that are represented within a single area on a slide glass. Moreover, we propose a project entitled 'comparative virology on cellular responses of infected hosts' that consists of multiple acquisition and integration of transcriptomes from a combination of several cells and viruses as a panel on the identical platform. The attempt should extract a novel concept in virology from investigation on differences and similarities among influence of a virus on a variety of different cells and conversely among responses of a species of cells against a variety of different viruses.

Watanabe, S., M. Hamano, et al. (2003). "Mannitol-1-phosphate dehydrogenase (MtlD) is required for mannitol and glucitol assimilation in Bacillus subtilis: possible cooperation of mtl and gut operons." J Bacteriol **185**(16): 4816-24.

We found that mannitol-1-phosphate dehydrogenase (MtlD), a component of the mannitol-

specific phosphotransferase system, is required for glucitol assimilation in addition to GutR, GutB, and GutP in Bacillus subtilis. Northern hybridization of total RNA and microarray studies of RNA from cells cultured on glucose, mannitol, and glucitol indicated that mannitol as the sole carbon source induced hyperexpression of the mtl operon, whereas glucitol induced both mtl and gut operons. The B. subtilis mtl operon consists of mtlA (encoding enzyme IICBA(mt1)) and mtlD, and its transcriptional regulator gene, mtlR, is located 14.4 kb downstream from the mtl operon on the chromosome. The mtlA, mtlD, and mtlR mutants disrupted by the introduction of the pMUTin derivatives MTLAd, MTLDd, and MTLRd, respectively, could not grow normally on either mannitol or glucitol. However, the growth of MTLAd on glucitol was enhanced by IPTG (isopropyl-beta-D-thiogalactopyranoside). This mutant has an IPTG-inducible promoter (Pspac promoter) located in mtlA, and this site corresponds to the upstream region of mtlD. Insertion mutants of mtlD harboring the chloramphenicol resistance gene also could not grow on either mannitol or glucitol. In contrast, an insertion mutant of mtlA could grow on glucitol but not on mannitol in the presence or absence of IPTG. MtlR bound to the promoter region of the mtl operon but not to a DNA fragment containing the gut promoter region.

Watanabe, T., Y. Murata, et al. (2004). "A new approach to species determination for yeast strains: DNA microarray-based comparative genomic hybridization using a yeast DNA microarray with 6000 genes." Yeast **21**(4): 351-65.

DNA-DNA hybridization is known as the superior method in the elucidation of relationships between closely related taxa, such as species and strain. For species determination we propose a new DNA-DNA hybridization method: the DNA microarray-based comparative genomic hybridization (CGH) method, using a yeast DNA microarray with approximately 6000 genes. The genome from a yeast strain as a sample strain (Sample) was labelled with Cy3-dye and hybridized to a single DNA microarray, together with the Cy5-labelled genome of S. cerevisiae S288C as a reference strain (Reference). The log2 ratio values [log2[Cy3(Sample)/Cy5(Reference)]: Ratio] of signal intensities of all the gene spots were estimated and divided into the following groups: Ratio < or = -1; -1 < Ratio < 1; 1 < or = Ratio. The hybridization profiles of the genomes of type strains belonging to the genus Saccharomyces were significantly different from that of S. cerevisiae S288C. The Ratio-based grouping allowed us to discriminate between some species from S. cerevisiae more clearly. Furthermore, cluster analysis discriminated between closely related species and strains. Using this method, we were able to not only perform species determination but also to obtain information on alternation in gene copy number of such gene amplifications and deletions with single-gene resolution. These observations indicated that DNA microarray-based CGH is a powerful system for species determination and comparative genome analysis.

Watterson, J. H., P. A. Piunno, et al. (2001). "Influences of non-selective interactions of nucleic acids on response rates of nucleic acid fiber optic biosensors." Fresenius J Anal Chem **369**(7-8): 601-8.

The immobilization of oligonucleotides to solid surfaces can provide a platform of chemistry that is suitable for the development of biosensor and microarray technologies. Experiments were performed using a fiber optic nucleic acid biosensor based on total internal reflection fluorescence to examine the effects of the presence of non-complementary DNA on the detection of hybridization of complementary target DNA. The work has focused on the rates and extent of hybridization in the presence and absence of non-selective adsorption using fluorescein-labeled DNA. A stop-flow system of 137 microL volume permitted rapid introduction and mixing of each sample. Response times measured were on the order of seconds to minutes. Non-selective adsorption of non-complementary oligonucleotides (ncDNA) was found to occur at a significantly faster rate than hybridization of complementary oligomers (cDNA) in all cases. The presence of ncDNA oligonucleotides did not inhibit selective interactions between immobilized DNA and cDNA in solution. The presence of high concentrations of non-complementary genomic DNA had little effect on the extent of hybridization of complementary oligonucleotides, but actually reduced the response times of sensors to cDNA oligonucleotides.

Wei, Y., J. M. Lee, et al. (2001). "High-density microarray-mediated gene expression profiling of Escherichia coli." J Bacteriol **183**(2): 545-56.

A nearly complete collection of 4,290 Escherichia coli open reading frames was amplified and arrayed in high density on glass slides. To exploit this reagent, conditions for RNA isolation from E. coli cells, cDNA production with attendant fluorescent dye incorporation, DNA-DNA hybridization, and hybrid quantitation have been established. A brief isopropyl-beta-D-thiogalactopyranoside (IPTG) treatment elevated lacZ, lacY, and lacA transcript content about 30-fold; in contrast, most other transcript titers remained unchanged. Distinct RNA expression patterns between E. coli cultures in the exponential and transitional phases of growth were catalogued, as were differences associated with culturing in minimal and rich media. The relative abundance of each transcript was estimated by using hybridization of a genomic DNA-derived, fluorescently labeled probe as a correction factor. This inventory provided a quantitative view of the steady-state level of each mRNA species. Genes the expression of which was detected by this method were enumerated, and results were compared with the current understanding of E. coli physiology.

Wei, Y., J. M. Lee, et al. (2001). "Global impact of sdiA amplification revealed by comprehensive gene expression profiling of Escherichia coli." J Bacteriol **183**(7): 2265-72.

In Escherichia coli the amplification of sdiA, a positive activator of ftsQAZ, genes that are essential for septation, results in mitomycin C resistance. To help us understand this resistance phenotype, genes whose expression was altered by increased sdiA dosage were identified using a DNA microarray-based, comprehensive transcript profiling method. The expression of 62 genes was reduced by more than threefold; of these, 41 are involved in motility and chemotaxis. Moreover, the expression of 75 genes, 36 of which had been previously characterized, was elevated at least threefold. As expected, increased sdiA dosage led to significantly elevated sdiA and 'ddlB-ftsQAZ-lpxC operon expression. Transcription of two genes, uvrY and uvrC, located downstream of sdiA and oriented in the same direction, was elevated about 10-fold, although the intervening gene, yecF, of opposite polarity was unaffected by increased sdiA dosage. Three genes (mioC and gidAB) flanking the replication origin, oriC, were transcribed more often when sdiA dosage was high, as were 12 genes within 1 min of a terminus of replication, terB. Transcription of the acrABDEF genes, mapping in three widely spaced loci, was elevated significantly, while several genes involved in DNA repair and replication (e.g., nei, recN, mioC, and mcrC) were moderately elevated in expression. Such global analysis provides a link between septation and the response to DNA-damaging agents.

Weidenhammer, E. M., B. F. Kahl, et al. (2002). "Multiplexed, targeted gene expression profiling and genetic analysis on electronic microarrays." Clin Chem **48**(11): 1873-82.

BACKGROUND: Electronic microarrays comprise independent microelectrode test sites that can be electronically biased positive or negative, or left neutral, to move and concentrate charged molecules such as DNA and RNA to one or more test sites. We developed a protocol for multiplexed gene expression profiling of mRNA targets that uses electronic field-facilitated hybridization on electronic microarrays. METHODS: A multiplexed, T7 RNA polymerase-mediated amplification method was used for expression profiling of target mRNAs from total cellular RNA; targets were detected by hybridization to sequence-specific capture oligonucleotides on electronic microarrays. Activation of individual test sites on the electronic microarray was used to target hybridization to designated subsets of sites and allow comparisons of target concentrations in different samples. We used multiplexed amplification and electronic field-facilitated hybridization to analyze expression of a model set of 10 target genes in the U937 cell line during lipopolysaccharide-mediated differentiation. Performance of multiple genetic analyses (single-nucleotide polymorphism detection, gene expression profiling, and splicing isoform detection) on a single electronic microarray was demonstrated using the ApoE and ApoER2 genes as a model system. RESULTS: Targets were detected after a 2-min hybridization reaction. With noncomplementary capture probes, no signal was detectable. Twofold changes in target concentration

were detectable throughout the (approximately 64-fold) range of concentrations tested. Levels of 10 targets were analyzed side by side across seven time points. By confining electronic activation to subsets of test sites, polymorphism detection, expression profiling, and splicing isoform analysis were performed on a single electronic microarray. CONCLUSIONS: Microelectronic array technology provides specific target detection and quantification with advantages over currently available methodologies for targeted gene expression profiling and combinatorial genomics testing.

Weiner, J., 3rd, C. U. Zimmerman, et al. (2003). "Transcription profiles of the bacterium Mycoplasma pneumoniae grown at different temperatures." Nucleic Acids Res **31**(21): 6306-20.

Applying microarray technology, we have investigated the transcriptome of the small bacterium Mycoplasma pneumoniae grown at three different temperature conditions: 32, 37 and 32 degrees C followed by a heat shock for 15 min at 43 degrees C, before isolating the RNA. From 688 proposed open-reading frames, 676 were investigated and 564 were found to be expressed ($P < 0.001$; 606 with $P < 0.01$) and at least 33 ($P < 0.001$; 77 at $P < 0.01$) regulated. By quantitative real-time PCR of selected mRNA species, the expression data could be linked to absolute molecule numbers. We found M.pneumoniae to be regulated at the transcriptional level. Forty-seven genes were found to be significantly up-regulated after heat shock ($P < 0.01$). Among those were the conserved heat shock genes like dnaK, lonA and clpB, but also several genes coding for ribosomal proteins and 10 genes of unassigned functions. In addition, 30 genes were found to be down-regulated under the applied heat shock conditions. Further more, we have compared different methods of cDNA synthesis (random hexamer versus gene-specific primers, different RNA concentrations) and various normalization strategies of the raw microarray data.

Wells, D. B., P. J. Tighe, et al. (2001). "Differential gene expression during meningeal-meningococcal interaction: evidence for self-defense and early release of cytokines and chemokines." Infect Immun **69**(4): 2718-22.

Using microarray technology, we studied the early differential expression of 3,528 genes in human meningothelial cells in response to meningococcal challenge. Thirty-two genes were up-regulated, and four were down-regulated. Those up-regulated included the tumor necrosis factor alpha, interleukin-6 (IL-6), and IL-8 (but not IL-1beta) genes, suggesting that meningeal cells may be a local and early source of these cytokines. Also, a trend in up-regulation of anti-apoptotic genes and down-regulation of pro-apoptotic genes was observed. This is the first evidence that meningothelial cells may mount cytoprotective responses to pathogenic bacteria.

Wen, Y. Y., T. Y. Chang, et al. (2003). "Comparative study of enterovirus 71 infection of human cell lines." J Med Virol **70**(1): 109-18.

The cell tropism of enterovirus 71 (Enteroviridae) in neuronal, glial and laryngeal cells. The 4643 strain, an enterovirus 71 isolate from a patient in Taiwan, was used to infect three human cell lines representing neuronal cells (SK-N-SH, neuroblastoma), glial cells (U373MG, glioblastoma), and laryngeal cells (HEp-2, larynx epidermoid carcinoma). Immunofluorescent staining and transmission electron microscopy (TEM) were used to detect mature enterovirus 71 4643 virions in these cell lines. The three cell lines were also compared for presence of virus-mediated cytopathic effect (CPE), synthesis of infected cell-specific proteins, viral (-) RNA, and virus replication rate. Virus particles were detected by TEM, and viral replication increased over time, indicating the existence and release of mature viruses from all three infected cell lines. The most severe CPE and the highest viral replication rate were observed in the SK-N-SH cells. Further screening of the infected cell lines by microarray analysis revealed that the neuron growth factor receptor (NGFR) gene was uniquely upregulated in infected SK-N-SH cells, implying that the receptor encoded by this gene may be involved in cell tropism. The data show that neurons are vulnerable to enterovirus 71 4643 infection and are consistent with the clinical observation that enterovirus 71 4643 targets mainly neuronal cells but is also found in many organs in conjunction with an inflammatory reaction.

Wendisch, V. F. (2003). "Genome-wide expression analysis in Corynebacterium glutamicum using DNA microarrays." J Biotechnol **104**(1-3): 273-85.

DNA microarray technology has become an important research tool for microbiology and biotechnology as it allows for comprehensive DNA and RNA analyses to characterize genetic diversity and gene expression in a genome-wide manner. DNA microarrays have been applied extensively to study the biology of many bacteria including Mycobacterium tuberculosis, but only recently have they been used for the related high-GC Gram-positive Corynebacterium glutamicum, which is widely used for biotechnological amino acid production. Besides the design and generation of microarrays as well as their use in hybridization experiments and subsequent data analysis, recent applications of DNA microarray technology in C. glutamicum including the characterization of ribose-specific gene expression and the valine stress response will be described. Emerging perspectives of functional genomics to enlarge our insight into fundamental biology of C. glutamicum and their impact on applied biotechnology will be discussed.

Wernisch, L., S. L. Kendall, et al. (2003). "Analysis of whole-genome microarray replicates using mixed models." Bioinformatics **19**(1): 53-61.

MOTIVATION: Microarray experiments are inherently noisy. Replication is the key to estimating realistic fold-changes despite such noise. In the analysis of the various sources of noise the dependency structure of the replication needs to be taken into account. RESULTS: We analyzed replicate data sets from a Mycobacterium tuberculosis trcS mutant in order to identify differentially expressed genes and suggest new methods for filtering and normalizing raw array data and for imputing missing values. Mixed ANOVA models are applied to quantify the various sources of error. Such analysis also allows us to determine the optimal number of samples and arrays. Significance values for differential expression are obtained by a hierarchical bootstrapping scheme on scaled residuals. Four highly upregulated genes, including bfrB, were analyzed further. We observed an artefact, where transcriptional readthrough from these genes led to apparent upregulation of adjacent genes. AVAILABILITY: All methods and data discussed are available in the package YASMAhttp://www.cryst.bbk.ac.uk/wernisch/yasma.html for the statistical data analysis system R (http://www.R-project.org).

Whitham, S. A., S. Quan, et al. (2003). "Diverse RNA viruses elicit the expression of common sets of genes in susceptible Arabidopsis thaliana plants." Plant J **33**(2): 271-83.

Systemic infections of plants by viruses require that viruses modify host cells in order to facilitate infections. These modifications include induction of host factors required for replication, propagation and movement, and suppression of host defense responses, which are likely to be associated with changes in host gene expression. Past studies of the effects of viral infection on gene expression in susceptible hosts have been limited to only a handful of genes. To gain broader insight into the responses elicited by viruses in susceptible hosts, high-density oligonucleotide probe microarray technology was used. Arabidopsis leaves were either mock inoculated or inoculated with cucumber mosaic cucumovirus, oil seed rape tobamovirus, turnip vein clearing tobamovirus, potato virus X potexvirus, or turnip mosaic potyvirus. Inoculated leaves were collected at 1, 2, 4, and 5 days after inoculation, total RNA was isolated, and samples were hybridized to Arabidopsis GeneChip microarrays (Affymetrix). Microarray hybridization revealed co-ordinated changes in gene expression in response to infection by diverse viruses. These changes include virus-general and virus-specific alterations in the expression of genes associated with distinct defense or stress responses. Analyses of the promoters of these genes further suggest that diverse RNA viruses elicit common responses in susceptible plant hosts through signaling pathways that have not been previously characterized.

Wichert, S., K. Fokianos, et al. (2004). "Identifying periodically expressed transcripts in microarray time series data." Bioinformatics **20**(1): 5-20.

MOTIVATION: Microarray experiments are now routinely used to collect large-scale time series data, for example to monitor gene expression during the cell cycle. Statistical analysis of this data poses many challenges, one being that it is hard to identify correctly the subset of genes with a clear periodic signature. This has lead to a controversial argument with regard to the suitability of both available methods and current microarray data. METHODS: We introduce two simple but efficient statistical methods for signal detection and gene selection in gene expression time series data. First, we suggest the average periodogram as an exploratory device for graphical assessment of the presence of periodic transcripts in the data. Second, we describe an exact statistical test to identify periodically expressed genes that allows one to distinguish periodic from purely random processes. This identification method is based on the so-called g-statistic and uses the false discovery rate approach to multiple testing. RESULTS: Using simulated data it is shown that the suggested method is capable of identifying cell-cycle-activated genes in a gene expression data set even if the number of the cyclic genes is very small and regardless the presence of a dominant non-periodic component in the data. Subsequently, we re-examine 12 large microarray time series data sets (in part controversially discussed) from yeast, human fibroblast, human HeLa and bacterial cells. Based on the statistical analysis it is found that a majority of these data sets contained little or no statistical significant evidence for genes with periodic variation linked to cell cycle regulation. On the other hand, for the remaining data the method extends the catalog of previously known cell-cycle-specific transcripts by identifying additional periodic genes not found by other methods. The problem of distinguishing periodicity due to generic cell cycle activity and to artifacts from synchronization is also discussed. AVAILABILITY: The approach has been implemented in the R package GeneTS available from http://www.stat.uni-muenchen.de/~strimmer/software.html under the terms of the GNU General Public License.

Wick, N., S. Luedemann, et al. (2003). "Induction of short interspersed nuclear repeat-containing transcripts in epithelial cells upon infection with a chicken adenovirus." J Mol Biol **328**(4): 779-90.

Chicken embryo lethal orphan adenovirus (CELO) is used as a vector for expression of exogenous genes in mammalian cells. Here, we analyzed transcriptional alterations in mouse epithelial host cells following infection with CELO using cDNA microarray analysis. Sequence data characterization revealed that a major portion of CELO-induced genes contained short interspersed nuclear elements of the B2 subclass (B2 SINEs). In fact, we could identify SINEs and other repetitive sequences as contributing significantly to the cDNAs used for microarray construction. Moreover, we found that the CELO protein Gam1 was able to mediate transcriptional activation of these B2 SINE-containing RNAs. We hypothesize that upregulation of B2-SINE-containing RNAs could be a novel contribution of Gam1 to CELO host cell infection.

Wildhaber, B. E., H. Yang, et al. (2003). "Gene alteration of intestinal intraepithelial lymphocytes with administration of total parenteral nutrition." J Pediatr Surg **38**(6): 840-3.

BACKGROUND: Total parenteral nutrition (TPN) is associated with sepsis and loss of immune reactivity. The authors previously have shown that changes in the intestinal mucosal immune system--ie, intraepithelial lymphocytes (IEL)--lead to a loss of epithelial barrier function. This may be a mechanism by which bacteria and toxins endanger individuals receiving TPN. To identify altered IEL gene expression during TPN administration, microarray assays were used. METHODS: Mice received oral feeding (control) or TPN for 7 days. Small bowel IEL were separated and retained, RNA purified, and microarray assays performed (Affymetrix system, 12,491 genes). Results were expressed as quantile-normalized trimmed-means. Significance equals a greater than 2-fold change (TPN v control), $P < .01$ (t test) or greater than 3-fold, $P < .05$. RESULTS: In the TPN group 88, IEL genes were significantly up regulated and 114 downregulated (v control). Of these genes, 4 were identified to have highest degree of upregulation (FK506-binding protein 5; mannose-binding lectin, metallothionein 1 and 2), 2 were highly downregulated (microsomal epoxide hydrolase 1 and cytochrome P450 1a1). These genes were found to have high potential for immune-modulatory effects. CONCLUSIONS: The observed alterations in IEL

gene expression may have an important role in the altered immune response with TPN and may relate to the increase in sepsis with TPN administration.

Willse, A., T. M. Straub, et al. (2004). "Quantitative oligonucleotide microarray fingerprinting of Salmonella enterica isolates." Nucleic Acids Res **32**(5): 1848-56.

We report on a genome-independent microbial fingerprinting method using nucleic acid microarrays for microbial forensics and epidemiology applications and demonstrate that the microarray method provides high resolution differentiation between closely related microorganisms, using Salmonella enterica strains as the test case. In replicate trials we used a simple 192 probe nonamer array to construct a fingerprint library of 25 closely related Salmonella isolates. Controlling false discovery rate for multiple testing at alpha = 0.05, at least 295 of 300 pairs of S.enterica isolate fingerprints were found to be statistically distinct using a modified Hotelling T2 test. Although most pairs of Salmonella fingerprints are found to be distinct, forensic applications will also require a protocol for library construction and reliable microbial classification against a fingerprint library. We outline additional steps required to produce such a protocol.

Wilson, J. W., C. M. Ott, et al. (2002). "Low-Shear modeled microgravity alters the Salmonella enterica serovar typhimurium stress response in an RpoS-independent manner." Appl Environ Microbiol **68**(11): 5408-16.

We have previously demonstrated that low-shear modeled microgravity (low-shear MMG) serves to enhance the virulence of a bacterial pathogen, Salmonella enterica serovar Typhimurium. The Salmonella response to low-shear MMG involves a signaling pathway that we have termed the low-shear MMG stimulon, though the identities of the low-shear MMG stimulon genes and regulatory factors are not known. RpoS is the primary sigma factor required for the expression of genes that are induced upon exposure to different environmental-stress signals and is essential for virulence in mice. Since low-shear MMG induces a Salmonella acid stress response and enhances Salmonella virulence, we reasoned that RpoS would be a likely regulator of the Salmonella low-shear MMG response. Our results demonstrate that low-shear MMG provides cross-resistance to several environmental stresses in both wild-type and isogenic rpoS mutant strains. Growth under low-shear MMG decreased the generation time of both strains in minimal medium and increased the ability of both strains to survive in J774 macrophages. Using DNA microarray analysis, we found no evidence of induction of the RpoS regulon by low-shear MMG but did find that other genes were altered in expression under these conditions in both the wild-type and rpoS mutant strains. Our results indicate that, under the conditions of these studies, RpoS is not required for transmission of the signal that induces the low-shear MMG stimulon. Moreover, our studies also indicate that low-shear MMG can be added to a short list of growth conditions that can serve to preadapt an rpoS mutant for resistance to multiple environmental stresses.

Wilson, J. W., R. Ramamurthy, et al. (2002). "Microarray analysis identifies Salmonella genes belonging to the low-shear modeled microgravity regulon." Proc Natl Acad Sci U S A **99**(21): 13807-12.

The low-shear environment of optimized rotation suspension culture allows both eukaryotic and prokaryotic cells to assume physiologically relevant phenotypes that have led to significant advances in fundamental investigations of medical and biological importance. This culture environment has also been used to model microgravity for ground-based studies regarding the impact of space flight on eukaryotic and prokaryotic physiology. We have previously demonstrated that low-shear modeled microgravity (LSMMG) under optimized rotation suspension culture is a novel environmental signal that regulates the virulence, stress resistance, and protein expression levels of Salmonella enterica serovar Typhimurium. However, the mechanisms used by the cells of any species, including Salmonella, to sense and respond to LSMMG and identities of the genes involved are unknown. In this study, we used DNA microarrays to elucidate the global transcriptional response of Salmonella to LSMMG. When compared with identical growth conditions under normal gravity (1 x g), LSMMG differentially regulated the expression of 163

genes distributed throughout the chromosome, representing functionally diverse groups including transcriptional regulators, virulence factors, lipopolysaccharide biosynthetic enzymes, iron-utilization enzymes, and proteins of unknown function. Many of the LSMMG-regulated genes were organized in clusters or operons. The microarray results were further validated by RT-PCR and phenotypic analyses, and they indicate that the ferric uptake regulator is involved in the LSMMG response. The results provide important insight about the Salmonella LSMMG response and could provide clues for the functioning of known Salmonella virulence systems or the identification of uncharacterized bacterial virulence strategies.

Wilson, K. H., W. J. Wilson, et al. (2002). "High-density microarray of small-subunit ribosomal DNA probes." Appl Environ Microbiol **68**(5): 2535-41.

Ribosomal DNA sequence analysis, originally conceived as a way to provide a universal phylogeny for life forms, has proven useful in many areas of biological research. Some of the most promising applications of this approach are presently limited by the rate at which sequences can be analyzed. As a step toward overcoming this limitation, we have investigated the use of photolithography chip technology to perform sequence analyses on amplified small-subunit rRNA genes. The GeneChip (Affymetrix Corporation) contained 31,179 20-mer oligonucleotides that were complementary to a subalignment of sequences in the Ribosomal Database Project (RDP) (B. L. Maidak et al., Nucleic Acids Res. 29:173-174, 2001). The chip and standard Affymetrix software were able to correctly match small-subunit ribosomal DNA amplicons with the corresponding sequences in the RDP database for 15 of 17 bacterial species grown in pure culture. When bacteria collected from an air sample were tested, the method compared favorably with cloning and sequencing amplicons in determining the presence of phylogenetic groups. However, the method could not resolve the individual sequences comprising a complex mixed sample. Given these results and the potential for future enhancement of this technology, it may become widely useful.

Wojtkowiak, A., A. Siek, et al. (2002). "RNAi and viral vectors as useful tools in the functional genomics of plants. Construction of BMV-based vectors for RNA delivery into plant cells." Cell Mol Biol Lett **7**(2A): 511-22.

The sequencing of several complete genomes and the development of a DNA microarray technology are among the most important achievements of molecular biology. They gave the proper grounds for the development of modern functional genomics. However, there is one additional condition which needs to be satisfied to truely enable the study of how a genome works: a suitable method of selectively inducing and silencing the expression of each individual gene. The methods used so far have usually only permitted the influencing of gene expression through genetic manipulations at the DNA level (genetically modified plants). The discovery of RNA interference (RNAi) opens up completely new possibilities of research on the functioning of particular plant genes, without the necessity of altering the genome structure. In this case, interference takes place at the transcript level. Thus, at any given moment during plant development, the expression of a specific gene (or several genes) can be inhibited, even if it is important for the survival of the organism under study. To this end, a double-stranded RNA inducing the RNAi phenomenon has to be delivered into the plant cell. Here we describe the construction of four brome mosaic virus-based vectors, which, as our preliminary data indicate, can be used to transfer RNA into barley cells.

Wolfgang, M. C., V. T. Lee, et al. (2003). "Coordinate regulation of bacterial virulence genes by a novel adenylate cyclase-dependent signaling pathway." Dev Cell **4**(2): 253-63.

Type III secretion systems (TTSSs) are utilized by numerous bacterial pathogens to inject effector proteins directly into host cells. Using a whole-genome microarray, we investigated the conditions and regulatory factors that control the expression of the Pseudomonas aeruginosa TTSS. The transcriptional response of known TTSS genes indicates a hierarchical pattern of expression in which a set of secretion

apparatus and regulatory genes is constitutively expressed. Further analysis of genes coordinately regulated with those encoding the TTSS led to the identification of a signaling pathway that originates from a membrane-associated adenylate cyclase and controls TTSS gene expression. Transcriptome analysis of mutants lacking the ability to synthesize cAMP or the cAMP binding protein Vfr implicated this pathway in the global regulation of host-directed virulence determinants, including the TTSS.

Wu, C. G., D. M. Salvay, et al. (2001). "Distinctive gene expression profiles associated with Hepatitis B virus x protein." Oncogene **20**(28): 3674-82.

Hepatitis B virus (HBV) is a major risk factor for the development of hepatocellular carcinoma (HCC). HBV encodes the potentially oncogenic HBx protein, which mainly functions as a transcriptional co-activator involving in multiple gene deregulations. However, mechanisms underlying HBx-mediated oncogenicity remain unclear. To determine the role(s) of HBx in the early genesis of HCC, we utilized the NCI Oncochip microarray that contains 2208 human cDNA clones to examine the gene expression profiles in either freshly isolated normal primary adult human hepatocytes (Hhep) or an HCC cell line (SK-Hep-1) ecotopically expressing HBx via an adenoviral system. The gene expression profiles also were determined in liver samples from HBV-infected chronic active hepatitis patients when compared with normal liver samples. The microarray results were validated through Northern blot analysis of the expression of selected genes. Using reciprocally labeling hybridizations, scatterplot analysis of gene expression ratios in human primary hepatocytes expressing HBx demonstrates that microarrays are highly reproducible. The comparison of gene expression profiles between HBx-expressing primary hepatocytes and HBV-infected liver samples shows a consistent alteration of many cellular genes including a subset of oncogenes (such as c-myc and c-myb) and tumor suppressor genes (such as APC, p53, WAF1 and WT1). Furthermore, clustering algorithm analysis showed distinctive gene expression profiles in Hhep and SK-Hep-1 cells. Our findings are consistent with the hypothesis that the deregulation of cellular genes by oncogenic HBx may be an early event that favors hepatocyte proliferation during liver carcinogenesis.

Wu, Q., P. Kirschmeier, et al. (2002). "Transcriptional regulation during p21WAF1/CIP1-induced apoptosis in human ovarian cancer cells." J Biol Chem **277**(39): 36329-37.

In this study we used adenovirus vector-mediated transduction of either the p53 gene (rAd-p53) or the p21(WAF1/CIP1) gene (rAd-p21) to mimic both p53-dependent and -independent up-regulation of p21(WAF1/CIP1) within a human ovarian cancer cell line, 2774, and the derivative cell lines, 2774qw1 and 2774qw2. We observed that rAd-p53 can induce apoptosis in both 2774 and 2774qw1 cells but not in 2774qw2 cells. Surprisingly, overexpression of p21(WAF1/CIP1) also triggered apoptosis within these two cell lines. Quantitative reverse transcription-PCR analysis revealed that the differential expression of BAX, BCL2, and caspase 3 genes, specific in rAd-p53-induced apoptotic cells, was not altered in rAd-p21-induced apoptotic cells, suggesting p21(WAF1/CIP1)-induced apoptosis through a pathway distinguishable from p53-induced apoptosis. Expression analysis of 2774qw1 cells infected with rAd-p21 on 60,000 cDNA microarrays identified 159 genes in response to p21(WAF1/CIP1) expression in at least one time point with 2.5-fold change as a cutoff. Integration of the data with the parallel microarray experiments with rAd-p53 infection allowed us to extract 66 genes downstream of both p53 and p21(WAF1/CIP1) and 93 genes in response to p21(WAF1/CIP1) expression in a p53-independent pathway. The genes in the former set may play a dual role in both p53-dependent and p53-independent pathways, and the genes in the latter set gave a mechanistic molecular explanation for p53-independent p21(WAF1/CIP1)-induced apoptosis. Furthermore, promoter sequence analysis suggested that transcription factor E2F family is partially responsible for the differential expression of genes following p21(WAF1/CIP1). This study has profound significance toward understanding the role of p21(WAF1/CIP1) in p53-independent apoptosis.

Xia, M., R. E. Bumgarner, et al. (2003). "Chlamydia trachomatis infection alters host cell transcription in diverse cellular pathways." J Infect Dis **187**(3): 424-34.

To study the responses of the host cell to chlamydial infection, differentially transcribed genes of the host cells were examined. Complementary DNA (cDNA) probes were made from messenger RNAs of HeLa cells infected with Chlamydia trachomatis and were hybridized to a high-density human DNA microarray of 15,000 genes and expressed sequence tags. C. trachomatis alters host cell transcription at both the early and middle phases of its developmental cycle. At 2 h after infection, 13 host genes showed mean expression ratios >/=2-fold. At 16 h after infection, 130 genes were differentially transcribed. These genes encoded factors inhibiting apoptosis and factors regulating cell differentiation, components of the cytoskeleton, transcription factors, and proinflammatory cytokines. This indicates that chlamydial infection, despite its intravacuolar location, alters the transcription of a broad range of host genes in diverse cellular pathways and provides a framework for future studies.

Xiao, W. W., W. L. Ma, et al. (2003). "[Bioinformatic analysis of dengue virus cDNAs and design of oligonucleotide probes for microarray detection of the virus]." Di Yi Jun Yi Da Xue Xue Bao **23**(9): 905-7.

OBJECTIVE: To design oligonucleotide probes for microarray detection of dengue virus. METHODS: By analyzing the cDNAs of dengue viruses of 4 different serotypes with BLAST program, a group of specific sequences for the candidate probes was acquired. Oligo6.0 software was applied to analyze the candidates to select the probes with high specificity, identical length and similar melting temperature (Tm). RESULT: Altogether 48 oligonucleotide probes were designed, and deposited on oligonucleotide chips as the microarray for dengue virus detection. CONCLUSION: BLAST program and Oligo6.0 software are simple and effective means for designing the oligonucleotide probes.

Xu, D., G. Li, et al. (2002). "PRIMEGENS: robust and efficient design of gene-specific probes for microarray analysis." Bioinformatics **18**(11): 1432-7.

MOTIVATION: DNA microarray is a powerful high-throughput tool for studying gene function and regulatory networks. Due to the problem of potential cross hybridization, using full-length genes for microarray construction is not appropriate in some situations. A bioinformatic tool, PRIMEGENS, has recently been developed for the automatic design of PCR primers using DNA fragments that are specific to individual open reading frames (ORFs). RESULTS: PRIMEGENS first carries out a BLAST search for each target ORF against all other ORFs of the genome to quickly identify possible homologous sequences. Then it performs optimal sequence alignment between the target ORF and each of its homologous ORFs using dynamic programming. PRIMEGENS uses the sequence alignments to select gene- specific fragments, and then feeds the fragments to the Primer3 program to design primer pairs for PCR amplification. PRIMEGENS can be run from the command line on Unix/Linux platforms as a stand-alone package or it can be used from a Web interface. The program runs efficiently, and it takes a few seconds per sequence on a typical workstation. PCR primers specific to individual ORFs from Shewanella oneidensis MR-1 and Deinococcus radiodurans R1 have been designed. The PCR amplification results indicate that this method is very efficient and reliable for designing specific probes for microarray analysis.

Xu, X. R., J. Huang, et al. (2001). "Insight into hepatocellular carcinogenesis at transcriptome level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding noncancerous liver." Proc Natl Acad Sci U S A **98**(26): 15089-94.

Human hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. In this work, we report on a comprehensive characterization of gene expression profiles of hepatitis B virus-positive HCC through the generation of a large set of 5'-read expressed sequence tag (EST) clusters (11,065 in total) from HCC and noncancerous liver samples, which then were applied to a cDNA microarray system containing 12,393 genes/ESTs and to comparison with a public database. The commercial cDNA microarray, which contains 1,176 known genes related to oncogenesis, was used also for profiling gene expression. Integrated data from the above approaches identified 2,253 genes/ESTs as

candidates with differential expression. A number of genes related to oncogenesis and hepatic function/differentiation were selected for further semiquantitative reverse transcriptase-PCR analysis in 29 paired HCC/noncancerous liver samples. Many genes involved in cell cycle regulation such as cyclins, cyclin-dependent kinases, and cell cycle negative regulators were deregulated in most patients with HCC. Aberrant expression of the Wnt-beta-catenin pathway and enzymes for DNA replication also could contribute to the pathogenesis of HCC. The alteration of transcription levels was noted in a large number of genes implicated in metabolism, whereas a profile change of others might represent a status of dedifferentiation of the malignant hepatocytes, both considered as potential markers of diagnostic value. Notably, the altered transcriptome profiles in HCC could be correlated to a number of chromosome regions with amplification or loss of heterozygosity, providing one of the underlying causes of the transcription anomaly of HCC.

Yamaguchi, K., I. Suzuki, et al. (2002). "A two-component Mn2+-sensing system negatively regulates expression of the mntCAB operon in Synechocystis." Plant Cell **14**(11): 2901-13.

Mn is an essential component of the oxygen-evolving machinery of photosynthesis and is an essential cofactor of several important enzymes, such as Mn-superoxide dismutase and Mn-catalase. The availability of Mn in the environment varies, and little is known about the mechanisms for maintaining cytoplasmic Mn(2+) ion homeostasis. Using a DNA microarray, we screened knockout libraries of His kinases and response regulators of Synechocystis sp PCC 6803 to identify possible participants in this process. We identified a His kinase, ManS, which might sense the extracellular concentration of Mn(2+) ions, and a response regulator, ManR, which might regulate the expression of the mntCAB operon for the ABC-type transporter of Mn(2+) ions. Furthermore, analysis with the DNA microarray and by reverse transcription PCR suggested that ManS produces a signal that activates ManR, which represses the expression of the mntCAB operon. At low concentrations of Mn(2+) ions, ManS does not generate a signal, with resulting inactivation of ManR and subsequent expression of the mntCAB operon.

Yamasaki, R., K. Yokota, et al. (2004). "Immune response in Helicobacter pylori-induced low-grade gastric-mucosa-associated lymphoid tissue (MALT) lymphoma." J Med Microbiol **53**(Pt 1): 21-9.

We have reported previously that heat-shock protein 60 kDa (hsp60) of Helicobacter pylori is an important antigen in the pathogenesis of gastric mucosa-associated lymphoid tissue (MALT) lymphoma. In order to investigate associations with host immune reactions and hsp60 antigen, CD40 ligand (CD40L) expression and cytokine production were analysed following stimulation with hsp60. To provide a clear antigen-driven immune response, peripheral blood mononuclear cells (PBMC) from patients with low-grade MALT lymphoma and gastritis and those from healthy volunteers were stimulated with recombinant H. pylori hsp60 and H. pylori cell lysate in the presence of cytokines (IL4 and granulocyte-macrophage colony-stimulating factor). mRNA expression was also analysed by a cDNA microarray containing 1100 genes. Expression of CD40L on PBMCs of patients with MALT lymphoma was increased by cytokines or by combination with stimulation with hsp60 antigens. The production of IL4 in PBMC cultures was increased in patients with MALT lymphoma; however, production of IFN-gamma was at low levels. DNA microarray analysis indicated increased levels of HLA-DR and integrin mRNAs. In cases of low-grade MALT lymphoma, adaptive immune responses against hsp60 may be enhanced by host factors, such as antigen presentation and T-cell activation, resulting in B-cell proliferation, which can be demonstrated during chronic H. pylori infection.

Yanagawa, T., H. Watanabe, et al. (2004). "Overexpression of autocrine motility factor in metastatic tumor cells: possible association with augmented expression of KIF3A and GDI-beta." Lab Invest **84**(4): 513-22.

Autocrine motility factor (AMF), which is identical to phosphohexose isomerase (PHI)/glucose-6-phosphate isomerase (GPI), a ubiquitous enzyme essential for glycolysis, neuroleukin (NLK), a neurotrophic growth factor, and maturation factor (MF) mediating the differentiation of human myeloid

cells, enhances the motility and metastatic ability of tumor cells. AMF/PHI activity is elevated in the serum or urine in patients with malignant tumors. Here, we constructed an amf/phi/nlk/mf gene using adenovirus vector and transfected into two tumor cell lines. Overexpression of AMF/PHI/NLK/MF enhanced AMF secretion into the culture media in both tumor cell lines. However, upregulation of motility and metastatic ability was found only in metastatic fibrosarcoma cells expressing an AMF receptor, gp78, and was not found in gp78-undetectable osteosarcoma cells. Thus, not only serum AMF activity but also gp78-expression in tumor cells may be required for metastasis-related motility induction. With the use of microarray analyses, we detected two augmented genes, rho GDP dissociation inhibitor beta and kinesin motor 3A, as well as AMF itself. The RNA message and protein expression of these two molecules was confirmed to be upregulated, suggesting a possible association with AMF-induced signaling for cell motility and metastasis.

Yang, R., F. M. Murillo, et al. (2004). "Papillomavirus-like particles stimulate murine bone marrow-derived dendritic cells to produce alpha interferon and Th1 immune responses via MyD88." J Virol **78**(20): 11152-60.

Dendritic cells (DCs) link innate and adaptive immunity by sensing pathogens or vaccinogens and signaling a variety of defense responses. Since human papillomavirus type 16 L1 virus-like particles (HPV16 VLPs) induce a potent, protective immune response after vaccination, we examined their recognition by DCs. HPV16 VLPs cause phenotypic maturation of murine bone marrow-derived DCs (BMDCs), and immunization of mice with HPV16 VLP-loaded BMDCs or HPV16 VLPs alone induced T helper 1 (Th1)-biased immune responses. Analysis of transcriptional responses of murine BMDCs by microarray suggested that alpha/beta interferon (IFN-alpha/beta) transcripts and numerous proinflammatory cytokines and chemokines are up regulated in response to HPV16 VLPs. Indeed, the induction of IFN-alpha, IFN-gamma, and interleukin-12 (IL-12) production by BMDCs after stimulation with HPV16 VLPs was demonstrated by quantitative enzyme-linked immunosorbent assay. Many microbial products that induce proinflammatory responses are recognized via Toll-like receptor (TLR) signaling through the key adaptor protein MyD88 and activation of NF-kappaB, nuclear factor of activated T cells (NF-AT), and activating protein 1 (AP-1). Reporter assays indicated that HPV16 VLPs activated NF-kappaB-, NF-AT-, and AP-1-dependent transcription in the RAW264.7 macrophage cell line. Knockdown of MyD88 transcripts by small interfering RNA in the RAW264.7 macrophage cell line inhibited the activation of NF-kappaB-, NF-AT- and AP-1-dependent transcription by HPV16 VLP. Furthermore, MyD88(-/-) BMDCs failed to up regulate IL-12 and IFN-alpha and -gamma in response to HPV16 VLPs. Finally, Th1-biased immune responses to HPV16 VLPs are dramatically impaired in MyD88 and IFN-alpha/beta receptor-deficient mice. This implicates TLR recognition as central to immune recognition of HPV16 L1 VLPs.

Ye, R. W., W. Tao, et al. (2000). "Global gene expression profiles of Bacillus subtilis grown under anaerobic conditions." J Bacteriol **182**(16): 4458-65.

Bacillus subtilis can grow under anaerobic conditions, either with nitrate or nitrite as the electron acceptor or by fermentation. A DNA microarray containing 4,020 genes from this organism was constructed to explore anaerobic gene expression patterns on a genomic scale. When mRNA levels of aerobic and anaerobic cultures during exponential growth were compared, several hundred genes were observed to be induced or repressed under anaerobic conditions. These genes are involved in a variety of cell functions, including carbon metabolism, electron transport, iron uptake, antibiotic production, and stress response. Among the highly induced genes are not only those responsible for nitrate respiration and fermentation but also those of unknown function. Certain groups of genes were specifically regulated during anaerobic growth on nitrite, while others were primarily affected during fermentative growth, indicating a complex regulatory circuitry of anaerobic metabolism.

Yim, S. H., J. M. Ward, et al. (2003). "Microarray analysis using amplified mRNA from laser capture

microdissection of microscopic hepatocellular precancerous lesions and frozen hepatocellular carcinomas reveals unique and consistent gene expression profiles." Toxicol Pathol **31**(3): 295-303.

The indirect labeling cDNA microarray technique was used to evaluate gene expression profiles of pure cell populations from frozen sections of carcinomas and adenomas harvested from precancerous hepatocellular lesions by using laser capture microdissection (LCM). The levels of differentially expressed genes were investigated using a cDNA microarray with 9,984 features with only 2 ug of two-round amplified aRNA, equivalent to 35 cells from LCM-adenomas and frozen samples of carcinomas from simian virus 40 (SV40) large T antigen transgenic rats. A total of 855 genes were identified as being 3-fold or more differentially expressed in carcinomas or adenomas as compared to normal tissue controls. Among these 855 genes, 71 genes were differentially expressed in both carcinomas and adenomas. Commonly up-regulated genes in both carcinoma and adenomas were 28 while 41 of the 71 genes were commonly down-regulated. Two genes, Igh1 (immunoglobulin heavy chain 1(Serum IgG2a), Image clone ID: 875880) and EST clone (AI893585, Image clone ID: 596604) were more than 7-fold up-regulated in carcinomas and 6-fold down-regulated in adenomas. In Cy5 and Cy3 reciprocal experiments for screening out false positive signals, the amplified carcinomas showed higher Pearson Correlation Coefficient values (-0.94 and -0.92) than the LCM-amplified adenoma samples (-0.79 and -0.84). LCM-amplified samples provided higher signal intensities over backgrounds and a greater average of Cy5:Cy3 ratios. Expression levels of mRNAs from selected genes, determined by using traditional dot blot analysis, revealed that 36 of 40 tested expression profiles were consistent with the microarray data. Thus, amplified aRNA harvested from homogeneous cell types using LCM can be applied to study gene expression profiles by use of microarray analysis.

Yin, Y., A. Zhu, et al. (2004). "Human RAD9 checkpoint control/proapoptotic protein can activate transcription of p21." Proc Natl Acad Sci U S A **101**(24): 8864-9.

When human cells incur DNA damage, two fundamental responses can follow, cell cycle arrest or apoptosis. Human RAD9 (hRAD9) and p53 function in both processes, but the mechanistic relationship between their activities is unknown. p53 mediates checkpoint control at G(1) by transcriptional regulation of p21. In this report, we show that hRAD9, like p53, can also regulate p21 at the transcriptional level. We demonstrate that overexpression of hRAD9 leads to increased p21 RNA and encoded protein levels. The promoter region of p21 fused to a luciferase reporter can be transactivated by either hRAD9 or p53, indicating that hRAD9 regulates the p21 promoter for transcriptional control of expression. Using an electrophoretic mobility-shift assay, we show that hRAD9 specifically binds to a p53-consensus DNA-binding sequence in the p21 promoter. Microarray screening coupled with Northern analysis reveals that hRAD9 regulates the abundance of other messages in addition to p21. Our data reveal a previously undescribed mechanism for regulation of p21 and demonstrate that hRAD9 can control gene transcription. We suggest that hRAD9 and p53 co-regulate p21 to direct cell cycle progression by similar molecular mechanisms. Furthermore, hRAD9 might regulate other cellular processes as well by modulating transcription of multiple down-stream target genes.

Yong Byon, J., T. Ohira, et al. (2005). "Use of a cDNA microarray to study immunity against viral hemorrhagic septicemia (VHS) in Japanese flounder (Paralichthys olivaceus) following DNA vaccination." Fish Shellfish Immunol **18**(2): 135-147.

Japanese flounder, Paralichthys olivaceus juveniles were vaccinated against viral hemorrhagic septicemia (VHS) by intramuscular injection of 10Î¼g of a plasmid DNA vector which encodes the viral hemorrhagic septicemia virus (VHSV) glycoprotein (G) gene under the control of the cytomegalovirus promoter. Experimental challenge of two viral doses (1x10(2) TCID(50) and 1x10(3) TCID(50)) one month post-vaccination revealed that the G gene was able to induce protective immunity against VHS and this lasted until 21 days after the challenge. The VHSV G-protein gene DNA vaccine had a high protective efficiency, giving relative percentage survival (RPS) values of at least 93%. The defense mechanisms activated by the DNA vaccine were further elucidated by microarray analysis. Non-specific

immune response genes such as NK, Kupffer cell receptor, MIP1-alpha and Mx1 protein gene were observed to be up-regulated by the VHSV G-protein DNA vaccine at 1 and 3 days post-immunization. Also, specific immune-related genes including the CD20 receptor, CD8 alpha chain, CD40 and B lymphocyte cell adhesion molecule were also up-regulated during that time. We observed significant up-regulation of some immune-related genes that are necessary for antiviral defense. Significant up- and/or down-regulation of unknown genes was also observed upon DNA vaccination. Our results confirm previous reports that the VHSV G gene elicits strong humoral and cellular immune responses which may play a pivotal role in protecting the fish during virus infections.

Yoshida, K., K. Kobayashi, et al. (2001). "Combined transcriptome and proteome analysis as a powerful approach to study genes under glucose repression in Bacillus subtilis." Nucleic Acids Res **29**(3): 683-92.

We used 2D protein gel electrophoresis and DNA microarray technologies to systematically analyze genes under glucose repression in B:acillus subtilis. In particular, we focused on genes expressed after the shift from glycolytic to gluconeogenic at the middle logarithmic phase of growth in a nutrient sporulation medium, which remained repressed by the addition of glucose. We also examined whether or not glucose repression of these genes was mediated by CcpA, the catabolite control protein of this bacterium. The wild-type and ccpA1 cells were grown with and without glucose, and their proteomes and transcriptomes were compared. 2D gel electrophoresis allowed us to identify 11 proteins, the synthesis of which was under glucose repression. Of these proteins, the synthesis of four (IolA, I, S and PckA) was under CcpA-independent control. Microarray analysis enabled us to detect 66 glucose-repressive genes, 22 of which (glmS, acoA, C, yisS, speD, gapB, pckA, yvdR, yxeF, iolA, B, C, D, E, F, G, H, I, J, R, S and yxbF) were at least partially under CcpA-independent control. Furthermore, we found that CcpA and IolR, a repressor of the iol divergon, were involved in the glucose repression of the synthesis of inositol dehydrogenase encoded by iolG included in the above list. The CcpA-independent glucose repression of the iol genes appeared to be explained by inducer exclusion.

Yoshida, K., Y. H. Ohki, et al. (2004). "Bacillus subtilis LmrA is a repressor of the lmrAB and yxaGH operons: identification of its binding site and functional analysis of lmrB and yxaGH." J Bacteriol **186**(17): 5640-8.

The Bacillus subtilis lmrAB operon is involved in multidrug resistance. LmrA is a repressor of its own operon, while LmrB acts as a multidrug efflux transporter. LmrA was produced in Escherichia coli cells and was shown to bind to the lmr promoter region, in which an LmrA-binding site was identified. Genome-wide screening involving DNA microarray analysis allowed us to conclude that LmrA also repressed yxaGH, which was not likely to contribute to the multidrug resistance. LmrA bound to a putative yxaGH promoter region, in which two tandem LmrA-binding sites were identified. The LmrA regulon was thus determined to comprise lmrAB and yxaGH. All three LmrA-binding sites contained an 18-bp consensus sequence, TAGACCRKTCWMTATAWT, which could play an important role in LmrA binding.

Yoshida, K., H. Yamaguchi, et al. (2003). "Identification of additional TnrA-regulated genes of Bacillus subtilis associated with a TnrA box." Mol Microbiol **49**(1): 157-65.

Bacillus subtilis TnrA is a global regulator that responds to the availability of nitrogen sources and both activates and represses many genes during nitrogen-limited growth. In order to obtain a holistic view of the gene regulation depending on TnrA, we performed a genome-wide screening for TnrA-regulated genes associated with a TnrA box. A combination of DNA microarray hybridization and a genome-wide search for TnrA boxes allowed us to find 36 TnrA-regulated transcription units associated with a putative TnrA box. Gel retardation assaying, using probes carrying at least one putative TnrA box and the deletion derivatives of each box, indicated that 17 out of 36 transcription units were likely TnrA targets associated with the TnrA boxes, two of which (nasA and nasBCDEF) possessed a common TnrA box. The sequences of these TnrA boxes contained a consensus one, TGTNANAWWWTMTNACA. The

TnrA targets detected in this study were nrgAB, pucJKLM, glnQHMP, nasDEF, oppABCDF, nasA, nasBCDEF and ywrD for positive regulation, and gltAB, pel, ywdIJK, yycCB, yttA, yxkC, ywlFG, yodF and alsT for negative regulation, nrgAB and gltAB being well-studied TnrA targets. It was unexpected that the negatively regulated TnrA targets were as many as the positively regulated targets. The physiological role of the TnrA regulon is discussed.

Yoshimura, H., S. Yanagisawa, et al. (2002). "Screening for the target gene of cyanobacterial cAMP receptor protein SYCRP1." Mol Microbiol **43**(4): 843-53.

The target genes for SYCRP1, a cyanobacterial cAMP receptor protein, were surveyed using a DNA microarray method. Total RNAs were extracted from a wild-type strain and a sycrp1 disruptant of Synechocystis sp. PCC 6803, and the respective gene expression levels were compared. The expression levels of six genes (slr1667, slr1168, slr2015, slr2016, slr2017 and slr2018) were clearly decreased by the disruption of the sycrp1 gene. The data suggest that slr1667 and slr1668 constitute one operon and the other four genes constitute another operon. Transcription start points for the first genes of these putative operons, which are slr1667 and slr2015, were determined by primer extension experiments. Gel mobility shift assays and DNase 1 footprint analyses were carried out to explore the binding of SYCRP1 to the putative promoter regions of slr1667 and slr2015. SYCRP1 bound to the specific site in the 5' upstream region of slr1667 from positions -170 to -155 relative to the transcription start point, while it did not bind to the 5' upstream region of slr2015. It was concluded that SYCRP1 regulates the expression of the slr1667 gene directly by binding to a specific site in its promoter.

Yoshimura, N. (2001). "[Retinal neuronal cell death: molecular mechanism and neuroprotection]." Nippon Ganka Gakkai Zasshi **105**(12): 884-902.

In retinitis pigmentosa, retinal detachment, age-related macular degeneration, and glaucoma, retinal neuronal cells are damaged by a common mechanism, apoptosis. Because apoptosis is an active process that requires de novo expression of a "death message", this process can be controlled by inhibiting the expression of the "death message". We first studied whether a retinal ischemia-reperfusion model can be used as a model for retinal neuronal apoptosis. In the retinal ischemia-reperfusion injuries, typical features of apoptosis, including TUNEL-positive cells, DNA ladder formation, and ultrastructural features of apoptosis were found. Using the model, systematic research to identify the "death message" was done by DNA microarray analysis. About 200 messages were found to be up- or down-regulated during the process of retinal ischemia-reperfusion. These genes were divided into four groups: (1) transcription factor genes, (2) cell cycle-related genes, (3) reactive oxygen scavenger genes and (4) molecular chaperon genes. The possible roles of such genes in neuronal apoptosis following retinal ischemia-reperfusion injury were studied. In the model, reactive oxygen species produced by reperfusion was found to generate lipid peroxides and induced up-regulation of a transcription factor, c-Jun, that further induced aberrant expression of cell cycle-related genes such as cyclin D1 in amacrine cells. However, because no controlled expression of cell cycle-related genes takes place in retinal neurons, amacrine cells died by a G1 arrest mechanism. On the other hand, horizontal cells never expressed cyclin D1 and the cells were found to die by necrosis. The study revealed a possible mechanism of retinal neuronal apoptosis and it also became apparent that different types of neurons use different "death messages". Furthermore, the possibility that inhibition of a "death message" sometimes induces necrosis rather than apoptosis was shown. This means that we need to try inhibition of the death mechanism upstream rather than downstream. Administration of thioredoxin, an endogenous reactive oxygen species that blocks generation of lipid peroxides and thus inhibits the death process upstream, was found to be neuroprotective against retinal ischemia-reperfusion injury. Aberrant expression of c-Jun and cyclin D1 was down-regulated by the treatment. Possible roles of caspases were also studied by using the ischemia-reperfusion injury, RCS rat, and excessive light exposure damage in wild type and caspase-1 deficient mice. Also, application of adeno-associated virus that carries Bcl-xL was tested to find possible neuroprotective effects on RCS rats. Our studies showed that caspase-1 played a more important role in

the retinal photoreceptors and caspase-3 was important in neurons in the inner nuclear layer. Caspase-2 was found to be a major caspase in the retinal ganglion cell layer. In agreement with the findings, caspase-1 deficient mice showed less prominent light damage than wild type mice. Gene therapy by Bcl-xL was effective to protect retinal photoreceptor damage in RCS rats.

Yost, C., L. Hauser, et al. (2003). "A computational study of Shewanella oneidensis MR-1: structural prediction and functional inference of hypothetical proteins." Omics **7**(2): 177-91.

The genomes of many organisms have been sequenced in the last 5 years. Typically about 30% of predicted genes from a newly sequenced genome cannot be given functional assignments using sequence comparison methods. In these situations three-dimensional structural predictions combined with a suite of computational tools can suggest possible functions for these hypothetical proteins. Suggesting functions may allow better interpretation of experimental data (e.g., microarray data and mass spectroscopy data) and help experimentalists design new experiments. In this paper, we focus on three hypothetical proteins of Shewanella oneidensis MR-1 that are potentially related to iron transport/metabolism based on microarray experiments. The threading program PROSPECT was used for protein structural predictions and functional annotation, in conjunction with literature search and other computational tools. Computational tools were used to perform transmembrane domain predictions, coiled coil predictions, signal peptide predictions, sub-cellular localization predictions, motif prediction, and operon structure evaluations. Combined computational results from all tools were used to predict roles for the hypothetical proteins. This method, which uses a suite of computational tools that are freely available to academic users, can be used to annotate hypothetical proteins in general.

Zabarovsky, E. R., L. Petrenko, et al. (2003). "Restriction site tagged (RST) microarrays: a novel technique to study the species composition of complex microbial systems." Nucleic Acids Res **31**(16): e95.

We have developed a new type of microarray, restriction site tagged (RST), for example NotI, microarrays. In this approach only sequences surrounding specific restriction sites (i.e. NotI linking clones) were used for generating microarrays. DNA was labeled using a new procedure, NotI representation, where only sequences surrounding NotI sites were labeled. Due to these modifications, the sensitivity of RST microarrays increases several hundred-fold compared to that of ordinary genomic microarrays. In a pilot experiment we have produced NotI microarrays from Gram-positive and Gram-negative bacteria and have shown that even closely related Escherichia coli strains can be easily discriminated using this technique. For example, two E.coli strains, K12 and R2, differ by less than 0.1% in their 16S rRNA sequences and thus the 16S rRNA sequence would not easily discriminate between these strains. However, these strains showed distinctly different hybridization patterns with NotI microarrays. The same technique can be adapted to other restriction enzymes as well. This type of microarray opens the possibility not only for studies of the normal flora of the gut but also for any problem where quantitative and qualitative analysis of microbial (or large viral) genomes is needed.

Zabierowski, S. and N. A. DeLuca (2004). "Differential cellular requirements for activation of herpes simplex virus type 1 early (tk) and late (gC) promoters by ICP4." J Virol **78**(12): 6162-70.

The herpes simplex virus type 1 immediate-early protein, ICP4, activates the transcription of viral early and late genes and is essential for viral growth. It has been shown to bind DNA and interact with components of the general transcription machinery to activate or repress viral transcription, depending upon promoter context. Since early and late gene promoters have different architectures and cellular metabolism may be very different at early and late times after infection, the cellular requirements for ICP4-mediated activation of early and late genes may differ. This hypothesis was tested using tk and gC as representative early and late promoters, respectively. Nuclear extracts and phosphocellulose column fractions derived from nuclear extracts were able to reconstitute basal and ICP4-activated transcription of both promoters in vitro. When examining the contribution of the general transcription factors on the

ability of ICP4 to activate transcription, the fraction containing the general transcription factor TFIIA was not essential for ICP4 activation of the gC promoter, but it was required for efficient activation of the tk promoter. The addition of recombinant TFIIA restored the ability of ICP4 to efficiently activate the tk promoter, but it had no net effect on activation of the gC promoter. The dispensability of TFIIA for ICP4 activation of the gC promoter required an intact INR element. In addition, microarray and Northern blot analysis indicated that TFIIA abundance may be reduced at late times of infection. This decrease in TFIIA expression during infection and its dispensability for activation of late but not early genes suggest one of possibly many mechanisms for the transition from viral early to late gene expression.

Zaheer, A., S. N. Mathur, et al. (2002). "Overexpression of glia maturation factor in astrocytes leads to immune activation of microglia through secretion of granulocyte-macrophage-colony stimulating factor." Biochem Biophys Res Commun **294**(2): 238-44.

We infected a mixed culture of primary rat astrocytes and microglia with a replication-defective adenovirus carrying the rat glia maturation factor (GMF) cDNA. Affymetrix microarray analysis showed a big increase in the expression of several major histocompatibility complex (MHC) class II proteins along with interleukin-1beta (IL-1beta). Subsequent study using reverse transcription-polymerase chain reaction (RT-PCR) yielded the same results with the mixed culture, but not with pure astrocytes or pure microglia. We also noticed that the GMF/virus construct infected only astrocytes but not microglia. This led us to suspect that overexpression of GMF in astrocytes resulted in the secretion of an active substance that stimulated the microglia to express MHC II and IL-1beta. We identified this substance as granulocyte-macrophage-colony stimulating factor (GM-CSF). MHC II are unique to antigen-presenting cells such as microglia and monocytes. The results suggest that GMF in astrocytes can initiate a series of events, leading to immune activation in the nervous system, and implicates its involvement in autoimmune diseases such as multiple sclerosis.

Zammatteo, N., L. Jeanmart, et al. (2000). "Comparison between different strategies of covalent attachment of DNA to glass surfaces to build DNA microarrays." Anal Biochem **280**(1): 143-50.

DNA microarray is a powerful tool allowing simultaneous detection of many different target molecules present in a sample. The efficiency of the array depends mainly on the sequence of the capture probes and the way they are attached to the support. The coupling procedure must be quick, covalent, and reproducible in order to be compatible with automatic spotting devices dispensing tiny drops of liquids on the surface. We compared several coupling strategies currently used to covalently graft DNA onto a glass surface. The results indicate that fixation of aminated DNA to an aldehyde-modified surface is a choice method to build DNA microarrays. Both the coupling procedure and the hybridization efficiency have been optimized. The detection limit of human cytomegalovirus target DNA amplicons on such DNA microarrays has been estimated to be 0.01 nM by fluorescent detection.

Zeng, H., A. Q. Carlson, et al. (2003). "Flagellin is the major proinflammatory determinant of enteropathogenic Salmonella." J Immunol **171**(7): 3668-74.

The gastroenteritis-causing pathogen Salmonella typhimurium induces profound transcriptional changes in intestinal epithelia resulting in the recruitment of neutrophils whose presence is the histopathologic hallmark of salmonellosis. Here we used cDNA microarray expression profiling to define the molecular determinants that mediate such changes in model intestinal epithelia. Enteropathogenic Salmonella induced a classical proinflammatory gene expression program similar to that activated by the canonical proinflammatory agonist TNF-alpha. Nonproinflammatory bacteria, both commensals (Escherichia coli) and systemic pathogens (S. typhi), did not activate this expression profile. While S. typhimurium strains lacking the SPI-1-encoded type III system were fully proinflammatory, strains lacking the genes for the flagellar structural component flagellin were nearly devoid of proinflammatory signaling. Lastly, the epithelial proinflammatory response could be largely recapitulated by basolateral addition of purified flagellin. Thus, S. typhimurium flagellin is the major molecular trigger by which this

pathogen activates gut epithelial proinflammatory gene expression.

Zeng, P., D. A. Vadnais, et al. (2004). "Refined glufosinate selection in Agrobacterium-mediated transformation of soybean [Glycine max (L.) Merrill]." Plant Cell Rep **22**(7): 478-82.

Modern genetic analysis and manipulation of soybean (Glycine max) depend heavily on an efficient and dependable transformation process, especially in public genotypes from which expressed sequence tag (EST), bacterial artificial chromosome and microarray data have been derived. Williams 82 is the subject of EST and functional genomics analyses. However, it has not previously been transformed successfully using either somatic embryogenesis-based or cotyledonary-node transformation methods, the two predominant soybean transformation systems. An advance has recently been made in using antioxidants to enhance Agrobacterium infection of soybean. Nonetheless, an undesirable effect of using these antioxidants is the compromised recovery of transgenic soybean when combined with the use of the herbicide glufosinate as a selective agent. Therefore, we optimized both Agrobacterium infection and glufosinate selection in the presence of L-cysteine for Williams 82. We have recovered transgenic lines of this genotype with an enhanced transformation efficiency using this herbicide selection system.

Zeng, Q., L. K. McCauley, et al. (2002). "Hepatocyte growth factor inhibits anoikis by induction of activator protein 1-dependent cyclooxygenase-2. Implication in head and neck squamous cell carcinoma progression." J Biol Chem **277**(51): 50137-42.

Anoikis, also called suspension-induced apoptosis, plays an important role in tumor development, progression, and metastasis. Recently we found that hepatocyte growth factor (HGF) inhibited anoikis of human head and neck squamous cell carcinoma (HNSCC) cells by activating the extracellular signal-regulated kinase (ERK)-signaling pathway. However, the anti-apoptotic effectors that were regulated by the ERK-signaling pathway were unknown. Here we report that HGF-mediated inhibition of anoikis was dependent on activator protein-1 activity through the activation of the ERK-signaling pathway. Using a combination of microarray analysis and Northern blot analysis, we found that an anti-apoptotic gene cyclooxygenase-2 (cox-2) was induced by HGF in an activator protein-1-dependent fashion. Inhibition of Cox-2 activity partially abolished HGF-mediated cell survival, and overexpression of Cox-2 in HNSCC cells provided resistance against anoikis. Moreover, HNSCC cells stably expressing Cox-2 had aggressive tumor growth in a nude mouse model compared with control cells. Taken together, our results demonstrate that Cox-2 plays an important role in HGF-mediated anoikis resistance. HGF may stimulate the progression and growth of HNSCC in vivo by induction of Cox-2.

Zhang, A., K. M. Wassarman, et al. (2003). "Global analysis of small RNA and mRNA targets of Hfq." Mol Microbiol **50**(4): 1111-24.

Hfq, a bacterial member of the Sm family of RNA-binding proteins, is required for the action of many small regulatory RNAs that act by basepairing with target mRNAs. Hfq binds this family of small RNAs efficiently. We have used co-immunoprecipitation with Hfq and direct detection of the bound RNAs on genomic microarrays to identify members of this small RNA family. This approach was extremely sensitive; even Hfq-binding small RNAs expressed at low levels were readily detected. At least 15 of 46 known small RNAs in E. coli interact with Hfq. In addition, high signals in other intergenic regions suggested up to 20 previously unidentified small RNAs bind Hfq; five were confirmed by Northern analysis. Strong signals within genes and operons also were detected, some of which correspond to known Hfq targets. Within the argX-hisR-leuT-proM operon, Hfq appears to compete with RNase E and modulate RNA processing and degradation. Thus Hfq immunoprecipitation followed by microarray analysis is a highly effective method for detecting a major class of small RNAs as well as identifying new Hfq functions.

Zhang, C., M. Zhang, et al. (2003). "Genome diversification in phylogenetic lineages I and II of Listeria monocytogenes: identification of segments unique to lineage II populations." J Bacteriol **185**(18): 5573-

84.

Thirteen different serotypes of Listeria monocytogenes can be distinguished on the basis of variation in somatic and flagellar antigens. Although the known virulence genes are present in all serotypes, greater than 90% of human cases of listeriosis are caused by serotypes 1/2a, 1/2b, and 4b and nearly all outbreaks of food-borne listeriosis have been caused by serotype 4b strains. Phylogenetic analysis of these three common clinical serotypes places them into two different lineages, with serotypes 1/2b and 4b belonging to lineage I and 1/2a belonging to lineage II. To begin examining evolution of the genome in these serotypes, DNA microarray analysis was used to identify lineage-specific and serotype-specific differences in genome content. A set of 44 strains representing serotypes 1/2a, 1/2b, and 4b was probed with a shotgun DNA microarray constructed from the serotype 1/2a strain 10403s. Clones spanning 47 different genes in 16 different contiguous segments relative to the lineage II 1/2a genome were found to be absent in all lineage I strains tested (serotype 4b and 1/2b) and an additional nine were altered exclusively in 4b strains. Southern hybridization confirmed that conserved alterations were, in all but two loci, due to absence of the segments from the genome. Genes within these contiguous segments comprise five functional categories, including genes involved in synthesis of cell surface molecules and regulation of virulence gene expression. Phylogenetic reconstruction and examination of compositional bias in the regions of difference are consistent with a model in which the ancestor of the two lineages had the 1/2 somatic serotype and the regions absent in the lineage I genome arose by loss of ancestral sequences.

Zhang, L., U. Srinivasan, et al. (2004). "Library on a slide for bacterial comparative genomics." BMC Microbiol **4**(1): 12.

BACKGROUND: We describe a novel application of microarray technology for comparative genomics of bacteria in which libraries of entire genomes rather than the sequence of a single genome or sets of genes are arrayed on the slide and then probed for the presence or absence of specific genes and/or gene alleles. RESULTS: We first adopted a 96-well high throughput working protocol to efficiently isolate high quality genomic DNA. We then optimized conditions to print genomic DNA onto a glass slide with high density (up to 15000 spots) and to sensitively detect gene targets in each genome spot using fluorescently labeled DNA probe. Finally, we created an E. coli reference collection array and probed it for the presence or absence of the hemolysin (hly) gene using a dual channel non-competing hybridization strategy. Results from the array hybridization matched perfectly with previous tests. CONCLUSIONS: This new form of microarray technology, Library on a Slide, is an efficient way for sharing and utilizing large strain collections in comparative genomic analyses.

Zhang, Z. C., S. J. Li, et al. (2004). "Microarray analysis of extracellular matrix genes expression in myocardium of mouse with Coxsackie virus B3 myocarditis." Chin Med J (Engl) **117**(8): 1228-31.

BACKGROUND: Extracellular matrix (ECM) orchestrates cell behaviour including growth, death, apoptosis, adhesion, migration, and invasion by activating several signalling pathways. Certain components of ECM, such as integrins, may act as receptors or co-receptors of enterovirus. ECM-activated gene expressions in myocardium of viral heart disease including myocarditis and partial cardiomyopathy remain elusive. This study was to investigate the expression of ECM-activated genes in myocardium of mouse with viral myocarditis. METHODS: BALB/c mice were infected with Coxsackie virus B3 (CVB3) to establish an animal model of myocarditis. Uninfected mice were also prepared and served as controls. Specific mRNA expression pattern in myocarditic mouse heart was analysed by an in-house cDNA microarray containing 8,192 genes. Overexpressed ECM genes were selected and subsequently confirmed by Northern blot analysis. RESULTS: Nine ECM genes were isolated, from the array of 8,192 genes, as overexpressed genes in hearts of myocarditic mice in comparison with controls. Subsequent Northern blot analysis confirmed that four of the nine genes were highly expressed. Expression of these four genes, Fin15, ILk, Lamr1 and ADAMTS-1, has not been reported previously to be induced by Coxsackie virus. CONCLUSION: CVB3-induced myocarditis is associated with gene

expression profiles of certain ECM components.

Zhao, H., F. Granberg, et al. (2003). "Strategic attack on host cell gene expression during adenovirus infection." J Virol **77**(20): 11006-15.

To understand the interaction between the virus and its host, we used three sources of cDNA microarrays to examine the expression of 12,309 unique genes at 6 h postinfection of HeLa cells with high multiplicities of adenovirus type 2. Seventy-six genes with significantly changed expression ratios were identified, suggesting that adenovirus only modulates expression of a limited set of cellular genes. Quantitative real-time PCR analyses on selected genes were performed to confirm the microarray results. Significantly, a pronounced transcriptional activation by the promiscuous E1A-289R transcriptional activator was not apparent. Instead, promoter sequences in 45% of the upregulated genes harbored a potential E2F binding site, suggesting that the ability of the amino-terminal domain of E1A to regulate E2F-dependent transcription may be a major pathway for regulation of cellular gene expression. CDC25A was the only upregulated gene directly involved in cell cycle control. In contrast, several genes implicated in cell growth arrest were repressed. The transforming growth factor beta superfamily was specifically affected in the expression of both the upstream ligand and an intracellular regulator. In agreement with previous reports, adenovirus also targeted the innate immune response by downregulating several cytokines, including CLL2, CXCL1, and interleukin-6. Finally, stress response genes encoding GADD45B, ATF3, and TP53AP1 were upregulated. Importantly, we also found a novel countermeasure-activation of the apoptosis inhibitor survivin.

Zheng, M., H. Qian, et al. (2003). "DNA microarray analysis of the uninoculated eye following anterior chamber inoculation of HSV-1." Ocul Immunol Inflamm **11**(3): 187-95.

PURPOSE: To use DNA microarray to analyze the expression patterns of genes in the uninoculated eye following uniocular anterior chamber inoculation of HSV-1. METHODS: On Day 9 following inoculation of 2 x 10(4) PFU of HSV-1 (KOS strain) or an equivalent volume of tissue culture medium into one anterior chamber of BALB/c mice, the uninoculated eyes were enucleated, pooled, and total RNA was isolated. cDNA was synthesized from the total RNA. The gene expression patterns were inferred based on the hybridization intensities of the probes on the cDNA array. The hybridization signals were globally normalized and filtered. The data were analyzed using hierarchical and gene tree clustering algorithms. Additional uninoculated eyes collected on Day 9 p.i. were stained for F4/80 and CD19. RESULTS: Compared with the uninoculated eye of control mice, 3800 genes were upregulated at least twofold in the contralateral eye of HSV-1-infected mice. Among the 10 most upregulated genes, T cell-specific protein, MHC II antigen A, and MHC II k region locus 2 were upregulated 179-, 164-, and 162-fold, respectively. Ten T-cell receptor-related genes, 61 cytokine and chemokine genes, and 16 MHC genes were upregulated. Furthermore, 11 immunoglobulin and B cell genes and 11 macrophage-related genes were also upregulated. F4/80+ and CD19+ cells were observed on Day 9 p.i. CONCLUSIONS: The DNA microarray results support the idea that T cells and immunomodulatory factors (cytokines, chemokines) are likely to be involved in HSV-1 retinitis. These results also suggest that B cells and/or macrophages play a role in the pathogenesis of HSV-1 retinitis.

Zheng, M., X. Wang, et al. (2001). "DNA microarray-mediated transcriptional profiling of the Escherichia coli response to hydrogen peroxide." J Bacteriol **183**(15): 4562-70.

The genome-wide transcription profile of Escherichia coli cells treated with hydrogen peroxide was examined with a DNA microarray composed of 4,169 E. coli open reading frames. By measuring gene expression in isogenic wild-type and oxyR deletion strains, we confirmed that the peroxide response regulator OxyR activates most of the highly hydrogen peroxide-inducible genes. The DNA microarray measurements allowed the identification of several new OxyR-activated genes, including the hemH heme biosynthetic gene; the six-gene suf operon, which may participate in Fe-S cluster assembly or repair; and four genes of unknown function. We also identified several genes, including uxuA, encoding mannonate

hydrolase, whose expression might be repressed by OxyR, since their expression was elevated in the DeltaoxyR mutant strain. In addition, the induction of some genes was found to be OxyR independent, indicating the existence of other peroxide sensors and regulators in E. coli. For example, the isc operon, which specifies Fe-S cluster formation and repair activities, was induced by hydrogen peroxide in strains lacking either OxyR or the superoxide response regulators SoxRS. These results expand our understanding of the oxidative stress response and raise interesting questions regarding the nature of other regulators that modulate gene expression in response to hydrogen peroxide.

Zhou, D., Y. Han, et al. (2004). "DNA microarray analysis of genome dynamics in Yersinia pestis: insights into bacterial genome microevolution and niche adaptation." J Bacteriol **186**(15): 5138-46.

Genomics research provides an unprecedented opportunity for us to probe into the pathogenicity and evolution of the world's most deadly pathogenic bacterium, Yersinia pestis, in minute detail. In our present work, extensive microarray analysis in conjunction with PCR validation revealed that there are considerable genome dynamics, due to gene acquisition and loss, in natural populations of Y. pestis. We established a genomotyping system to group homologous isolates of Y. pestis, based on profiling or gene acquisition and loss in their genomes, and then drew an outline of parallel microevolution of the Y. pestis genome. The acquisition of a number of genomic islands and plasmids most likely induced Y. pestis to evolve rapidly from Yersinia pseudotuberculosis to a new, deadly pathogen. Horizontal gene acquisition also plays a key role in the dramatic evolutionary segregation of Y. pestis lineages (biovars and genomovars). In contrast to selective genome expansion by gene acquisition, genome reduction occurs in Y. pestis through the loss of DNA regions. We also theorized about the links between niche adaptation and genome microevolution. The transmission, colonization, and expansion of Y. pestis in the natural foci of endemic plague are parallel and directional and involve gradual adaptation to the complex of interactions between the environment, the hosts, and the pathogen itself. These adaptations are based on the natural selections against the accumulation of genetic changes within genome. Our data strongly support that the modern plague originated from Yunnan Province in China, due to the arising of biovar orientalis from biovar antiqua rather than mediaevalis.

Zhou, J. (2003). "Microarrays for bacterial detection and microbial community analysis." Curr Opin Microbiol **6**(3): 288-94.

Several types of microarrays have recently been developed and evaluated for bacterial detection and microbial community analysis. These studies demonstrated that specific, sensitive and quantitative detection could be obtained with both functional gene arrays and community genome arrays. Although single-base mismatch can be differentiated with phylogenetic oligonucleotide arrays, reliable specific detection at the single-base level is still problematic. Microarray-based hybridization approaches are also useful for defining genome diversity and bacterial relatedness. However, more rigorous and systematic assessment and development are needed to realize the full potential of microarrays for microbial detection and community analysis.

Zhou, L., X. H. Lei, et al. (2003). "Phenotype microarray analysis of Escherichia coli K-12 mutants with deletions of all two-component systems." J Bacteriol **185**(16): 4956-72.

Two-component systems are the most common mechanism of transmembrane signal transduction in bacteria. A typical system consists of a histidine kinase and a partner response regulator. The histidine kinase senses an environmental signal, which it transmits to its partner response regulator via a series of autophosphorylation, phosphotransfer, and dephosphorylation reactions. Much work has been done on particular systems, including several systems with regulatory roles in cellular physiology, communication, development, and, in the case of bacterial pathogens, the expression of genes important for virulence. We used two methods to investigate two-component regulatory systems in Escherichia coli K-12. First, we systematically constructed mutants with deletions of all two-component systems by using a now-standard technique of gene disruption (K. A. Datsenko and B. L. Wanner, Proc. Natl. Acad. Sci.

USA 97:6640-6645, 2000). We then analyzed these deletion mutants with a new technology called Phenotype MicroArrays, which permits assays of nearly 2,000 growth phenotypes simultaneously. In this study we tested 100 mutants, including mutants with individual deletions of all two-component systems and several related genes, including creBC-regulated genes (cbrA and cbrBC), phoBR-regulated genes (phoA, phoH, phnCDEFGHIJKLMNOP, psiE, and ugpBAECQ), csgD, luxS, and rpoS. The results of this battery of nearly 200,000 tests provided a wealth of new information concerning many of these systems. Of 37 different two-component mutants, 22 showed altered phenotypes. Many phenotypes were expected, and several new phenotypes were also revealed. The results are discussed in terms of the biological roles and other information concerning these systems, including DNA microarray data for a large number of the same mutants. Other mutational effects are also discussed.

Zhu, H., H. Zhao, et al. (2003). "Gene expression associated with interferon alfa antiviral activity in an HCV replicon cell line." Hepatology **37**(5): 1180-8.

Interferon alfa (IFN-alpha)-based treatment is the only therapeutic option for chronic hepatitis C viral infection. However, the molecular mechanisms of IFN-alpha antiviral activity are not completely understood. The recent development of an HCV replicon cell culture system provides a feasible experimental model to investigate the molecular details of IFN-induced direct antiviral activity in hepatocytes. In this report, we show that IFN-alpha can effectively inhibit HCV subgenomic RNA replication and suppress viral nonstructural protein synthesis. Using cDNA microarray analysis, we also show that the replicon cells have different gene expression profile compared with the parental hepatoma cells (Huh7). IFN-alpha can induce a number of responsive genes in the replicon cells. One of the genes, 6-16 (G1P3), can enhance IFN-alpha antiviral efficacy. In addition, we demonstrate that IFN-alpha can significantly activate STAT3 in hepatoma cells, suggesting that this pathway plays a role in IFN-alpha signaling. In conclusion, our results indicate that IFN-alpha antiviral activity is associated with activation of STAT3-signaling pathway and intracellular gene activation. Our results also suggest that IFN-alpha-induced target genes may play an important role in IFN-alpha anti-HCV activity.

Zhukovskaya, N. V., M. Fukuzawa, et al. (2004). "Dd-STATb, a Dictyostelium STAT protein with a highly aberrant SH2 domain, functions as a regulator of gene expression during growth and early development." Development **131**(2): 447-58.

Dictyostelium, the only known non-metazoan organism to employ SH2 domain:phosphotyrosine signaling, possesses STATs (signal transducers and activators of transcription) and protein kinases with orthodox SH2 domains. Here, however, we describe a novel Dictyostelium STAT containing a remarkably divergent SH2 domain. Dd-STATb displays a 15 amino acid insertion in its SH2 domain and the conserved and essential arginine residue, which interacts with phosphotyrosine in all other known SH2 domains, is substituted by leucine. Despite these abnormalities, Dd-STATb is biologically functional. It has a subtle role in growth, so that Dd-STATb-null cells are gradually lost from the population when they are co-cultured with parental cells, and microarray analysis identified several genes that are either underexpressed or overexpressed in the Dd-STATb null strain. The best characterised of these, discoidin 1, is a marker of the growth-development transition and it is overexpressed during growth and early development of Dd-STATb null cells. Dimerisation of STAT proteins occurs by mutual SH2 domain:phosphotyrosine interactions and dimerisation triggers STAT nuclear accumulation. Despite its aberrant SH2 domain, the Dd-STATb protein sediments at the size expected for a homodimer and it is constitutively enriched in the nucleus. Moreover, these properties are retained when the predicted site of tyrosine phosphorylation is substituted by phenylalanine. These observations suggest a non-canonical mode of activation of Dd-STATb that does not rely on orthodox SH2 domain:phosphotyrosine interactions.

Zhu-Salzman, K., R. A. Salzman, et al. (2004). "Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid." Plant Physiol **134**(1): 420-31.

When attacked by a phloem-feeding greenbug aphid (Schizaphis graminum), sorghum (Sorghum bicolor) activates jasmonic acid (JA)- and salicylic acid (SA)-regulated genes, as well as genes outside known wounding and SA signaling pathways. A collection of 672 cDNAs was obtained by differential subtraction with cDNAs prepared from sorghum seedlings infested by greenbug aphids and those from uninfested seedlings. Subsequent expression profiling using DNA microarray and northern-blot analyses identified 82 transcript types from this collection responsive to greenbug feeding, methyl jasmonate (MeJA), or SA application. DNA sequencing analyses indicated that these encoded proteins functioning in direct defense, defense signaling, oxidative burst, secondary metabolism, abiotic stress, cell maintenance, and photosynthesis, as well as proteins of unknown function. In response to insect feeding, sorghum increased transcript abundance of numerous defense genes, with some SA-dependent pathogenesis-related genes responding to greenbug more strongly than to SA. In contrast, only weak induction of MeJA-regulated defense genes was observed after greenbug treatment. However, infestation tests confirmed that JA-regulated pathways were effective in plant defense against greenbugs. Activation of certain transcripts exclusively by greenbug infestation was observed, and may represent unique signal transduction events independent of JA- and SA-regulated pathways. Results indicate that plants coordinately regulate defense gene expression when attacked by phloem-feeding aphids, but also suggest that aphids are able to avoid triggering activation of some otherwise potentially effective plant defensive machinery, possibly through their particular mode of feeding.

Zimmermann, K., T. Eiter, et al. (2003). "Consecutive analysis of bacterial PCR samples on a single electronic microarray." J Microbiol Methods **55**(2): 471-4.

For routine mass screening, the use of microarrays is hampered because one chip can only analyze one sample. 16S rRNA gene PCR products of several bacterial strains or mixtures thereof were consecutively loaded on a single electronic microarray and successfully analyzed using probes specific for the bacterial strains.

Zwiesler-Vollick, J., A. E. Plovanich-Jones, et al. (2002). "Identification of novel hrp-regulated genes through functional genomic analysis of the Pseudomonas syringae pv. tomato DC3000 genome." Mol Microbiol **45**(5): 1207-18.

Pseudomonas syringae pv. tomato (Pst) strain DC3000 infects the model plants Arabidopsis thaliana and tomato, causing disease symptoms characterized by necrotic lesions surrounded by chlorosis. One mechanism used by Pst DC3000 to infect host plants is the type III protein secretion system, which is thought to deliver multiple effector proteins to the plant cell. The exact number of type III effectors in Pst DC3000 or any other plant pathogenic bacterium is not known. All known type III effector genes of P. syringae are regulated by HrpS, an NtrC family protein, and the HrpL alternative sigma factor, which presumably binds to a conserved cis element (called the "hrp box") in the promoters of type III secretion-associated genes. In this study, we designed a search motif based on the promoter sequences conserved in 12 published hrp operons and putative effector genes in Pst DC3000. Seventy-three predicted genes were retrieved from the January 2001 release of the Pst DC3000 genome sequence, which had 95% genome coverage. The expression of the 73 genes was analysed by microarray and Northern blotting, revealing 24 genes/operons (including eight novel genes), the expression of which was consistently higher in hrp-inducing minimal medium than in nutrient-rich Luria-Bertani broth. Expression of all eight genes was dependent on the hrpS gene. Most were also dependent on the hrpL gene, but at least one was dependent on the hrpS gene, but not on the hrpL gene. An AvrRpt2-based type III translocation assay provides evidence that some of the hrpS-regulated novel genes encode putative effector proteins.

Clinical Applications

A-H

Aguilar, J. S., D. Roy, et al. (2002). "Dimethyl sulfoxide blocks herpes simplex virus-1 productive infection in vitro acting at different stages with positive cooperativity. Application of micro-array analysis." BMC Infect Dis **2**(1): 9.

BACKGROUND: Dimethyl sulfoxide (DMSO) is frequently used at a concentration of up to 95% in the formulation of antiherpetic agents because of its properties as a skin penetration enhancer. Here, we have analyzed the effect of DMSO on several parameters of Herpes Simplex Virus replication. METHODS: Productive infection levels of HSV-1 were determined by plaque assay or by reporter gene activity, and its DNA replication was estimated by PCR. Transcript levels were evaluated with HSV-specific DNA micro-arrays. RESULTS: DMSO blocks productive infection in vitro in different cell types with a 50% inhibitory concentration (IC50) from 0.7 to 2% depending upon the multiplicity of infection. The concentration dependence exhibits a Hill coefficient greater than 1, indicating that DMSO blocks productive infection by acting at multiple different points (mechanisms of action) with positive cooperativity. Consistently, we identified at least three distinct temporal target mechanisms for inhibition of virus growth by DMSO. At late stages of infection, DMSO reduces virion infectivity, and markedly inhibits viral DNA replication. A third mode of action was revealed using an oligonucleotide-based DNA microarray system for HSV. These experiments showed that DMSO reduced the transcript levels of many HSV-1 genes; including several genes coding for proteins involved in forming and assembling the virion. Also, DMSO markedly inhibited some but not all early transcripts indicating a previously unknown mode for inhibiting the early phase of HSV transcription-replication cycle. CONCLUSION: These observations suggest that DMSO itself may have a role in the anti-herpetic activity of formulations utilizing it as a dispersant.

Ahn, S. K., T. B. Choe, et al. (2003). "The gene expression profile of human umbilical vein endothelial cells stimulated with lipopolysaccharide using cDNA microarray analysis." Int J Mol Med **12**(2): 231-6.

Vascular endothelium, situated at the boundary between blood and tissues, is now known to play a critical role in the inflammatory process through recruiting immune cells into tissues and sites of inflammation. Lipopolysaccharide (LPS), endotoxic component extracted from the cell wall of gram-negative bacteria, stimulates endothelial cells to activate the nuclear transcription factor NF-kappaB and induce various adhesion molecules and inflammatory mediators. Among the anti-apoptotic genes activated by NF-kappaB, transcripts for inhibitor of apoptosis proteins (IAPs) are rapidly induced in response to LPS and delay apoptosis through direct and indirect inhibition of caspase activity. In the present study we carried out cDNA microarray analysis to elucidate how LPS alters program of gene expression of human umbilical vein endothelial cells (HUVECs) and to identify genes that are differentially expressed in HUVECs cultured with LPS as a mimickappaing of pathologic and physiologic inflammatory conditions in vitro. From the analysis of cDNA microarray together with Northern blotting and semi-quantitative RT-PCR, we identified dramatically upregulated both previously reported and undiscovered transcripts for adhesion molecules, inflammation/chemokappaines, transcription factors and anti-apoptotic proteins in LPS-stimulated HUVECs. In addition, we simultaneously identified anti-inflammatory, anti-oxidative stress and pro-apoptotic genes highly upregulated by LPS treatment in HUVECs. Surprisingly, although cIAP-1, cIAP-2 and XIAP transcripts were highly upregulated, their expression of endogenous proteins were not increased in HUVECs stimulated with LPS indicating the existence of yet undiscovered transcriptional or translational mechanisms may control expression and regulation of IAPs. The data presented here therefore suggest that when endothelial cells are challenged by inflammatory stimuli such as LPS, they undergo functional changes not only for the proinflammatory but also for the anti-inflammatory states and these may further be controlled by particular cellular or environmental signals in vascular pathological and physiological diseases.

Ahn, W. S., S. M. Bae, et al. (2004). "Searching for pathogenic gene functions to cervical cancer." Gynecol Oncol **93**(1): 41-8.

OBJECTIVES: Molecular pathology of cervical cancers associated with human papillomavirus (HPV) infection is presently unclear. In an effort to clarify this issue, we investigated gene expression profiles and pathogenic cellular processes of cervical cancer lesions. METHODS: Tissues of 11 patients (invasive cancer stages Ib-IIIa) were investigated by a cDNA microarray of 4700 genes, hierarchical clustering and the Gene Ontology (GO). RESULTS: We identified 74 genes showing a more than 2-fold difference in their expression in at least 8 out of 11 patients. Among these, 33 genes were up-regulated, in contrast, 41 genes were down-regulated. The gene expression profiles were classified into mutually dependent 345 function sets, resulting in 611 cellular processes according to the GO. The GO analysis showed that cervical carcinogenesis underwent complete down-regulation of cell death, protein biosynthesis, and nucleic acid metabolism. Also, genes belonging to nucleic acid binding and structural molecule activity were significantly down-regulated. In contrast, significant up-regulation was shown in skeletal development, immune response, and extracellular activity. CONCLUSIONS: These data suggest that the regulated genes and cellular processes could be further used for predicting prognosis and diagnosis of cervical cancer patients, and further investigation and functional characterization of the identified genes is warranted.

Ahn, W. S., S. W. Huh, et al. (2003). "A major constituent of green tea, EGCG, inhibits the growth of a human cervical cancer cell line, CaSki cells, through apoptosis, G(1) arrest, and regulation of gene expression." DNA Cell Biol **22**(3): 217-24.

A constituent of green tea, (-)-epigallocatechin-3-gallate (EGCG) has been known to possess antiproliferative properties. In this study, we investigated the anticancer effects of EGCG in human papillomavirus (HPV)-16 associated cervical cancer cell line, CaSki cells. The growth inhibitory mechanism(s) and regulation of gene expression by EGCG were also evaluated. EGCG showed growth inhibitory effects in CaSki cells in a dose-dependent fashion, with an inhibitory dose (ID)(50) of approximately 35 microM. When CaSki cells were further tested for EGCG-induced apoptosis, apoptotic cells were significantly observed after 24 h at 100 microM EGCG. In contrast, an insignificant induction of apoptotic cells was observed at 35 microM EGCG. However, cell cycles at the G1 phase were arrested at 35 microM EGCG, suggesting that cell cycle arrests might precede apoptosis. When CaSki cells were tested for their gene expression using 384 cDNA microarray, an alteration in the gene expression was observed by EGCG treatment. EGCG downregulated the expression of 16 genes over time more than twofold. In contrast, EGCG upregulated the expression of four genes more than twofold, suggesting a possible gene regulatory role of EGCG. This data supports that EGCG can inhibit cervical cancer cell growth through induction of apoptosis and cell cycle arrest as well as regulation of gene expression in vitro. Furthermore, in vivo antitumor effects of EGCG were also observed. Thus, EGCG likely provides an additional option for a new and potential drug approach for cervical cancer patients.

Ahren, I. L., E. Eriksson, et al. (2003). "Nontypeable Haemophilus influenzae activates human eosinophils through beta-glucan receptors." Am J Respir Cell Mol Biol **29**(5): 598-605.

Eosinophils are a characteristic component of the inflammatory response seen in several diseases, including allergic asthma and chronic obstructive pulmonary disease. After activation, eosinophil-derived products may exert proinflammatory effects and cause considerable tissue damage. In the present study, we investigated innate interactions between the respiratory tract pathogen nontypeable Haemophilus influenzae (NTHi) and human eosinophils. Bacterial binding to eosinophils was dependent on (1-3)-beta-D-glucan receptors, as deduced from blocking experiments using the soluble glucan derivatives laminarin and scleroglucan. In addition, expression of the beta-glucan receptor dectin-1 was shown in eosinophils by reverse transcriptase-polymerase chain reaction. Activation of the beta-glucan receptors by bacteria elicited a time- and dose-dependent respiratory burst in eosinophils. NTHi caused increased expression of the proinflammatory chemokine interleukin-8 as measured by reverse transcriptase-polymerase chain reaction and enzyme-linked immunosorbent assay. Incubation of eosinophils in the presence of NTHi for

4.5 h revealed upregulation of 245 different genes as detected by microarray. Signal transduction-related transcripts were most strongly upregulated, followed by cytokine mRNAs. Our findings suggest that NTHi can induce an innate inflammatory response in eosinophils that is mainly mediated via beta-glucan receptors. This points to possible pathophysiologic mechanisms involving innate recognition of NTHi by eosinophils during infection of the airways, thus promoting inflammation in chronic pulmonary disease.

Aizaki, H., T. Harada, et al. (2002). "Expression profiling of liver cell lines expressing entire or parts of hepatitis C virus open reading frame." Hepatology **36**(6): 1431-8.

Although hepatitis C virus (HCV) is a causative agent of liver diseases, its mechanism of pathogenesis is still unclear, mainly because of the lack of adequate cell culture systems to support HCV infection and replication. In this report, we describe development and characterization of human hepatoma cell lines constitutively expressing entire (Hep394) or parts (Hep352, Hep3294) of the HCV open reading frame (ORF). The viral and cellular proteolytic machinery involved in the viral precursor processing was consistently functional, and processed HCV proteins were synthesized in these established cell lines. By using a cDNA microarray analysis coupled with semiquantitative reverse-transcription polymerase chain reaction (RT-PCR), we identified 12 genes up-regulated and 4 genes down-regulated in Hep394 cells. With regard to genes related to cell growth regulation, we found up-regulation of forkhead transcription factor FREAC-1, poly (A) binding protein PABP2, and Ras suppressor Rsu-1. Another category of changes in gene expression includes MHC antigens, which play an important role in the T-cell-mediated immune reaction in the liver. In conclusion, functional genomic approaches comparing expression among the different cell lines expressing parts of the HCV genome may promote our understanding of the molecular basis of pathogenicity of HCV infection.

Afonso, C. L., M. E. Piccone, et al. (2004). "African swine fever virus multigene family 360 and 530 genes affect host interferon response." J Virol **78**(4): 1858-64.

African swine fever virus (ASFV) multigene family 360 and 530 (MGF360/530) genes affect viral growth in macrophage cell cultures and virulence in pigs (L. Zsak, Z. Lu, T. G. Burrage, J. G. Neilan, G. F. Kutish, D. M. Moore, and D. L. Rock, J. Virol. 75:3066-3076, 2001). The mechanism by which these novel genes affect virus-host interactions is unknown. To define MGF360/530 gene function, we compared macrophage transcriptional responses following infection with parental ASFV (Pr4) and an MGF360/530 deletion mutant (Pr4 Delta 35). A swine cDNA microarray containing 7,712 macrophage cDNA clones was used to compare the transcriptional profiles of swine macrophages infected with Pr4 and Pr4 Delta 35 at 3 and 6 h postinfection (hpi). While at 3 hpi most (7,564) of the genes had similar expression levels in cells infected with either virus, 38 genes had significantly increased (>2.0-fold, $P < 0.05$) mRNA levels in Pr4 Delta 35-infected macrophages. Similar up-regulation of these genes was observed at 6 hpi. Viral infection was required for this induced transcriptional response. Most Pr Delta 35 up-regulated genes were part of a type I interferon (IFN) response or were genes that are normally induced by double-stranded RNA and/or viral infection. These included monocyte chemoattractant protein, transmembrane protein 3, tetratricopeptide repeat protein 1, a ubiquitin-like 17-kDa protein, ubiquitin-specific protease ISG43, an RNA helicase DEAD box protein, GTP-binding MX protein, the cytokine IP-10, and the PKR activator PACT. Differential expression of IFN early-response genes in Pr4 Delta 35 relative to Pr4 was confirmed by Northern blot analysis and real-time PCR. Analysis of IFN-alpha mRNA and secreted IFN-alpha levels at 3, 8, and 24 hpi revealed undetectable IFN-alpha in mock- and Pr4-infected macrophages but significant IFN-alpha levels at 24 hpi in Pr4 Delta 35-infected macrophages. The absence of IFN-alpha in Pr4-infected macrophages suggests that MGF360/530 genes either directly or indirectly suppress a type I IFN response. An inability to suppress host type I IFN responses may account for the growth defect of Pr4 Delta 35 in macrophages and its attenuation in swine.

Alexander, J. J., A. K. Saxena, et al. (2002). "Prominent renal expression of a murine leukemia retrovirus in experimental systemic lupus erythematosus." J Am Soc Nephrol **13**(12): 2869-77.

A role for retroviruses in human systemic lupus erythematosus (SLE) and in mouse lupus models such as the New Zealand Black and White mice (NZB/W) strain has been postulated. This study compared the gene profile of nephritic NZB/W kidney with nondiseased NZW controls. The most highly upregulated gene (5.5-fold) hybridized with an expressed sequence tag on a cDNA microarray, which was sequenced and found to correspond with an endogenous murine retrovirus related to the Duplan strain (EDV, L08395). NZB/W kidney contained the full-length 4.2-kb EDV transcript. By 4 wk of age in NZB/W mice, an age preceding renal histologic disease, the EDV transcript was more than threefold increased relative to NZB or NZW control strains. This upregulated expression tended to fall with progression of renal histologic disease. By in situ hybridization, the EDV transcript was highly expressed in tubules of NZB/W mice. There was also upregulated expression of EDV transcript in NZB/W lung and brain, sites of inflammation in this strain, but not in spleen or liver. Thus, using microarrays, the most highly expressed gene in mouse lupus nephritis corresponded to an endogenous retrovirus. This retroviral transcript was highly expressed in the kidneys of lupus mice and tended to decline with advancement of disease. The remarkable upregulation of the EDV transcript only in the setting of active disease suggests this transcript is involved in inflammatory disease.

Almeida, R., A. Norrish, et al. (2002). "From genomes to vaccines: Leishmania as a model." Philos Trans R Soc Lond B Biol Sci **357**(1417): 5-11.

The 35 Mb genome of Leishmania should be sequenced by late 2002. It contains approximately 8500 genes that will probably translate into more than 10 000 proteins. In the laboratory we have been piloting strategies to try to harness the power of the genome-proteome for rapid screening of new vaccine candidate. To this end, microarray analysis of 1094 unique genes identified using an EST analysis of 2091 cDNA clones from spliced leader libraries prepared from different developmental stages of Leishmania has been employed. The plan was to identify amastigote-expressed genes that could be used in high-throughput DNA-vaccine screens to identify potential new vaccine candidates. Despite the lack of transcriptional regulation that polycistronic transcription in Leishmania dictates, the data provide evidence for a high level of post-transcriptional regulation of RNA abundance during the developmental cycle of promastigotes in culture and in lesion-derived amastigotes of Leishmania major. This has provided 147 candidates from the 1094 unique genes that are specifically upregulated in amastigotes and are being used in vaccine studies. Using DNA vaccination, it was demonstrated that pooling strategies can work to identify protective vaccines, but it was found that some potentially protective antigens are masked by other disease-exacerbatory antigens in the pool. A total of 100 new vaccine candidates are currently being tested separately and in pools to extend this analysis, and to facilitate retrospective bioinformatic analysis to develop predictive algorithms for sequences that constitute potentially protective antigens. We are also working with other members of the Leishmania Genome Network to determine whether RNA expression determined by microarray analyses parallels expression at the protein level. We believe we are making good progress in developing strategies that will allow rapid translation of the sequence of Leishmania into potential interventions for disease control in humans.

Alsaker, K. V., T. R. Spitzer, et al. (2004). "Transcriptional analysis of spo0A overexpression in Clostridium acetobutylicum and its effect on the cell's response to butanol stress." J Bacteriol **186**(7): 1959-71.

Spo0A is the regulator of stationary-phase events and is required for transcription of solvent formation genes in Clostridium acetobutylicum. In order to elucidate the role of spo0A in differentiation, we performed transcriptional analysis of 824(pMSPOA) (a spo0A-overexpressing C. acetobutylicum strain with enhanced sporulation) against a plasmid control strain. DNA microarray data were contrasted to data from a spo0A knockout strain (SKO1) that neither sporulates nor produces solvents. Transcripts of fatty acid metabolism genes, motility and chemotaxis genes, heat shock protein genes, and genes encoding the Fts family of cell division proteins were differentially expressed in the two strains, suggesting that these genes play roles in sporulation and the solvent stress response. 824(pMSPOA) alone

showed significant downregulation of many glycolytic genes in stationary phase, which is consistent with metabolic flux analysis data. Surprisingly, spo0A overexpression resulted in only nominal transcriptional changes of regulatory genes (abrB and sigF) whose expression was significantly altered in SKO1. Overexpression of spo0A imparted increased tolerance and prolonged metabolism in response to butanol stress. While most of the differentially expressed genes appear to be part of a general stress response (similar to patterns in two plasmid control strains and a groESL-overexpressing strain), several genes were expressed at higher levels at early time points after butanol challenge only in 824(pMSPOA). Most of these genes were related to butyryl coenzyme A and butyrate formation and/or assimilation, but they also included the cell division gene ftsX, the gyrase subunit-encoding genes gyrB and gyrA, DNA synthesis and repair genes, and fatty acid synthesis genes, all of which might play a role in the immediate butanol stress response, and thus in enhanced butanol tolerance.

Amexis, G., S. Rubin, et al. (2002). "Sequence diversity of Jeryl Lynn strain of mumps virus: quantitative mutant analysis for vaccine quality control." Virology **300**(2): 171-9.

The Jeryl Lynn strain of mumps vaccine live (MVL) was developed in 1966 by Merck Co. and has been widely used in the U.S. and other countries since the early 1970s. Partial sequencing has recently shown that the vaccine contains a mixture of two substrains with substantially different nucleotide sequences. We have determined the complete genomic sequences of both substrains and identified 414 nucleotide differences (2.69%), leading to 87 amino acid substitutions (1.67%). We used this information to develop methods for quantification of the substrain components in vaccine samples based on PCR and restriction enzyme cleavage and oligonucleotide microarray hybridization and monitored their dynamics in viral populations propagated in different conditions. Passaging Jeryl Lynn strain in Vero or CEF cell cultures resulted in rapid selection of the major component JL1, while growth in embryonated chicken eggs (ECE) favored accumulation of the minor component JL2. Based on the findings presented here, it is proposed that the substrain composition of Jeryl Lynn vaccine can be monitored as a part of its quality control to ensure consistency of the vaccine.

An, H. J., N. H. Cho, et al. (2003). "Correlation of cervical carcinoma and precancerous lesions with human papillomavirus (HPV) genotypes detected with the HPV DNA chip microarray method." Cancer **97**(7): 1672-80.

BACKGROUND: Human papillomavirus (HPV) infection is considered to play an important role in the development of cervical carcinoma, and it is known that certain HPV types, such as HPV-16 and HPV-18, are highly associated with cervical carcinoma. However, the pathologic behavior of other HPV types remains unclear. Recently, a new HPV detection technique, the HPV DNA chip, was introduced. The HPV DNA chip harbors 22 HPV probes and has the advantage of being able to detect 22 HPV types simultaneously. To evaluate the quality of the HPV DNA chip method and to identify HPV types related to cervical carcinoma and precancerous lesions, the authors performed HPV typing in cervical specimens from 1983 patients and compared their cytologic and histologic diagnoses. METHODS: The HPV DNA chip was used for HPV typing. Among 1983 patients who were tested for HPV types, cervical smear cytology was performed in 1650 patients, and 677 of those patients underwent cervical biopsy. RESULTS: Among the 1650 smears that were examined cytologically, 92.7% (114 of 123 smears) of low-grade squamous intraepithelial lesions (LSILs), 98.1% (106 of 108 smears) of high-grade squamous intraepithelial lesions (HSILs), and 96.3% (51 of 53 smears) of carcinomas were HPV positive, compared with only 35.1% of smears with normal cytology that were HPV positive. HPV-16 was the most prevalent type (chi-square test; $P < 0.01$) in LSILs (28.5%), in HSILs (51.9%), and in carcinomas (62.5%) followed by HPV-58 and a group of low-risk types (HPV-6, HPV-11, HPV-34, HPV-40, HPV-42, HPV-43,and HPV-44) in LSILs. HPV-58 (15.7%), HPV-18 (6.7%), and HPV-52 (4.6%) were the next most prevalent types after HPV-16 in HSILs. HPV-18 (11.4%) and HPV-58 (11.4%) were the second most common types in carcinomas. HPV-58 had the highest positive predictive value (54.9%) for the detection of histologically confirmed HSIL or carcinoma, whereas HPV 16 had the highest negative predictive value

(80.6%). The sensitivity (96.0%) of the HPV test using the DNA chip method for detecting HSIL or carcinoma was superior compared with the sensitivity of cytologic diagnosis (83.6%). CONCLUSIONS: The HPV DNA chip provides a very sensitive method for detecting 22 HPV genotypes with reasonable sensitivity (96.0%) and reasonable negative predictive value (96.9%), and it overcomes the low sensitivity of cytologic screening for the detection of HSIL or carcinoma. HPV-58, HPV-52, and HPV-56, as well as HPV-16 and HPV-18, were associated highly with HSIL and carcinoma in the current large series. In addition, multiple HPV infection was associated less frequently with cervical carcinoma and with precancerous lesions compared with normal cytology.

Anders, R. A., L. M. Yerian, et al. (2003). "cDNA microarray analysis of macroregenerative and dysplastic nodules in end-stage hepatitis C virus-induced cirrhosis." Am J Pathol **162**(3): 991-1000.

Hepatocellular carcinoma is a common malignancy causing significant morbidity and mortality worldwide. In this study we use expression microarray technology to identify novel genes that consistently displayed altered expression levels in the earliest identifiable precursors to hepatocellular carcinoma, dysplastic and macroregenerative nodules. The gene expression profiles from nine patients with end-stage hepatitis C cirrhosis that contained a combined 11 dysplastic or macroregenerative nodules were compared to the patient's matched cirrhotic liver tissue. A total of 53 genes were consistently dysregulated in the patient liver specimens. Six of seven genes were validated by quantitative real-time reverse transcriptase-polymerase chain reaction, or by immunohistochemical studies performed on an independent set of lesions. The novel genes, including caveolin-1, semaphorin E, and FMS-like tyrosine kinase 3 ligand, have putative roles in carcinogenesis but have not been reported in hepatocellular carcinogenesis. Microarray expression analysis of dysplastic and macroregenerative liver nodules provide insight into the earliest changes in hepatocellular carcinogenesis

Araki, M., K. Hiratsuka, et al. (2004). "Monitoring of dnaK gene expression in Porphyromonas gingivalis by oxygen stress using DNA microarray." J Oral Sci **46**(2): 93-100.

Porphyromonas gingivalis, a Gram-negative anaerobe associated with adult periodontitis, expresses numerous potential virulence factors. dnaK, a member of the heat shock protein family, functions as a molecular chaperone and plays a role in microbial pathogenicity. However, little is known regarding its gene expression caused by oxygen stress in P. gingivalis. In the present study, a custom-made DNA microarray was designed and used to monitor dnaK gene expression in P. gingivalis caused by oxygen stress. The results demonstrated that dnaK mRNA was up-regulated in a short time, and the DNA microarray results were confirmed by real-time polymerase chain reaction analysis. These findings suggest that oxygen stress stimulates gene expression of dnaK and may have a relationship to the aerotolerance activity of this organism as well as its expression of pathogenesis.

Arias, L. F., S. Hernandez, et al. (2003). "Epstein-Barr virus latency in kidney specimens from transplant recipients." Nephrol Dial Transplant **18**(12): 2638-43.

BACKGROUND: Epstein-Barr virus (EBV) infection is common in immunosuppressed patients and can lead to life threatening lymphoproliferative diseases. Small numbers of cells infected by EBV have been detected in human tissues, transplanted or non-transplanted. Little is known about EBV latency in the allograft kidneys of patients without post-transplant lymphoproliferative disease (PTLD). The aims of this study were to look for the presence of EBV-encoded small RNAs (EBER) in allograft kidneys and to quantify their expression. METHODS: We analysed 62 allograft nephrectomies and 20 native kidneys to determine the presence of EBV; we also quantified its expression and calculated its ratios to CD45 and CD20 cells. The techniques used were: tissue microarray, EBER-1- and 2-specific in situ hybridization and immunohistochemistry. RESULTS: EBER expression was detected in 30.6% of transplanted kidneys and 5% of non-transplanted kidneys. In the positive specimens, a mean of 8.2 cells/1.57 mm(2) expressed the EBERs (range 1-38 cells). The ratios of EBER-positive (+) cells to CD45 or CD20 cells were 1.7 +/- 2.4% (range 0.1-8.1%) and 8.4 +/- 10.9% (range 0.5-34.4%), respectively. No relationship was found

between anti-T-cell treatment and EBER expression in the failed allografts. CONCLUSIONS: In failed kidney allografts, a small number of lymphocytes can express EBV latency. The number of EBER+ cells is smaller than in PTLD. Studies of functioning grafts are necessary to better understand the clinical relevance of this expression.

Arias, M., B. Lahme, et al. (2002). "Adenoviral delivery of an antisense RNA complementary to the 3' coding sequence of transforming growth factor-beta1 inhibits fibrogenic activities of hepatic stellate cells." Cell Growth Differ **13**(6): 265-73.

Liver fibrosis occurs as a consequence of the transdifferentiation of hepatic stellate cells into myofibroblasts and is associated with an increased expression and activation of transforming growth factor (TGF)-beta1. This pluripotent, profibrogenic cytokine stimulates matrix synthesis and decreases matrix degradation, resulting in fibrosis. Thus, blockade of synthesis or sequestering of mature TGF-beta1 is a primary target for the development of antifibrotic approaches. The purpose of this study was to investigate whether the administration of adenoviruses constitutively expressing an antisense mRNA complementary to the 3' coding sequence of TGF-beta1 is able to suppress the synthesis of TGF-beta1 in culture-activated hepatic stellate cells. We demonstrate that the adenoviral vehicle directs high-level expression of the transgene and proved that the transduced antisense is biologically active by immunoprecipitation, Western blot, quantitative TGF-beta1 ELISA, and cell proliferation assays. Additionally, the biological function of the transgene was confirmed by analysis of differential activity of TGF-beta1-responsive genes using cell ELISA, Northern blotting, and by microarray technology, respectively. Furthermore, we examined the effects of that transgene on the expression of TGF-beta2, TGF-beta3, collagen type alpha1(I), latent transforming growth factor binding protein 1, types I and II TGF-beta receptors, and alpha-smooth muscle actin. Our results indicate that the administration of antisense mRNA offers a feasible approach to block autocrine TGF-beta1 signaling in hepatic stellate cells and may be useful and applicable in future to the treatment of fibrosis in chronic liver diseases.

Bacarese-Hamilton, T., L. Mezzasoma, et al. (2004). "Serodiagnosis of infectious diseases with antigen microarrays." J Appl Microbiol **96**(1): 10-7.

AIMS: To generate protein microarrays by printing microbial antigens on slides to enable the simultaneous determination in human sera of antibodies directed against Toxoplasma gondii, rubella virus, cytomegalovirus and herpes simplex virus (HSV) types 1 and 2. METHODS AND RESULTS: Antigens were printed on activated glass slides using high-speed robotics. The slides were incubated with serum samples and subsequently with fluorescently labelled secondary antibodies. Human IgG and IgM bound to the printed antigens were detected using confocal scanning microscopy and quantified with internal calibration curves. The microarray assay could detect as little as 0.5 pg of both IgG and IgM bound onto the glass surface. Precision profiles ranged from 1.7 to 18.5% for all the antigens. Microarrays and commercial ELISAs were utilized to detect serum antibodies against the ToRCH antigens in a panel of characterized human sera. Overall >80% concordance was obtained between microarray and ELISA kits in the classification of sera. CONCLUSIONS: These results indicate that the microarray is a suitable assay format for the serodiagnosis of infectious diseases. SIGNIFICANCE AND IMPACT OF STUDY: Antigen microarrays can be optimized for clinical use, their performance is equivalent to ELISA but they offer significant advantages in throughput, convenience and cost.

Bagge, N., M. Schuster, et al. (2004). "Pseudomonas aeruginosa biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production." Antimicrob Agents Chemother **48**(4): 1175-87.

The lungs of cystic fibrosis (CF) patients are commonly colonized with Pseudomonas aeruginosa biofilms. Chronic endobronchial P. aeruginosa infections are impossible to eradicate with antibiotics, but intensive suppressive antibiotic therapy is essential to maintain the lung function of CF patients. The treatment often includes beta-lactam antibiotics. How these antibiotics influence gene expression in the

surviving biofilm population of P. aeruginosa is not clear. Thus, we used the microarray technology to study the effects of subinhibitory concentrations of a beta-lactam antibiotic, imipenem, on gene expression in biofilm populations. Many genes showed small but statistically significant differential expression in response to imipenem. We identified 34 genes that were induced or repressed in biofilms exposed to imipenem more than fivefold compared to the levels of induction or repression for the controls. As expected, the most strongly induced gene was ampC, which codes for chromosomal beta-lactamase. We also found that genes coding for alginate biosynthesis were induced by exposure to imipenem. Alginate production is correlated to the development of impaired lung function, and P. aeruginosa strains isolated from chronically colonized lungs of CF patients are nearly always mucoid due to the overproduction of alginate. Exposure to subinhibitory concentrations of imipenem caused structural changes in the biofilm, e.g., an increased biofilm volume. Increased levels of alginate production may be an unintended adverse consequence of imipenem treatment in CF patients.

Bahl, A., B. Brunk, et al. (2002). "PlasmoDB: the Plasmodium genome resource. An integrated database providing tools for accessing, analyzing and mapping expression and sequence data (both finished and unfinished)." Nucleic Acids Res **30**(1): 87-90.

PlasmoDB (http://PlasmoDB.org) is the official database of the Plasmodium falciparum genome sequencing consortium. This resource incorporates finished and draft genome sequence data and annotation emerging from Plasmodium sequencing projects. PlasmoDB currently houses information from five parasite species and provides tools for cross-species comparisons. Sequence information is also integrated with other genomic-scale data emerging from the Plasmodium research community, including gene expression analysis from EST, SAGE and microarray projects. The relational schemas used to build PlasmoDB [Genomics Unified Schema (GUS) and RNA Abundance Database (RAD)] employ a highly structured format to accommodate the diverse data types generated by sequence and expression projects. A variety of tools allow researchers to formulate complex, biologically based queries of the database. A version of the database is also available on CD-ROM (Plasmodium GenePlot), facilitating access to the data in situations where Internet access is difficult (e.g. by malaria researchers working in the field). The goal of PlasmoDB is to enhance utilization of the vast quantities of data emerging from genome-scale projects by the global malaria research community.

Bahl, A., B. Brunk, et al. (2003). "PlasmoDB: the Plasmodium genome resource. A database integrating experimental and computational data." Nucleic Acids Res **31**(1): 212-5.

PlasmoDB (http://PlasmoDB.org) is the official database of the Plasmodium falciparum genome sequencing consortium. This resource incorporates the recently completed P. falciparum genome sequence and annotation, as well as draft sequence and annotation emerging from other Plasmodium sequencing projects. PlasmoDB currently houses information from five parasite species and provides tools for intra- and inter-species comparisons. Sequence information is integrated with other genomic-scale data emerging from the Plasmodium research community, including gene expression analysis from EST, SAGE and microarray projects and proteomics studies. The relational schema used to build PlasmoDB, GUS (Genomics Unified Schema) employs a highly structured format to accommodate the diverse data types generated by sequence and expression projects. A variety of tools allow researchers to formulate complex, biologically-based, queries of the database. A stand-alone version of the database is also available on CD-ROM (P. falciparum GenePlot), facilitating access to the data in situations where internet access is difficult (e.g. by malaria researchers working in the field). The goal of PlasmoDB is to facilitate utilization of the vast quantities of genomic-scale data produced by the global malaria research community. The software used to develop PlasmoDB has been used to create a second Apicomplexan parasite genome database, ToxoDB (http://ToxoDB.org).

Barker, K. S., S. Crisp, et al. (2004). "Genome-wide expression profiling reveals genes associated with amphotericin B and fluconazole resistance in experimentally induced antifungal resistant isolates of

Candida albicans." J Antimicrob Chemother **54**(2): 376-85.

OBJECTIVES: The aim of this study was to identify changes in the gene expression profile of Candida albicans associated with the acquisition of experimentally induced resistance to amphotericin B and fluconazole. METHODS: C. albicans SC5314 was passed in increasing concentrations of amphotericin B to generate isolate SC5314-AR. Susceptibility testing by Etest revealed SC5314-AR to be highly resistant to both amphotericin B and fluconazole. The gene expression profile of SC5314-AR was compared with that of SC5314 using DNA microarray analysis. Sterol composition was determined for both strains. RESULTS: Upon examination of MICs of antifungal compounds, it was found that SC5314-AR was resistant to both amphotericin B and fluconazole. By microarray analysis a total of 134 genes were found to be differentially expressed, that is up-regulated or down-regulated by at least 50%, in SC5314-AR. In addition to the cell stress genes DDR48 and RTA2, the ergosterol biosynthesis genes ERG5, ERG6 and ERG25 were up-regulated. Several histone genes, protein synthesis genes and energy generation genes were down-regulated. Sterol analysis revealed the prevalence of sterol intermediates eburicol and lanosterol in SC5314-AR, whereas ergosterol was the predominant sterol in SC5314. CONCLUSION: Along with changes in expression of these ergosterol biosynthesis genes was the accumulation of sterol intermediates in the resistant strain, which would account for the decreased affinity of amphotericin B for membrane sterols and a decreased requirement for lanosterol demethylase activity in membrane sterol production. Furthermore, other genes are implicated as having a potential role in the polyene and azole antifungal resistant phenotype.

Baskin, C. R., A. Garcia-Sastre, et al. (2004). "Integration of clinical data, pathology, and cDNA microarrays in influenza virus-infected pigtailed macaques (Macaca nemestrina)." J Virol **78**(19): 10420-32.

For most severe viral pandemics such as influenza and AIDS, the exact contribution of individual viral genes to pathogenicity is still largely unknown. A necessary step toward that understanding is a systematic comparison of different influenza virus strains at the level of transcriptional regulation in the host as a whole and interpretation of these complex genetic changes in the context of multifactorial clinical outcomes and pathology. We conducted a study by infecting pigtailed macaques (Macaca nemestrina) with a genetically reconstructed strain of human influenza H1N1 A/Texas/36/91 virus and hypothesized not only that these animals would respond to the virus similarly to humans, but that gene expression patterns in the lungs and tracheobronchial lymph nodes would fit into a coherent and complete picture of the host-virus interactions during infection. The disease observed in infected macaques simulated uncomplicated influenza in humans. Clinical signs and an antibody response appeared with induction of interferon and B-cell activation pathways, respectively. Transcriptional activation of inflammatory cells and apoptotic pathways coincided with gross and histopathological signs of inflammation, with tissue damage and concurrent signs of repair. Additionally, cDNA microarrays offered new evidence of the importance of cytotoxic T cells and natural killer cells throughout infection. With this experiment, we confirmed the suitability of the nonhuman primate model in the quest for understanding the individual and joint contributions of viral genes to influenza virus pathogenesis by using cDNA microarray technology and a reverse genetics approach.

Baumgarth, N., R. Szubin, et al. (2004). "Highly tissue substructure-specific effects of human papilloma virus in mucosa of HIV-infected patients revealed by laser-dissection microscopy-assisted gene expression profiling." Am J Pathol **165**(3): 707-18.

Human papilloma virus (HPV) causes focal infections of epithelial layers in skin and mucosa. HIV-infected patients on highly active antiretroviral therapy (HAART) appear to be at increased risk of developing HPV-induced oral warts. To identify the mechanisms that allow long-term infection of oral epithelial cells in these patients, we used a combination of laser-dissection microscopy (LDM) and highly sensitive and quantitative, non-biased, two-step multiplex real-time RT-PCR to study pathogen-induced alterations of specific tissue subcompartments. Expression of 166 genes was compared in three distinct

epithelial and subepithelial compartments isolated from biopsies of normal mucosa from HIV-infected and non-infected patients and of HPV32-induced oral warts from HIV-infected patients. In contrast to the underlying HIV infection and/or HAART, which did not significantly elaborate tissue substructure-specific effects, changes in oral warts were strongly tissue substructure-specific. HPV 32 seems to establish infection by selectively enhancing epithelial cell growth and differentiation in the stratum spinosum and to evade the immune system by actively suppressing inflammatory responses in adjacent underlying tissues. With this highly sensitive and quantitative method tissue-specific expression of hundreds of genes can be studied simultaneously in a few cells. Because of its large dynamic measurement range it could also become a method of choice to confirm and better quantify results obtained by microarray analysis.

Behr, M. A., M. A. Wilson, et al. (1999). "Comparative genomics of BCG vaccines by whole-genome DNA microarray." Science **284**(5419): 1520-3.

Bacille Calmette-Guerin (BCG) vaccines are live attenuated strains of Mycobacterium bovis administered to prevent tuberculosis. To better understand the differences between M. tuberculosis, M. bovis, and the various BCG daughter strains, their genomic compositions were studied by performing comparative hybridization experiments on a DNA microarray. Regions deleted from BCG vaccines relative to the virulent M. tuberculosis H37Rv reference strain were confirmed by sequencing across the missing segment of the H37Rv genome. Eleven regions (encompassing 91 open reading frames) of H37Rv were found that were absent from one or more virulent strains of M. bovis. Five additional regions representing 38 open reading frames were present in M. bovis but absent from some or all BCG strains; this is evidence for the ongoing evolution of BCG strains since their original derivation. A precise understanding of the genetic differences between closely related Mycobacteria suggests rational approaches to the design of improved diagnostics and vaccines.

Bekal, S., R. Brousseau, et al. (2003). "Rapid identification of Escherichia coli pathotypes by virulence gene detection with DNA microarrays." J Clin Microbiol **41**(5): 2113-25.

One approach to the accurate determination of the pathogenic potential (pathotype) of isolated Escherichia coli strains would be through a complete assessment of each strain for the presence of all known E. coli virulence factors. To accomplish this, an E. coli virulence factor DNA microarray composed of 105 DNA PCR amplicons printed on glass slides and arranged in eight subarrays corresponding to different E. coli pathotypes was developed. Fluorescently labeled genomic DNAs from E. coli strains representing known pathotypes were initially hybridized to the virulence gene microarrays for both chip optimization and validation. Hybridization pattern analysis with clinical isolates permitted a rapid assessment of their virulence attributes and determination of the pathogenic group to which they belonged. Virulence factors belonging to two different pathotypes were detected in one human E. coli isolate (strain H87-5406). The microarray was also tested for its ability to distinguish among phylogenetic groups of genes by using gene probes derived from the attaching-and-effacing locus (espA, espB, tir). After hybridization with these probes, we were able to distinguish E. coli strains harboring espA, espB, and tir sequences closely related to the gene sequences of an enterohemorrhagic strain (EDL933), a human enteropathogenic strain (E2348/69), or an animal enteropathogenic strain (RDEC-1). Our results show that the virulence factor microarray is a powerful tool for diagnosis-based studies and that the concept is useful for both gene quantitation and subtyping. Additionally, the multitude of virulence genes present on the microarray should greatly facilitate the detection of virulence genes acquired by horizontal transfer and the identification of emerging pathotypes.

Ben Mamoun, C., I. Y. Gluzman, et al. (2001). "Co-ordinated programme of gene expression during asexual intraerythrocytic development of the human malaria parasite Plasmodium falciparum revealed by microarray analysis." Mol Microbiol **39**(1): 26-36.

Plasmodium falciparum is a protozoan parasite responsible for the most severe forms of human

malaria. All the clinical symptoms and pathological changes seen during human infection are caused by the asexual blood stages of Plasmodium. Within host red blood cells, the parasite undergoes enormous developmental changes during its maturation. In order to analyse the expression of genes during intraerythrocytic development, DNA microarrays were constructed and probed with stage-specific cDNA. Developmental upregulation of specific mRNAs was found to cluster into functional groups and revealed a co-ordinated programme of gene expression. Those involved in protein synthesis (ribosomal proteins, translation factors) peaked early in development, followed by those involved in metabolism, most dramatically glycolysis genes. Adhesion/invasion genes were turned on later in the maturation process. At the end of intraerythrocytic development (late schizogony), there was a general shut-off of gene expression, although a small set of genes, including a number of protein kinases, were turned on at this stage. Nearly all genes showed some regulation over the course of development. A handful of genes remained constant and should be useful for normalizing mRNA levels between stages. These data will facilitate functional analysis of the P. falciparum genome and will help to identify genes with a critical role in parasite progression and multiplication in the human host.

Beres, S. B., G. L. Sylva, et al. (2004). "Genome-wide molecular dissection of serotype M3 group A Streptococcus strains causing two epidemics of invasive infections." Proc Natl Acad Sci U S A **101**(32): 11833-8.

Molecular factors that contribute to the emergence of new virulent bacterial subclones and epidemics are poorly understood. We hypothesized that analysis of a population-based strain sample of serotype M3 group A Streptococcus (GAS) recovered from patients with invasive infection by using genome-wide investigative methods would provide new insight into this fundamental infectious disease problem. Serotype M3 GAS strains (n = 255) cultured from patients in Ontario, Canada, over 11 years and representing two distinct infection peaks were studied. Genetic diversity was indexed by pulsed-field gel electrophoresis, DNA-DNA microarray, whole-genome PCR scanning, prophage genotyping, targeted gene sequencing, and single-nucleotide polymorphism genotyping. All variation in gene content was attributable to acquisition or loss of prophages, a molecular process that generated unique combinations of proven or putative virulence genes. Distinct serotype M3 genotypes experienced rapid population expansion and caused infections that differed significantly in character and severity. Molecular genetic analysis, combined with immunologic studies, implicated a 4-aa duplication in the extreme N terminus of M protein as a factor contributing to an epidemic wave of serotype M3 invasive infections. This finding has implications for GAS vaccine research. Genome-wide analysis of population-based strain samples cultured from clinically well defined patients is crucial for understanding the molecular events underlying bacterial epidemics.

Bernardini, G., G. Renzone, et al. (2004). "Proteome analysis of Neisseria meningitidis serogroup A." Proteomics **4**(10): 2893-926.

Neisseria meningitidis is an encapsulated Gram-negative bacterium responsible for significant morbidity and mortality worldwide. Meningococci are opportunistic pathogens, carried in the nasopharynx of approximately 10% of asymptomatic adults. Occasionally they enter the bloodstream to cause septicaemia and meningitis. Meningococci are classified into serogroups on the basis of polysaccharide capsule diversity, and serogroup A strains have caused major epidemics mainly in the developing world. Here we describe a two-dimensional gel electrophoresis protein map of the serogroup A strain Z4970, a clinical isolate classified as ancestral to several pandemic waves. To our knowledge this is the first systematically annotated proteomic map for N. meningitidis. Total protein samples from bacteria grown on GC-agar were electrophoretically separated and protein species were identified by matrix-assisted laser desorption/ionization time of flight spectrometry. We identified the products of 273 genes, covering several functional classes, including 94 proteins so far considered as hypothetical. We also describe several protein species encoded by genes reported by DNA microarray studies as being regulated in physiological conditions which are relevant to natural meningococcal pathogenicity. Since

menA differs from other serogroups by having a fairly stable clonal population structure (i.e. with a low degree of variability), we envisaged comparative mapping as a useful tool for microevolution studies, in conjunction with established genotyping methods. As a proof of principle, we performed a comparative analysis on the B subunit of the meningococcal transferrin receptor, a vaccine candidate encoded by the tbpB gene, and a known marker of population diversity in meningococci. The results show that TbpB spot pattern variation observed in the maps of nine clinical isolates from diverse epidemic spreads, fits previous analyses based on allelic variations of the tbpB gene.

Berthier, D., R. Quere, et al. (2003). "Serial analysis of gene expression (SAGE) in bovine trypanotolerance: preliminary results." Genet Sel Evol **35 Suppl 1**: S35-47.

In Africa, trypanosomosis is a tsetse-transmitted disease which represents the most important constraint to livestock production. Several indigenous West African taurine Bos taurus) breeds, such as the Longhorn (N'Dama) cattle are well known to control trypanosome infections. This genetic ability named "trypanotolerance" results from various biological mechanisms under multigenic control. The methodologies used so far have not succeeded in identifying the complete pool of genes involved in trypanotolerance. New post genomic biotechnologies such as transcriptome analyses are efficient in characterising the pool of genes involved in the expression of specific biological functions. We used the serial analysis of gene expression (SAGE) technique to construct, from Peripheral Blood Mononuclear Cells of an N'Dama cow, 2 total mRNA transcript libraries, at day 0 of a Trypanosoma congolense experimental infection and at day 10 post-infection, corresponding to the peak of parasitaemia. Bioinformatic comparisons in the bovine genomic databases allowed the identification of 187 up- and down- regulated genes, EST and unknown functional genes. Identification of the genes involved in trypanotolerance will allow to set up specific microarray sets for further metabolic and pharmacological studies and to design field marker-assisted selection by introgression programmes.

Betts, J. C., P. T. Lukey, et al. (2002). "Evaluation of a nutrient starvation model of Mycobacterium tuberculosis persistence by gene and protein expression profiling." Mol Microbiol **43**(3): 717-31.

The search for new TB drugs that rapidly and effectively sterilize the tissues and are thus able to shorten the duration of chemotherapy from the current 6 months has been hampered by a lack of understanding of the metabolism of the bacterium when in a 'persistent' or latent form. Little is known about the condition in which the bacilli survive, although laboratory models have shown that Mycobacterium tuberculosis can exist in a non-growing, drug-resistant state that may mimic persistence in vivo. Using nutrient starvation, we have established a model in which M. tuberculosis arrests growth, decreases its respiration rate and is resistant to isoniazid, rifampicin and metronidazole. We have used microarray and proteome analysis to investigate the response of M. tuberculosis to nutrient starvation. Proteome analysis of 6-week-starved cultures revealed the induction of several proteins. Microarray analysis enabled us to monitor gene expression during adaptation to nutrient starvation and confirmed the changes seen at the protein level. This has provided evidence for slowdown of the transcription apparatus, energy metabolism, lipid biosynthesis and cell division in addition to induction of the stringent response and several other genes that may play a role in maintaining long-term survival within the host. Thus, we have generated a model with which we can search for agents active against persistent M. tuberculosis and revealed a number of potential targets expressed under these conditions.

Betts, J. C., A. McLaren, et al. (2003). "Signature gene expression profiles discriminate between isoniazid-, thiolactomycin-, and triclosan-treated Mycobacterium tuberculosis." Antimicrob Agents Chemother **47**(9): 2903-13.

Genomic technologies have the potential to greatly increase the efficiency of the drug development process. As part of our tuberculosis drug discovery program, we used DNA microarray technology to profile drug-induced effects in Mycobacterium tuberculosis. Expression profiles of M. tuberculosis treated with compounds that inhibit key metabolic pathways are required as references for

the assessment of novel antimycobacterial agents. We have studied the response of M. tuberculosis to treatment with the mycolic acid biosynthesis inhibitors isoniazid, thiolactomycin, and triclosan. Thiolactomycin targets the beta-ketoacyl-acyl carrier protein (ACP) synthases KasA and KasB, while triclosan inhibits the enoyl-ACP reductase InhA. However, controversy surrounds the precise mode of action of isoniazid, with both InhA and KasA having been proposed as the primary target. We have shown that although the global response profiles of isoniazid and thiolactomycin are more closely related to each other than to that of triclosan, there are differences that distinguish the mode of action of these two drugs. In addition, we have identified two groups of genes, possibly forming efflux and detoxification systems, through which M. tuberculosis may limit the effects of triclosan. We have developed a mathematical model, based on the expression of 21 genes, which is able to perfectly discriminate between isoniazid-, thiolactomycin-, or triclosan-treated M. tuberculosis. This model is likely to prove invaluable as a tool to improve the efficiency of our drug development programs by providing a means to rapidly confirm the mode of action of thiolactomycin analogues or novel InhA inhibitors as well as helping to translate enzyme activity into whole-cell activity.

Bigger, C. B., K. M. Brasky, et al. (2001). "DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection." J Virol **75**(15): 7059-66.

Hepatitis C virus (HCV) poses a worldwide health problem in that the majority of individuals exposed to HCV become chronically infected and are predisposed for developing significant liver disease. DNA microarray technology provides an opportunity to survey transcription modulation in the context of an infectious disease and is a particularly attractive approach in characterizing HCV-host interactions, since the mechanisms underlying viral persistence and disease progression are not understood and are difficult to study. Here, we describe the changes in liver gene expression during the course of an acute-resolving HCV infection in a chimpanzee. Clearance of viremia in this animal occurred between weeks 6 and 8, while clearance of residual infected hepatocytes did not occur until 14 weeks postinfection. The most notable changes in gene expression occurred in numerous interferon response genes (including all three classical interferon antiviral pathways) that increased dramatically, some as early as day 2 postinfection. The data suggest a biphasic mechanism of viral clearance dependent on both the innate and adaptive immune responses and provide insight into the response of the liver to a hepatotropic viral infection.

Bina, J., J. Zhu, et al. (2003). "ToxR regulon of Vibrio cholerae and its expression in vibrios shed by cholera patients." Proc Natl Acad Sci U S A **100**(5): 2801-6.

Toxigenic Vibrio cholerae cause cholera, a severe diarrheal disease responsible for significant morbidity and mortality worldwide. Two determinants, cholera enterotoxin (CT) and toxin coregulated pilus (TCP) are critical factors responsible for this organism's virulence. The genes for these virulence determinants belong to a network of genes (the ToxR regulon) whose expression is modulated by transcriptional regulators encoded by the toxRS, tcpPH, and toxT genes. To define the ToxR regulon more fully, mutants defective in these regulatory genes were transcriptionally profiled by using V. cholerae genomic microarrays. This study identified 13 genes that were transcriptionally repressed by the toxT mutation (all involved in CT and TCP biogenesis), and 27 and 60 genes that were transcriptionally repressed by the tcpPH and toxRS mutations, respectively. During the course of this analysis, we validated the use of a genomic DNA-based reference sample as a means to standardize and normalize data obtained in different microarray experiments. This method allowed the accurate transcriptional profiling of V. cholerae cells present in stools from cholera patients and the comparison of these profiles to those of wild-type and mutant strains of V. cholerae grown under optimal conditions for CT and TCP expression. Our results suggest that vibrios present in cholera stools carry transcripts for these two virulence determinants, albeit at relatively low levels compared with optimal in vitro conditions. The transcriptional profile of vibrios present in cholera stools also suggests that the bacteria experienced conditions of anaerobiosis, iron limitation, and nutrient deprivation within the human gastrointestinal tract.

Binnicker, M. J., R. D. Williams, et al. (2003). "Infection of human urethral epithelium with Neisseria gonorrhoeae elicits an upregulation of host anti-apoptotic factors and protects cells from staurosporine-induced apoptosis." Cell Microbiol **5**(8): 549-60.

In order to better understand the host response to an infection with Neisseria gonorrhoeae, microarray technology was used to analyse the gene expression profile between uninfected and infected human urethral epithelium. The anti-apoptotic genes bfl-1, cox-2 and c-IAP-2 were identified to be upregulated approximately eight-, four- or twofold, respectively, following infection. Subsequent assays including RT-PCR, real time RT-PCR and RNase protection confirmed the increased expression of these apoptotic regulators, and identified that a fourth anti-apoptotic factor, mcl-1, is also upregulated. RT-PCR and RNase protection also showed that key pro-apoptotic factors including bax, bad and bak do not change in expression. Furthermore, our studies demonstrated that infection with the gonococcus partially protects urethral epithelium from apoptosis induced by the protein kinase inhibitor, staurosporine (STS). This work shows that following infection with Neisseria gonorrhoeae, several host anti-apoptotic factors are upregulated. In addition, a gonococcal infection protects host cells from subsequent STS-induced death. The regulation of host cell death by the gonococcus may represent a mechanism employed by this pathogen to survive and proliferate in host epithelium.

Bischoff, M., P. Dunman, et al. (2004). "Microarray-based analysis of the Staphylococcus aureus sigmaB regulon." J Bacteriol **186**(13): 4085-99.

Microarray-based analysis of the transcriptional profiles of the genetically distinct Staphylococcus aureus strains COL, GP268, and Newman indicate that a total of 251 open reading frames (ORFs) are influenced by sigmaB activity. While sigmaB was found to positively control 198 genes by a factor of > or =2 in at least two of the three genetic lineages analyzed, 53 ORFs were repressed in the presence of sigmaB. Gene products that were found to be influenced by sigmaB are putatively involved in all manner of cellular processes, including cell envelope biosynthesis and turnover, intermediary metabolism, and signaling pathways. Most of the genes and/or operons identified as upregulated by sigmaB were preceded by a nucleotide sequence that resembled the sigmaB consensus promoter sequence of Bacillus subtilis. A conspicuous number of virulence-associated genes were identified as regulated by sigmaB activity, with many adhesins upregulated and prominently represented in this group, while transcription of various exoproteins and toxins were repressed. The data presented here suggest that the sigmaB of S. aureus controls a large regulon and is an important modulator of virulence gene expression that is likely to act conversely to RNAIII, the effector molecule of the agr locus. We propose that this alternative transcription factor may be of importance for the invading pathogen to fine-tune its virulence factor production in response to changing host environments.

Bjorkholm, B., A. Lundin, et al. (2001). "Comparison of genetic divergence and fitness between two subclones of Helicobacter pylori." Infect Immun **69**(12): 7832-8.

Helicobacter pylori has a very plastic genome, reflecting its high rate of recombination and point mutation. This plasticity promotes divergence of the population by the development of subclones and presumably enhances adaptation to host niches. We have investigated the genotypic and phenotypic characteristics of two such subclones isolated from one patient as well as the genetic evolution of these isolates during experimental infection. Whole-genome genotyping of the isolates using DNA microarrays revealed that they were more similar to each other than to a panel of other genotyped strains recovered from different hosts. Nonetheless, they still showed significant differences. For example, one isolate (67:21) contained the entire Cag pathogenicity island (PAI), whereas the other (67:20) had excised the PAI. Phenotypic studies disclosed that both isolates expressed adhesins that recognized human histo-blood group Lewis(b) glycan receptors produced by gastric pit and surface mucus cells. In addition, both isolates were able to colonize, to equivalent density and with similar efficiency, germ-free transgenic mice genetically engineered to synthesize Lewis(b) glycans in their pit cells (12 to 14 mice/isolate). Remarkably, the Cag PAI-negative isolate was unable to colonize conventionally raised Lewis(b)

transgenic mice harboring a normal gastric microflora, whereas the Cag PAI-positive isolate colonized 74% of the animals (39 to 40 mice/isolate). The genomic evolution of both isolates during the infection of conventionally raised and germ-free mice was monitored over the course of 3 months. The Cag PAI-positive isolate was also surveyed after a 10 month colonization of conventionally raised transgenic animals (n = 9 mice). Microarray analysis of the Cag PAI and sequence analysis of the cagA, recA, and 16S rRNA genes disclosed no changes in recovered isolates. Together, these results reveal that the H. pylori population infecting one individual can undergo significant divergence, creating stable subclones with substantial genotypic and phenotypic differences.

Bjorkholm, B. M., J. L. Guruge, et al. (2002). "Colonization of germ-free transgenic mice with genotyped Helicobacter pylori strains from a case-control study of gastric cancer reveals a correlation between host responses and HsdS components of type I restriction-modification systems." J Biol Chem **277**(37): 34191-7.

Helicobacter pylori infects the stomachs of half of all humans. It has a relatively benign relationship with most hosts but produces severe pathology, including gastric cancer, in others. Identifying the relative contributions of host, microbial, and environmental factors to the outcome of infection has been challenging. Here we describe one approach for identifying microbial genes that affect the magnitude of host responses to infection. Single colony purified H. pylori isolates were obtained from 25 cases and 71 controls in a Swedish case-control study of gastric cancer. Strains were first phenotyped based on their ability to produce adhesins that recognize two classes of human gastric epithelial receptors. Thirteen binding strains and two non-binding controls were then subjected to whole genome genotyping using H. pylori DNA microarrays. A cohort of "variable" genes was identified based on a microarray-determined call of "absent" in at least one member of the strain panel. Each strain was subsequently introduced into two types of germ-free transgenic mice, each programmed to express a different host factor postulated to pose increased risk for development of severe pathology. Expression of biomarkers of host defense was quantitated 4 weeks after inoculation, and the magnitude of the response correlated with bacterial genotype. The proportion of genes encoding HsdS homologs (specificity subunit of hetero-oligomeric type I restriction-modification systems) was significantly higher in the pool of 18 variable genes whose presence directly correlated with a robust host response than their proportion in the remaining 352 members of the variable gene pool. This suggests that the functions of these HsdS homologs may include control of expression of microbial determinants that affect the extent of gastric responses to this potentially virulent pathogen.

Blader, I. J., I. D. Manger, et al. (2001). "Microarray analysis reveals previously unknown changes in Toxoplasma gondii-infected human cells." J Biol Chem **276**(26): 24223-31.

Cells infected with the intracellular protozoan parasite Toxoplasma gondii undergo up-regulation of pro-inflammatory cytokines, organelle redistribution, and protection from apoptosis. To examine the molecular basis of these and other changes, gene expression profiles of human foreskin fibroblasts infected with Toxoplasma were studied using human cDNA microarrays consisting of approximately 22,000 known genes and uncharacterized expressed sequence tags. Early during infection (1-2 h), <1% of all genes show a significant change in the abundance of their transcripts. Of the 63 known genes in this group, 27 encode proteins associated with the immune response. These genes are also up-regulated by secreted, soluble factors from extracellular parasites indicating that the early response does not require parasite invasion. Later during infection, genes involved in numerous host cell processes, including glucose and mevalonate metabolism, are modulated. Many of these late genes are dependent on the direct presence of the parasite; i.e. secreted products from either the parasite or infected cells are insufficient to induce these changes. These results reveal several previously unknown effects on the host cell and lay the foundation for detailed analysis of their role in the host-pathogen interaction.

Bodrossy, L. and A. Sessitsch (2004). "Oligonucleotide microarrays in microbial diagnostics." Curr Opin

Microbiol **7**(3): 245-54.

Oligonucleotide microarrays offer a fast, high-throughput alternative for the parallel detection of microbes from virtually any sample. The application potential spreads across most sectors of life sciences, including environmental microbiology and microbial ecology; human, veterinary, food and plant diagnostics; water quality control; industrial microbiology, and so on. The past two years have witnessed a rapid increase of research in this field. Many alternative techniques were developed and validated as seen in 'proof-of-concept' articles. Publications reporting on the application of oligonucleotide microarray technology for microbial diagnostics in microbiology driven projects have just started to appear. Current and future technical and bioinformatics developments will inevitably improve the potential of this technology further.

Bohn, E., S. Muller, et al. (2004). "Gene expression patterns of epithelial cells modulated by pathogenicity factors of Yersinia enterocolitica." Cell Microbiol **6**(2): 129-41.

Epithelial cells express genes whose products signal the presence of pathogenic microorganisms to the immune system. Pathogenicity factors of enteric bacteria modulate host cell gene expression. Using microarray technology we have profiled epithelial cell gene expression upon interaction with Yersinia enterocolitica. Yersinia enterocolitica wild-type and isogenic mutant strains were used to identify host genes modulated by invasin protein (Inv), which is involved in enteroinvasion, and Yersinia outer protein P (YopP) which inhibits innate immune responses. Among 22 283 probesets (14,239 unique genes), we found 193 probesets (165 genes) to be regulated by Yersinia infection. The majority of these genes were induced by Inv, whose recognition leads to expression of NF-kappa B-regulated factors such as cytokines and adhesion molecules. Yersinia virulence plasmid (pYV)-encoded factors counter regulated Inv-induced gene expression. Thus, YopP repressed Inv-induced NF-kappa B regulated genes at 2 h post infection whereas other pYV-encoded factors repressed host cell genes at 4 and 8 h post infection. Chromosomally encoded factors of Yersinia, other than Inv, induced expression of genes known to be induced by TGF-beta receptor signalling. These genes were also repressed by pYV-encoded factors. Only a few host genes were exclusively induced by pYV-encoded factors. We hypothesize that some of these genes may contribute to pYV-mediated silencing of host cells. In conclusion, the data demonstrates that epithelial cells express a limited number of genes upon interaction with enteric Yersinia. Both Inv and YopP appear to modulate gene expression in order to subvert epithelial cell functions involved in innate immunity.

Bolt, G., K. Berg, et al. (2002). "Measles virus-induced modulation of host-cell gene expression." J Gen Virol **83**(Pt 5): 1157-65.

The influence of measles virus (MV) infection on gene expression by human peripheral blood mononuclear cells (PBMCs) was examined with cDNA microarrays. The mRNA levels of more than 3000 cellular genes were compared between uninfected PBMCs and cells infected with either the Edmonston MV strain or a wild-type MV isolate. The MV-induced upregulation of individual genes identified by microarray analyses was confirmed by RT-PCR. In the present study, a total of 17 genes was found to be upregulated by MV infection. The Edmonston strain grew better in the PBMC cultures than the wild-type MV, and the Edmonston strain was a stronger inducer of the upregulated host cell genes than the wild-type virus. The anti-apoptotic B cell lymphoma 3 (Bcl-3) protein and the transcription factor NF-kappaB p52 subunit were upregulated in infected PBMCs both at the mRNA and at the protein level. Several genes of the interferon system including that for interferon regulatory factor 7 were upregulated by MV. The genes for a number of chaperones, transcription factors and other proteins of the endoplasmic reticulum stress response were also upregulated. These included the gene for the pro-apoptotic and growth arrest-inducing CHOP/GADD153 protein. Thus, the present study demonstrated the activation by MV of cellular mechanisms and pathways that may play a role in the pathogenesis of measles.

Bonnah, R. A., M. U. Muckenthaler, et al. (2004). "Expression of epithelial cell iron-related genes upon infection by Neisseria meningitidis." Cell Microbiol **6**(5): 473-84.

Infection by the obligate human pathogens Neisseria meningitidis (MC) and Neisseria gonorrhoeae (GC) reduces the expression of host epithelial cell transferrin receptor 1 (TfR-1) (Bonnah et al., 2000, Cellular Microbiology 2: 207-218). In addition, the rate and pattern of TfR-1 cycling is altered, leading to diminished uptake of Tf-iron by infected host cells. As Tf-iron is important for maintaining iron homeostasis in the eukaryotic cell, these findings raised the possibility that Neisseria infection might affect further pathways of epithelial cell iron metabolism. We used a specialized cDNA microarray platform, the 'IronChip', to investigate the expression of genes involved in iron transport, storage and regulation. We show that mRNA expression of several host genes involved in iron homeostasis is altered. Surprisingly, the general mRNA expression profile of infected cells closely resembled that of uninfected cells grown in an iron-limited environment. An important exception to this profile is TfR-1, the mRNA level of which is strongly reduced. Low TfR-1 expression may be explained in part by decreased activity of the iron-regulatory proteins (IRPs) in MC-infected cells, which may result in the destabilization of TfR-1 mRNA. Intriguingly, low IRP activity contrasts with the decrease in H-ferritin protein levels in infected cells. This finding suggests that low IRP activity may be responsible in part for the decrease in TfR-1 mRNA levels. A discussion of these novel findings in relation to MC infection and virulence is provided.

Booth, S. A., M. A. Drebot, et al. (2003). "Design of oligonucleotide arrays to detect point mutations: molecular typing of antibiotic resistant strains of Neisseria gonorrhoeae and hantavirus infected deer mice." Mol Cell Probes **17**(2-3): 77-84.

Microarrays are promising tools for use in molecular diagnostics due to their ability to perform a multitude of tests simultaneously. In the case of genotyping many such tests will require discrimination of sequence at the single nucleotide level. A number of challenges exist including binding of optimal quantities of probe to the chip surface, the use of uniform hybridization conditions across the chip and the generation of labeled target. We investigated two model systems to test out the efficacy and ease with which probes can be designed for this purpose. In the first of these we designed primers to identify five mutations found in two genes from N. gonohorroeae, gyrA and parC that have been implicated in ciprofloxacin resistance. In the second system we used a similar strategy to identify four mutations in AT rich mitochondrial DNA from deer mice. These mutations are associated with deer mice subspecies that originate from different geographical regions of Canada and harbor different hantavirus strains. In every case we were able to design probes that could discriminate mutations in the target sequences under uniform hybridization conditions, even when targets were fairly long in length, up to 400 bp. Our results suggest that microarray analysis of point mutations might be very useful for automated identification and characterization of pathogens and their hosts.

Borucki, M. K., M. J. Krug, et al. (2003). "Discrimination among Listeria monocytogenes isolates using a mixed genome DNA microarray." Vet Microbiol **92**(4): 351-62.

Listeria monocytogenes can cause serious illness in humans, usually following the ingestion of contaminated food. Epidemiologic investigation requires identification of specific isolates, usually done by a combination of serotyping and subtyping using pulsed-field gel electrophoresis (PFGE). DNA microarrays provide a new format to resolve genetic differences among isolates and, unlike PFGE, to identify specific genes associated with the infecting pathogen. A 585 probe, mixed genome microarray was constructed and 24 strains of L. monocytogenes were hybridized to the array. Microarray analysis allowed discrimination among L. monocytogenes isolates within a serotype and obtained from similar geographic and epidemiologic sources. Importantly, the microarray results preserved previously described phylogenetic relationships between major serogroups and, in a limited comparison, agreed with PFGE subtypes. The association of individual probes with isolates allowed identification of specific genes. Sequencing of 10 polymorphic probes identified nine matches with previously described bacterial genes

including several suspected virulence factors. These results demonstrate that mixed genomic microarrays are useful for differentiating among closely related L. monocytogenes isolates and identifying genetic markers that can be used in epidemiologic and possibly pathogenesis studies.

Boshoff, H. I., T. G. Myers, et al. (2004). "The transcriptional responses of Mycobacterium tuberculosis to inhibitors of metabolism: novel insights into drug mechanisms of action." J Biol Chem **279**(38): 40174-84.

The differential transcriptional response of Mycobacterium tuberculosis to drugs and growth-inhibitory conditions was monitored to generate a data set of 430 microarray profiles. Unbiased grouping of these profiles independently clustered agents of known mechanism of action accurately and was successful at predicting the mechanism of action of several unknown agents. These predictions were validated biochemically for two agents of previously uncategorized mechanism, pyridoacridones and phenothiazines. Analysis of this data set further revealed 150 underlying clusters of coordinately regulated genes offering the first glimpse at the full metabolic potential of this organism. A signature subset of these gene clusters was sufficient to classify all known agents as to mechanism of action. Transcriptional profiling of both crude and purified natural products can provide critical information on both mechanism and detoxification prior to purification that can be used to guide the drug discovery process. Thus, the transcriptional profile generated by a crude marine natural product recapitulated the mechanistic prediction from the pure active component. The underlying gene clusters further provide fundamental insights into the metabolic response of bacteria to drug-induced stress and provide a rational basis for the selection of critical metabolic targets for screening for new agents with improved activity against this important human pathogen.

Boyce, J. D., I. Wilkie, et al. (2002). "Genomic scale analysis of Pasteurella multocida gene expression during growth within the natural chicken host." Infect Immun **70**(12): 6871-9.

Little is known about the genomic-scale transcriptional responses of bacteria during natural infections. We used whole-genome microarray analysis to assess the transcriptional state of the gram-negative pathogen Pasteurella multocida, the causative agent of fowl cholera, during infection in the natural chicken host. We compared the expression profiles of bacteria harvested from the blood of septicemic chickens experiencing late-stage fowl cholera with those from bacteria grown in rich medium. Independent analysis of bacterial expression profiles from the infection of three individual chickens indicated that 40 genes were differentially expressed in all three individuals, 126 were differentially expressed in two of the three individuals, and another 372 were differentially expressed in one individual. Real-time reverse transcription-PCR assays were used to confirm the expression ratios for a number of genes. Of the 40 genes differentially expressed in all three individuals, 17 were up-regulated and 23 were down-regulated in the host compared with those grown in rich medium. The majority (10 of 17) of the up-regulated genes were involved in amino acid transport and metabolism and energy production and conversion, clearly indicating how P. multocida alters its biosynthetic and energy production pathways to cope with the host environment. In contrast, the majority (15 of 23) of down-regulated genes were of unknown or poorly characterized functions. There were clear differences in gene expression between the bacteria isolated from each of the three chickens, a finding consistent with individual host variation being an important factor in determining pathogen gene expression. Interestingly, bacteria from only two of the three infected animals had a gene expression profile highly similar to that observed during growth under iron-limiting conditions, suggesting that severe iron starvation may not always occur during P. multocida infection.

Boyd, E. F., S. Porwollik, et al. (2003). "Differences in gene content among Salmonella enterica serovar typhi isolates." J Clin Microbiol **41**(8): 3823-8.

We used a nonredundant microarray of the Salmonella enterica serovar Typhimurium LT2 and Typhi CT18 genomes to assess the genomic content of a diverse set of isolates of serovar Typhi.

Comparative genomic hybridization revealed 13 regions of absent or divergent gene content in the eight Typhi strains examined compared to Typhi CT18. In particular, two Typhi CT18 prophage regions, STY1048 to STY1077 and STY2038 to STY2077, as well as a five-gene islet (STY3188 to STY3193) were absent or divergent in all other Typhi strains examined. Seven Typhi strains lacked most or all of the IS1 elements present in strain CT18, and three Typhi strains lacked a P4-like phage (STY4821 to STY4834). One strain was devoid of a 149-gene region (STY4521 to STY4680), which encodes numerous phage genes and the Vi antigen biosynthesis and export gene cluster, a type IV pilus, and numerous phage genes. In Typhi strain 26T25, an amplification of an entire inter-ribosomal region encompassing 31 genes has occurred. Furthermore, a 257-gene region (STY1360 to STY1639) showed an aberrant replication pattern in three Typhi isolates. Overall, these differences in gene content indicate that even within a highly clonal bacterial population the genomic reservoir is unstable.

Bozdech, Z., J. Zhu, et al. (2003). "Expression profiling of the schizont and trophozoite stages of Plasmodium falciparum with a long-oligonucleotide microarray." Genome Biol **4**(2): R9.

BACKGROUND: The worldwide persistence of drug-resistant Plasmodium falciparum, the most lethal variety of human malaria, is a global health concern. The P. falciparum sequencing project has brought new opportunities for identifying molecular targets for antimalarial drug and vaccine development. RESULTS: We developed a software package, ArrayOligoSelector, to design an open reading frame (ORF)-specific DNA microarray using the publicly available P. falciparum genome sequence. Each gene was represented by one or more long 70 mer oligonucleotides selected on the basis of uniqueness within the genome, exclusion of low-complexity sequence, balanced base composition and proximity to the 3' end. A first-generation microarray representing approximately 6,000 ORFs of the P. falciparum genome was constructed. Array performance was evaluated through the use of control oligonucleotide sets with increasing levels of introduced mutations, as well as traditional northern blotting. Using this array, we extensively characterized the gene-expression profile of the intraerythrocytic trophozoite and schizont stages of P. falciparum. The results revealed extensive transcriptional regulation of genes specialized for processes specific to these two stages. CONCLUSIONS: DNA microarrays based on long oligonucleotides are powerful tools for the functional annotation and exploration of the P. falciparum genome. Expression profiling of trophozoites and schizonts revealed genes associated with stage-specific processes and may serve as the basis for future drug targets and vaccine development

Broekhuijsen, M., P. Larsson, et al. (2003). "Genome-wide DNA microarray analysis of Francisella tularensis strains demonstrates extensive genetic conservation within the species but identifies regions that are unique to the highly virulent F. tularensis subsp. tularensis." J Clin Microbiol **41**(7): 2924-31.

Francisella tularensis is a potent pathogen and a possible bioterrorism agent. Little is known, however, to explain the molecular basis for its virulence and the distinct differences in virulence found between the four recognized subspecies, F. tularensis subsp. tularensis, F. tularensis subsp. mediasiatica, F. tularensis subsp. holarctica, and F. tularensis subsp. novicida. We developed a DNA microarray based on 1,832 clones from a shotgun library used for sequencing of the highly virulent strain F. tularensis subsp. tularensis Schu S4. This allowed a genome-wide analysis of 27 strains representing all four subspecies. Overall, the microarray analysis confirmed a limited genetic variation within the species F. tularensis, and when the strains were compared, at most 3.7% of the probes showed differential hybridization. Cluster analysis of the hybridization data revealed that the causative agents of type A and type B tularemia, i.e., F. tularensis subsp. tularensis and F. tularensis subsp. holarctica, respectively, formed distinct clusters. Despite marked differences in their virulence and geographical origin, a high degree of genomic similarity between strains of F. tularensis subsp. tularensis and F. tularensis subsp. mediasiatica was apparent. Strains from Japan clustered separately, as did strains of F. tularensis subsp. novicida. Eight regions of difference (RD) 0.6 to 11.5 kb in size, altogether comprising 21 open reading frames, were identified that distinguished strains of the moderately virulent subspecies F. tularensis subsp.

holarctica and the highly virulent subspecies F. tularensis subsp. tularensis. One of these regions, RD1, allowed for the first time the development of an F. tularensis-specific PCR assay that discriminates each of the four subspecies.

Brooks, C. S., P. S. Hefty, et al. (2003). "Global analysis of Borrelia burgdorferi genes regulated by mammalian host-specific signals." Infect Immun **71**(6): 3371-83.

Lyme disease is a tick-borne infection that can lead to chronic, debilitating problems if not recognized or treated appropriately. Borrelia burgdorferi, the causative agent of Lyme disease, is maintained in nature by a complex enzootic cycle involving Ixodes ticks and mammalian hosts. Many previous studies support the notion that B. burgdorferi differentially expresses numerous genes and proteins to help it adapt to growth in the mammalian host. In this regard, several studies have utilized a dialysis membrane chamber (DMC) cultivation system to generate "mammalian host-adapted" spirochetes for the identification of genes selectively expressed during mammalian infection. Here, we have exploited the DMC cultivation system in conjunction with microarray technology to examine the global changes in gene expression that occur in the mammalian host. To identify genes regulated by only mammal-specific signals and not by temperature, borrelial microarrays were hybridized with cDNA generated either from organisms temperature shifted in vitro from 23 degrees C to 37 degrees C or from organisms cultivated by using the DMC model system. Statistical analyses of the combined data sets revealed that 125 genes were expressed at significantly different levels in the mammalian host, with almost equivalent numbers of genes being up- or down-regulated by B. burgdorferi within DMCs compared to those undergoing temperature shift. Interestingly, during DMC cultivation, the vast majority of genes identified on the plasmids were down-regulated (79%), while the differentially expressed chromosomal genes were almost entirely up-regulated (93%). Global analysis of the upstream promoter regions of differentially expressed genes revealed that several share a common motif that may be important in transcriptional regulation during mammalian infection. Among genes with known or putative functions, the cell envelope category, which includes outer membrane proteins, was found to contain the most differentially expressed genes. The combined findings have generated a subset of genes that can now be further characterized to help define their role or roles with regard to B. burgdorferi virulence and Lyme disease pathogenesis.

Brunelle, B. W., T. L. Nicholson, et al. (2004). "Microarray-based genomic surveying of gene polymorphisms in Chlamydia trachomatis." Genome Biol **5**(6): R42.

By comparing two fully sequenced genomes of Chlamydia trachomatis using competitive hybridization on DNA microarrays, a logarithmic correlation was demonstrated between the signal ratio of the arrays and the 75-99% range of nucleotide identities of the genes. Variable genes within 14 uncharacterized strains of C. trachomatis were identified by array analysis and verified by DNA sequencing. These genes may be crucial for understanding chlamydial virulence and pathogenesis.

Bryant, P. A., D. Venter, et al. (2004). "Chips with everything: DNA microarrays in infectious diseases." Lancet Infect Dis **4**(2): 100-11.

Two developments are set to revolutionise research in and clinical management of infectious diseases. First, the completion of the human genome project together with the sequencing of many pathogen genomes, and second, the development of microarray technology. This review explains the principles underlying DNA microarrays and highlights the uses to which they are being put to investigate the molecular basis of infectious diseases. Pathogen studies enable identification of both known and novel organisms, understanding of genetic evolution, and investigation of determinants of pathogenicity. Host studies show the complexities of development and activation of both innate and adaptive immunity. Host-pathogen studies allow global analysis of gene expression during pathogenesis. Microarray technology will accelerate our understanding of the complex genetic processes underlying the interaction between microorganisms and the host, with consequent improvements in the diagnosis, treatment, and prevention of infectious diseases.

Call, D. R., M. K. Borucki, et al. (2003). "Mixed-genome microarrays reveal multiple serotype and lineage-specific differences among strains of Listeria monocytogenes." J Clin Microbiol **41**(2): 632-9.

Epidemiological studies and analysis of putative virulence genes have shown that Listeria monocytogenes has diverged into several phylogenetic divisions. We hypothesize that similar divergence has occurred for many genes that influence niche-specific fitness and virulence and that identifying these differences may offer new opportunities for the detection, treatment, and control of this important pathogen. To explore this issue further, we developed a microarray composed of fragmented DNA taken from 10 strains of L. monocytogenes. We then hybridized genomic DNA from 50 different strains to replicate arrays and analyzed the resulting hybridization patterns. A simple Euclidean distance metric permitted the reconstruction of previously described genetic relationships between serotypes, and only four microarray probes were needed to discriminate between the most important serotypes (1/2a, 1/2b, 1/2c, and 4). We calculated an index of linkage equilibrium from the microarray data and confirmed that L. monocytogenes has a strongly clonal population structure (I(A) = 3.85). Twenty-nine informative probes were retrieved from the library and sequenced. These included genes associated with repairing UV-damaged DNA, salt tolerance, biofilm formation, heavy metal transport, ferrous iron transport, and teichoic acid synthesis. Several membrane-bound lipoproteins and one internalin were identified, plus three phage sequences and six sequences with unknown function. Collectively, these data confirm that many genes have diverged between lineages of L. monocytogenes. Furthermore, these results demonstrate the value of mixed-genome microarrays as a tool for deriving biologically useful information and for identifying and screening genetic markers for clinically important microbes.

Chan, K., S. Baker, et al. (2003). "Genomic comparison of Salmonella enterica serovars and Salmonella bongori by use of an S. enterica serovar typhimurium DNA microarray." J Bacteriol **185**(2): 553-63.

The genus Salmonella consists of over 2,200 serovars that differ in their host range and ability to cause disease despite their close genetic relatedness. The genetic factors that influence each serovar's level of host adaptation, how they evolved or were acquired, their influence on the evolution of each serovar, and the phylogenic relationships between the serovars are of great interest as they provide insight into the mechanisms behind these differences in host range and disease progression. We have used an Salmonella enterica serovar Typhimurium spotted DNA microarray to perform genomic hybridizations of various serovars and strains of both S. enterica (subspecies I and IIIa) and Salmonella bongori to gain insight into the genetic organization of the serovars. Our results are generally consistent with previously published DNA association and multilocus enzyme electrophoresis data. Our findings also reveal novel information. We observe a more distant relationship of serovar Arizona (subspecies IIIa) from the subspecies I serovars than previously measured. We also observe variability in the Arizona SPI-2 pathogenicity island, indicating that it has evolved in a manner distinct from the other serovars. In addition, we identify shared genetic features of S. enterica serovars Typhi, Paratyphi A, and Sendai that parallel their unique ability to cause enteric fever in humans. Therefore, whereas the taxonomic organization of Salmonella into serogroups provides a good first approximation of genetic relatedness, we show that it does not account for genomic changes that contribute to a serovar's degree of host adaptation.

Chan, V. L. (2003). "Bacterial genomes and infectious diseases." Pediatr Res **54**(1): 1-7.

The genome sequencing approach has proved to be highly effective and invaluable for gaining an insight on structure of bacteria genomes and the biology and evolution of bacteria. The diversity of bacteria genomes is beyond expectation. Gaining a full understanding of the biology and pathogenic mechanisms of these pathogens will be a major task because on an average only approximately 69% of the encoded proteins in each genome have known functions. Genome sequence analyses have identified novel putative virulence genes, vaccine candidates, targets for antibacterial drugs, and specific diagnostic probes. Microarray technology that makes use of the genomic sequences of human and bacterial pathogens will be a major tool for gaining full understanding of the complexity of host-pathogen interactions and mechanisms of pathogenesis.

Chattopadhyay, S., N. E. Muzaffar, et al. (2000). "The yeast model for batten disease: mutations in BTN1, BTN2, and HSP30 alter pH homeostasis." J Bacteriol **182**(22): 6418-23.

The BTN1 gene product of the yeast Saccharomyces cerevisiae is 39% identical and 59% similar to human CLN3, which is associated with the neurodegenerative disorder Batten disease. Furthermore, btn1-Delta strains have an elevated activity of the plasma membrane H(+)-ATPase due to an abnormally high vacuolar acidity during the early phase of growth. Previously, DNA microarray analysis revealed that btn1-Delta strains compensate for the altered plasma membrane H(+)-ATPase activity and vacuolar pH by elevating the expression of the two genes HSP30 and BTN2. We now show that deletion of either HSP30 or BTN2 in either BTN1(+) or btn1-Delta strains does not alter vacuolar pH but does lead to an increased activity of the vacuolar H(+)-ATPase. Deletion of BTN1, BTN2, or HSP30 does not alter cytosolic pH but diminishes pH buffering capacity and causes poor growth at low pH in a medium containing sorbic acid, a condition known to result in disturbed intracellular pH homeostasis. Btn2p was localized to the cytosol, suggesting a role in mediating pH homeostasis between the vacuole and plasma membrane H(+)-ATPase. Increased expression of HSP30 and BTN2 in btn1-Delta strains and diminished growth of btn1-Delta, hsp30-Delta, and btn2-Delta strains at low pH reinforce our view that altered pH homeostasis is the underlying cause of Batten disease.

Chaussabel, D., R. T. Semnani, et al. (2003). "Unique gene expression profiles of human macrophages and dendritic cells to phylogenetically distinct parasites." Blood **102**(2): 672-81.

Monocyte-derived dendritic cells (DCs) and macrophages (Ms) generated in vitro from the same individual blood donors were exposed to 5 different pathogens, and gene expression profiles were assessed by microarray analysis. Responses to Mycobacterium tuberculosis and to phylogenetically distinct protozoan (Leishmania major, Leishmania donovani, Toxoplasma gondii) and helminth (Brugia malayi) parasites were examined, each of which produces chronic infections in humans yet vary considerably in the nature of the immune responses they trigger. In the absence of microbial stimulation, DCs and Ms constitutively expressed approximately 4000 genes, 96% of which were shared between the 2 cell types. In contrast, the genes altered transcriptionally in DCs and Ms following pathogen exposure were largely cell specific. Profiling of the gene expression data led to the identification of sets of tightly coregulated genes across all experimental conditions tested. A newly devised literature-based clustering algorithm enabled the identification of functionally and transcriptionally homogenous groups of genes. A comparison of the responses induced by the individual pathogens by means of this strategy revealed major differences in the functionally related gene profiles associated with each infectious agent. Although the intracellular pathogens induced responses clearly distinct from the extracellular B malayi, they each displayed a unique pattern of gene expression that would not necessarily be predicted on the basis of their phylogenetic relationship. The association of characteristic functional clusters with each infectious agent is consistent with the concept that antigen-presenting cells have prewired signaling patterns for use in the response to different pathogens.

Chemlal, K. and F. Portaels (2003). "Molecular diagnosis of nontuberculous mycobacteria." Curr Opin Infect Dis **16**(2): 77-83.

PURPOSE OF REVIEW: Diagnosis of infection due to nontuberculous mycobacteria is not easy, as it must be distinguished from colonization or contamination by other nontuberculous mycobacteria. Molecular methods offer many advantages over conventional methods of identification. The results are obtained rapidly, are reliable and reproducible, and even mixed or contaminated cultures can be examined. This review highlights the recent advances in molecular techniques for identification of nontuberculous mycobacteria. RECENT FINDINGS: Nontuberculous mycobacteria are ubiquitous towards the environment and have the potential to colonize and cause serious infection. An increasing number of species and clinical presentations are being described, and progress has been made towards the understanding of the underlying predisposing factors. Disease caused by nontuberculous mycobacteria is

often associated with various forms of immunosuppression, particularly HIV infection, whereas mild forms of immune defects have been observed in some patients who, apart from their nontuberculous mycobacterial disease, seem to be healthy on initial examination. Molecular techniques have shown their usefulness for the identification of most mycobacteria. Probes are widely used in clinical laboratories for the identification of the most common mycobacterial species. Because automated DNA sequencing and the programs for analysing sequence data have become technically simpler, polymerase chain reaction-based sequencing is now used in many mycobacterial reference laboratories as a routine method for species identification. SUMMARY: Significant advances have been made with molecular tools for diagnosis of mycobacteria. The DNA microarray technique holds great promise for the future because it is easy to perform, it can be readily automated, and it allows the identification of a large number of mycobacterial species in one reaction.

Chen, Z., D. Pei, et al. (2004). "Antigenicity analysis of different regions of the severe acute respiratory syndrome coronavirus nucleocapsid protein." Clin Chem **50**(6): 988-95.

BACKGROUND: The widespread threat of severe acute respiratory syndrome (SARS) to human health has made urgent the development of fast and accurate analytical methods for its early diagnosis and a safe and efficient antiviral vaccine for preventive use. For this purpose, we investigated the antigenicity of different regions of the SARS coronavirus (SARS-CoV) nucleocapsid (N) protein. METHODS: The cDNA for full-length N protein and its various regions from the SARS-CoV was cloned and expressed in Escherichia coli. After purification, all of the protein fragments were printed on glass slides to fabricate a protein microarray and then probed with the sera from SARS patients to determine the reactivity of these protein fragments. RESULTS: The full-length protein and two other fragments reacted with all 52 sera tested. Four important regions with possible epitopes were identified and named as EP1 (amino acids 51-71), EP2 (134-208), EP3 (249-273), and EP4 (349-422), respectively. EP2 and EP4 possessed linear epitopes, whereas EP1 and EP2 were able to form conformational epitopes that could react with most (>80%) of the tested sera. EP3 and EP4 also formed conformational epitopes, and antibodies against these epitopes existed in all 52 of the sera tested. CONCLUSION: The N protein is a highly immunogenic protein of the SARS-CoV. Conformational epitopes are important for this protein, and antigenicity of the COOH terminus is higher than that of the NH(2) terminus. The N protein is a potential diagnostic antigen and vaccine candidate for SARS-CoV.

Cherkasova, E., M. Laassri, et al. (2003). "Microarray analysis of evolution of RNA viruses: evidence of circulation of virulent highly divergent vaccine-derived polioviruses." Proc Natl Acad Sci U S A **100**(16): 9398-403.

Two approaches based on hybridization of viral probes with oligonucleotide microarrays were developed for rapid analysis of genetic variations during microevolution of RNA viruses. Microarray analysis of viral recombination and microarray for resequencing and heterogeneity analysis were able to generate instant genetic maps of vaccine-derived polioviruses (VDPVs) and reveal the degree of their evolutionary divergence. Unlike conventional methods based on cDNA sequencing and restriction fragment length polymorphism, the microarray approaches are better suited for analysis of heterogeneous populations and mixtures of different strains. The microarray hybridization profile is very sensitive to the cumulative presence of small quantities of different mutations, including those that cannot be revealed by sequencing, making this approach useful for characterization of profiles of nucleotide sequence diversity in viral populations. By using these methods, we identified a type-3 VDPV isolated from a healthy person and missed by conventional methods of screening. The mutational profile of the polio strain was consistent with >1 yr of circulation in human population and was highly virulent in transgenic mice, confirming the ability of VDPV to persist in communities despite high levels of immunity. The proposed methods for fine genotyping of heterogeneous viral populations can also have utility for a variety of other applications in studies of genetic changes in viruses, bacteria, and genes of higher organisms.

Cheung, S. T., X. Chen, et al. (2002). "Identify metastasis-associated genes in hepatocellular carcinoma through clonality delineation for multinodular tumor." Cancer Res **62**(16): 4711-21.

Disease recurrence and metastasis are frequently observed in many successfully treated localized cancers, including hepatocellular carcinoma in which intrahepatic and extrahepatic recurrence (metastasis) are frequently observed after curative resection. The present study aimed at identifying metastasis-associated genes through delineation of the clonality for multinodular liver cancer. The clonal relationship of 22 tumor foci from six patients was investigated by the genome-wide expression profile via cDNA microarray consisting of 23,000 genes. Tumor molecular properties including p53 protein overexpression and gene mutation, hepatitis B virus integration pattern, and genetic alteration examined by comparative genomic hybridization were compared. Results indicated that gene expression patterns could serve as the molecular fingerprint for clonality identification. Together with the molecular data from p53, hepatitis B virus integration and comparative genomic hybridization profiles, tumor nodules from five patients were confirmed with clonal relationship, and the expression profiles of the primary nodules were compared with their corresponding intrahepatic metastatic nodules. A total of 90 clones were found to be correlated with intrahepatic metastasis by Student's t test ($P < 0.05$). With reference to the primary tumor, 63 clones (39 known genes and 24 express sequence tags) were down-regulated whereas 27 clones (14 known genes and 13 express sequence tags) were up-regulated in the metastatic nodules. These metastasis-associated genes may provide clues to reveal patients with increased risk of developing metastasis, and to identify novel therapeutic targets for the treatment of metastasis.

Chizhikov, V., M. Wagner, et al. (2002). "Detection and genotyping of human group A rotaviruses by oligonucleotide microarray hybridization." J Clin Microbiol **40**(7): 2398-407.

A rapid and reliable method for the identification of five clinically relevant G genotypes (G1 to G4 and G9) of human rotaviruses based on oligonucleotide microarray hybridization has been developed. The genotype-specific oligonucleotides immobilized on the surface of glass slides were selected to bind to the multiple target regions within the VP7 gene that are highly conserved among individual rotavirus genotypes. Rotavirus cDNA was amplified in a PCR with primers common to all group A rotaviruses. A second round of nested PCR amplification was performed in the presence of indodicarbocyanine-dCTP and another pair of degenerate primers also broadly specific for all genotypes. The use of one primer containing 5'-biotin allowed us to prepare fluorescently labeled single-stranded hybridization probe by binding of another strand to magnetic beads. The identification of rotavirus genotype was based on hybridization with several individual genotype-specific oligonucleotides. This approach combines the high sensitivity of PCR with the selectivity of DNA-DNA hybridization. The specificity of oligonucleotide microchip hybridization was evaluated by testing 20 coded rotavirus isolates from different geographic areas for which genotypes were previously determined by conventional methods. Analysis of the coded specimens showed that this microarray-based method is capable of unambiguous identification of all rotavirus strains. Because of the presence of random mutations, each individual virus isolate produced a unique hybridization pattern capable of distinguishing different isolates of the same genotype and, therefore, subgenotype differentiation. This strain information indicates one of several advantages that microarray technology has over conventional PCR techniques

Cho, N. H., H. J. An, et al. (2003). "Genotyping of 22 human papillomavirus types by DNA chip in Korean women: comparison with cytologic diagnosis." Am J Obstet Gynecol **188**(1): 56-62.

OBJECTIVE: More sensitive and reliable methods than individual testing (such as polymerase chain reaction, restriction fragment length polymorphism, and Southern blot) should be developed as screening tools for the detection of latent human papillomavirus. Today, the new Bethesda system recommends human papillomavirus testing as an adjuvant to the conventional Papanicolaou smear for more comprehensive identification of women at certain risk of cervical neoplasia. We performed human papillomavirus genotyping with the newly designed human papillomavirus DNA chip, which is based on polymerase chain reaction for high-throughput screening power, and compared the results with the results

of a Papanicolaou smear according to the new Bethesda system. STUDY DESIGN: Polymerase chain reaction amplifications of the human papillomavirus L1 region from biologic samples were hybridized to silanized glass slides by a microarrayer, which comprised 22 specific oligonucleotide probes to their genotypes, consisting of 15 high-risk and 7 low-risk types. Two cervical cancer cell lines and 20 plasmids that contained each type of the human papillomavirus whole genome were used for the evaluation of this method; in all cases, the cancer cell lines and plasmids showed clear positive signals on their corresponding positions. A comparative study that used 685 cervicovaginal swabs was performed by human papillomavirus DNA chip microarray together with Papanicolaou diagnosis. RESULTS: Human papillomavirus was identified as positive in 31.9% of the 414 control samples and in 78.6% of the 271 neoplastic lesions. The major prevailing human papillomavirus genotypes were human papillomavirus types 16, 58, and 18, in descending order of incidence (average overall, 78.8%). Almost all of the remaining cases were comprised of human papillomavirus types 39, 52, 56, and 51. The frequency of multiple infection of human papillomavirus was highest in low-grade squamous intraepithelial lesion but was lowest in squamous cell carcinoma. All cases that exhibited infection of single human papillomavirus type 58 were squamous cell carcinoma. CONCLUSION: Human papillomavirus types 16, 18, and 58 were confirmed to be major causative factors for cervical carcinogenesis. Low-grade squamous intraepithelial lesion is a heterogeneous entity that is composed of different human papillomavirus subtypes and prevails in younger women (<40 years old). The human papillomavirus chip has potential use as a high-throughput screening test.

Choi, B. S., O. Kim, et al. (2003). "Genital human papillomavirus genotyping by HPV oligonucleotide microarray in Korean commercial sex workers." J Med Virol **71**(3): 440-5.

Because of the diversity in human papillomavirus (HPV) distribution, according to the population and region, detailed investigations of HPV genotypes are important in designing more effective HPV vaccines for any given country. HPV DNA oligonucleotide microarray was used to investigate the distribution of HPV genotypes among commercial sex workers. The prevalence of HPV in Korean commercial sex workers was 47%, with HPV-16 and HPV-51 as the dominant genotypes. HPV subtypes in 148 commercial sex workers comprised 70 with one genotype, 42 with two genotypes, 17 with three genotypes, and 19 with four or more genotypes. HPV-40, the most dominant low-risk genotype, was not detected in single-infection commercial sex workers. All women with multiple infections of low-risk genotypes had the HPV-40 genotype. This molecular epidemiological study of genital HPV will be useful for the development of a favorable strategy to prevent the spread of this potentially serious infection.

Chopra, P., L. S. Meena, et al. (2003). "New drug targets for Mycobacterium tuberculosis." Indian J Med Res **117**: 1-9.

In spite of the availability of effective chemotherapy and Bacille-Calmette-Guerin (BCG) vaccine, tuberculosis remains a leading infectious killer world-wide. Many factors such as, human immunodeficiency virus (HIV) co-infection, drug resistance, lack of patient compliance with chemotherapy, delay in diagnosis, variable efficacy of BCG vaccine and various other factors contribute to the mortality due to tuberculosis. In spite of the new advances in understanding the biology of Mycobacterium tuberculosis, and availability of functional genomic tools, such as microarray and proteomics, in combination with modern approaches, no new drug has been developed in the past 30 yr. Therefore, there is an urgent need to identify new drug targets in mycobacteria and eventually, develop new drugs. The release of the complete genome sequence of M. tuberculosis has facilitated a more rational, and directional approach to search for new drug targets. In general, gene products involved in mycobacterial metabolism, persistence, transcription, cell wall synthesis and virulence would be possible targets for the development of new drugs. The exploitation of host cell signaling pathways for the benefit of the pathogen is a phenomenon that deserves to be looked into with a new perspective in the current scenario to combat M. tuberculosis. Reversible phosphorylation and dephosphorylation, which are carried out by specific protein kinases and phosphatases have been shown to modify the host proteins and help in

the establishment of disease by several pathogenic bacteria. In this review, we discuss some possible drug targets for M. tuberculosis.

Chou, H. C., C. Z. Lee, et al. (2004). "Isolation of a chromosomal region of Klebsiella pneumoniae associated with allantoin metabolism and liver infection." Infect Immun **72**(7): 3783-92.

Klebsiella pneumoniae liver abscess with metastatic complications is an emerging infectious disease in Taiwan. To identify genes associated with liver infection, we used a DNA microarray to compare the transcriptional profiles of three strains causing liver abscess and three strains not associated with liver infection. There were 13 clones that showed higher RNA expression levels in the three liver infection strains, and 3 of these 13 clones contained a region that was absent in MGH 78578. Sequencing of the clones revealed the replacement of 149 bp of MGH 78578 with a 21,745-bp fragment in a liver infection strain, NTUH-K2044. This 21,745-bp fragment contained 19 open reading frames, 14 of which were proven to be associated with allantoin metabolism. The K2044 (DeltaallS) mutant showed a significant decrease of virulence in intragastric inoculation of BALB/c mice, and the prevalence of this chromosomal region was significantly higher in strains associated with liver abscess than in those that were not (19 or 32 versus 2 of 94; P = 0.0001 [chi(2) test]). Therefore, the 22-kb region may play a role in K. pneumoniae liver infection and serve as a marker for rapid identification.

Chun, T. W., J. S. Justement, et al. (2003). "Gene expression and viral prodution in latently infected, resting CD4+ T cells in viremic versus aviremic HIV-infected individuals." Proc Natl Acad Sci U S A **100**(4): 1908-13.

The presence of HIV-1 in latently infected, resting CD4(+) T cells has been clearly demonstrated in infected individuals; however, the extent of viral expression and the underlying mechanisms of the persistence of HIV-1 in this viral reservoir have not been fully delineated. Here, we show that resting CD4(+) T cells from the majority of viremic patients are capable of producing cell-free HIV-1 spontaneously ex vivo. The levels of HIV-1 released by resting CD4(+) T cells were not significantly reduced in the presence of inhibitors of cellular proliferation and viral replication. However, resting CD4(+) T cells from the majority of aviremic patients failed to produce virions, despite levels of HIV-1 proviral DNA and cell-associated HIV-1 RNA comparable to viremic patients. The DNA microarray analysis demonstrated that a number of genes involving transcription regulation, RNA processing and modification, and protein trafficking and vesicle transport were significantly upregulated in resting CD4(+) T cells of viremic patients compared to those of aviremic patients. These results suggest that active viral replication has a significant impact on the physiologic state of resting CD4(+) T cells in infected viremic patients and, in turn, allows release of HIV-1 without exogenous activation stimuli. In addition, given that no quantifiable virions were produced by the latent viral reservoir in the majority of aviremic patients despite the presence of cell-associated HIV-1 RNA, evidence for transcription of HIV-1 RNA in resting CD4(+) T cells of aviremic patients should not necessarily be taken as direct evidence for ongoing viral replication during effective therapy.

Clements, M. O., S. Eriksson, et al. (2002). "Polynucleotide phosphorylase is a global regulator of virulence and persistency in Salmonella enterica." Proc Natl Acad Sci U S A **99**(13): 8784-9.

For many pathogens, the ability to regulate their replication in host cells is a key element in establishing persistency. Here, we identified a single point mutation in the gene for polynucleotide phosphorylase (PNPase) as a factor affecting bacterial invasion and intracellular replication, and which determines the alternation between acute or persistent infection in a mouse model for Salmonella enterica infection. In parallel, with microarray analysis, PNPase was found to affect the mRNA levels of a subset of virulence genes, in particular those contained in Salmonella pathogenicity islands 1 and 2. The results demonstrate a connection between PNPase and Salmonella virulence and show that alterations in PNPase activity could represent a strategy for the establishment of persistency.

Conacci-Sorrell, M. E., T. Ben-Yedidia, et al. (2002). "Nr-CAM is a target gene of the beta-catenin/LEF-1 pathway in melanoma and colon cancer and its expression enhances motility and confers tumorigenesis." Genes Dev **16**(16): 2058-72.

beta-catenin and plakoglobin (gamma-catenin) are homologous molecules involved in cell adhesion, linking cadherin receptors to the cytoskeleton. beta-catenin is also a key component of the Wnt pathway by being a coactivator of LEF/TCF transcription factors. To identify novel target genes induced by beta-catenin and/or plakoglobin, DNA microarray analysis was carried out with RNA from cells overexpressing either protein. This analysis revealed that Nr-CAM is the gene most extensively induced by both catenins. Overexpression of either beta-catenin or plakoglobin induced Nr-CAM in a variety of cell types and the LEF/TCF binding sites in the Nr-CAM promoter were required for its activation by catenins. Retroviral transduction of Nr-CAM into NIH3T3 cells stimulated cell growth, enhanced motility, induced transformation, and produced rapidly growing tumors in nude mice. Nr-CAM and LEF-1 expression was elevated in human colon cancer tissue and cell lines and in human malignant melanoma cell lines but not in melanocytes or normal colon tissue. Dominant negative LEF-1 decreased Nr-CAM expression and antibodies to Nr-CAM inhibited the motility of B16 melanoma cells. The results indicate that induction of Nr-CAM transcription by beta-catenin or plakoglobin plays a role in melanoma and colon cancer tumorigenesis, probably by promoting cell growth and motility.

Cowell, J. K., Y. D. Wang, et al. (2004). "Identification and characterisation of constitutional chromosome abnormalities using arrays of bacterial artificial chromosomes." Br J Cancer **90**(4): 860-5.

Constitutional chromosome deletions and duplications frequently predispose to the development of a wide variety of cancers. We have developed a microarray of 6000 bacterial artificial chromosomes for array-based comparative genomic hybridisation, which provides an average resolution of 750 kb across the human genome. Using these arrays, subtle gains and losses of chromosome regions can be detected in constitutional cells, following a single overnight hybridisation. In this report, we demonstrate the efficiency of this procedure in identifying constitutional deletions and duplications associated with predisposition to retinoblastoma, Wilms tumour and Beckwith-Wiedemann syndrome.

Cummings, C. A. and D. A. Relman (2000). "Using DNA microarrays to study host-microbe interactions." Emerg Infect Dis **6**(5): 513-25.

Complete genomic sequences of microbial pathogens and hosts offer sophisticated new strategies for studying host-pathogen interactions. DNA microarrays exploit primary sequence data to measure transcript levels and detect sequence polymorphisms, for every gene, simultaneously. The design and construction of a DNA microarray for any given microbial genome are straightforward. By monitoring microbial gene expression, one can predict the functions of uncharacterized genes, probe the physiologic adaptations made under various environmental conditions, identify virulence-associated genes, and test the effects of drugs. Similarly, by using host gene microarrays, one can explore host response at the level of gene expression and provide a molecular description of the events that follow infection. Host profiling might also identify gene expression signatures unique for each pathogen, thus providing a novel tool for diagnosis, prognosis, and clinical management of infectious disease.

Daiba, A., N. Inaba, et al. (2004). "A low-density cDNA microarray with a unique reference RNA: pattern recognition analysis for IFN efficacy prediction to HCV as a model." Biochem Biophys Res Commun **315**(4): 1088-96.

We have designed and established a low-density (295 genes) cDNA microarray for the prediction of IFN efficacy in hepatitis C patients. To obtain a precise and consistent microarray data, we collected a data set from three spots for each gene (mRNA) and using three different scanning conditions. We also established an artificial reference RNA representing pseudo-inflammatory conditions from established hepatocyte cell lines supplemented with synthetic RNAs to 48 inflammatory genes. We also developed a novel algorithm that replaces the standard hierarchical-clustering method and allows handling of the large

data set with ease. This algorithm utilizes a standard space database (SSDB) as a key scale to calculate the Mahalanobis distance (MD) from the center of gravity in the SSDB. We further utilized sMD (divided by parameter k: MD/k) to reduce MD number as a predictive value. The efficacy prediction of conventional IFN mono-therapy was 100% for non-responder (NR) vs. transient responder (TR)/sustained responder (SR) ($P < 0.0005$). Finally, we show that this method is acceptable for clinical application.

Danelishvili, L., J. McGarvey, et al. (2003). "Mycobacterium tuberculosis infection causes different levels of apoptosis and necrosis in human macrophages and alveolar epithelial cells." Cell Microbiol **5**(9): 649-60.

Mycobacterium tuberculosis interacts with macrophages and epithelial cells in the alveolar space of the lung, where it is able to invade and replicate in both cell types. M. tuberculosis-associated cytotoxicity to these cells has been well documented, but the mechanisms of host cell death are not well understood. We examined the induction of apoptosis and necrosis of human macrophages (U937) and type II alveolar epithelial cells (A549) by virulent (H37Rv) and attenuated (H37Ra) M. tuberculosis strains. Apoptosis was determined by both enzyme-linked immunosorbent assay (ELISA) and TdT-mediated dUTP nick end labelling (TUNEL) assay, whereas necrosis was evaluated by the release of lactate dehydrogenase (LDH). Both virulent and attenuated M. tuberculosis induced apoptosis in macrophages; however, the attenuated strain resulted in significantly more apoptosis than the virulent strain after 5 days of infection. In contrast, cytotoxicity of alveolar cells was the result of necrosis, but not apoptosis. Although infection with M. tuberculosis strains resulted in apoptosis of 14% of the cells on the monolayer, cell death associated with necrosis was observed in 59% of alveolar epithelial cells after 5 days of infection. Infection with M. tuberculosis suppressed apoptosis of alveolar epithelial cells induced by the kinase inhibitor, staurosporine. Because our findings suggest that M. tuberculosis can modulate the apoptotic response of macrophages and epithelial cells, we carried out an apoptosis pathway-specific cDNA microarray analysis of human macrophages and alveolar epithelial cells. Whereas the inhibitors of apoptosis, bcl-2 and Rb, were upregulated over 2.5-fold in infected (48 h) alveolar epithelial cells, the proapoptotic genes, bad and bax, were downregulated. The opposite was observed when U937 macrophages were infected with M. tuberculosis. Upon infection of alveolar epithelial cells with M. tuberculosis, the generation of apoptosis, as determined by the expression of caspase-1, caspase-3 and caspase-10, was inhibited. Inhibition of replication of intracellular bacteria resulted in an increase in apoptosis in both cell types. Our results showed that the differential induction of apoptosis between macrophages and alveolar epithelial cells represents specific strategies of M. tuberculosis for survival in the host.

Day, W. A., Jr., R. E. Fernandez, et al. (2001). "Pathoadaptive mutations that enhance virulence: genetic organization of the cadA regions of Shigella spp." Infect Immun **69**(12): 7471-80.

Pathoadaptive mutations improve the fitness of pathogenic species by modification of traits that interfere with factors (virulence and ancestral) required for survival in host tissues. A demonstrated pathoadaptive mutation is the loss of lysine decarboxylase (LDC) expression in Shigella species that have evolved from LDC-expressing Escherichia coli. Previous studies demonstrated that the product of LDC activity, cadaverine, blocks the action of Shigella enterotoxins and that the gene encoding LDC, cadA, was abolished by large chromosomal deletions in each Shigella species. To better understand the nature and evolution of these pathoadaptive mutations, remnants of the cad region were sequenced from the four Shigella species. These analyses reveal novel gene arrangements in this region of the pathogens' chromosomes. Insertion sequences, a phage genome, and/or loci from different positions on the ancestral E. coli chromosome displaced the cadA locus to form distinct genetic linkages that are unique to each Shigella species. Hybridization studies, using an E. coli K-12 microarray, indicated that the genes displaced to form the novel linkages still remain in the Shigella genomes. None of these novel gene arrangements were observed in representatives of all E. coli phylogenies. Collectively, these observations indicate that inactivation of the cadA antivirulence gene occurred independently in each Shigella species.

The convergent evolution of these pathoadaptive mutations demonstrates that, following evolution from commensal E. coli, strong pressures in host tissues selected Shigella clones with increased fitness and virulence through the loss of an ancestral trait (LDC). These observations strongly support the role of pathoadaptive mutation as an important pathway in the evolution of pathogenic organisms.

DeBiasi, R. L., P. Clarke, et al. (2003). "Reovirus-induced alteration in expression of apoptosis and DNA repair genes with potential roles in viral pathogenesis." J Virol **77**(16): 8934-47.

Reoviruses are a leading model for understanding cellular mechanisms of virus-induced apoptosis. Reoviruses induce apoptosis in multiple cell lines in vitro, and apoptosis plays a key role in virus-induced tissue injury of the heart and brain in vivo. The activation of transcription factors NF-kappaB and c-Jun are key events in reovirus-induced apoptosis, indicating that new gene expression is critical to this process. We used high-density oligonucleotide microarrays to analyze cellular transcriptional alterations in HEK293 cells after infection with reovirus strain T3A (i.e., apoptosis inducing) compared to infection with reovirus strain T1L (i.e., minimally apoptosis inducing) and uninfected cells. These strains also differ dramatically in their potential to induce apoptotic injury in hearts of infected mice in vivo-T3A is myocarditic, whereas T1L is not. Using high-throughput microarray analysis of over 12,000 genes, we identified differential expression of a defined subset of genes involved in apoptosis and DNA repair after reovirus infection. This provides the first comparative analysis of altered gene expression after infection with viruses of differing apoptotic phenotypes and provides insight into pathogenic mechanisms of virus-induced disease.

Debnath, A., P. Das, et al. (2004). "Identification of genomic responses to collagen binding by trophozoites of Entamoeba histolytica." J Infect Dis **190**(3): 448-57.

Attachment of Entamoeba histolytica trophozoites to collagen is a known stimulus for parasite activation, leading to subsequent tissue destruction and invasion. To identify cellular mechanisms of trophozoite activation, we assessed global variations in gene expression during collagen interaction with E. histolytica. A shotgun DNA microarray was constructed by use of 9600 random inserts from an E. histolytica genomic DNA library. Through differential hybridization, key differences between gene expression in collagen-activated trophozoites and that in nonactivated trophozoites were identified. Fourteen differentially regulated clones were reproducibly identified and selected for sequencing. Among the genes identified were those coding for (1) components of a signaling cascade that had been previously hypothesized to transmit responses to cell attachment, (2) adapter proteins for vesicle formation, and (3) proteins that are implicated in cytoskeletal reorganization and locomotion. Two known virulence-factor genes--those for cysteine proteinases and amebapore--also were up-regulated in response to collagen stimulation. These results provide important new clues about how a pathogen orchestrates responses to the host environment as well as a new tool for the analysis of other aspects of Entamoeba species infection and pathogenicity.

Debouck, C. and P. N. Goodfellow (1999). "DNA microarrays in drug discovery and development." Nat Genet **21**(1 Suppl): 48-50.

DNA microarrays can be used to measure the expression patterns of thousands of genes in parallel, generating clues to gene function that can help to identify appropriate targets for therapeutic intervention. They can also be used to monitor changes in gene expression in response to drug treatments. Here, we discuss the different ways in which microarray analysis is likely to affect drug discovery.

Delehanty, J. B. and F. S. Ligler (2002). "A microarray immunoassay for simultaneous detection of proteins and bacteria." Anal Chem **74**(21): 5681-7.

We report the development and characterization of an antibody microarray biosensor for the rapid detection of both protein and bacterial analytes under flow conditions. Using a noncontact microarray printer, biotinylated capture antibodies were immobilized at discrete locations on the surface of an avidin-

coated glass microscope slide. Preservation of capture antibody function during the deposition process was accomplished with the use of a low-salt buffer containing sucrose and bovine serum albumin. The slide was fitted with a six-channel flow module that conducted analyte-containing solutions over the array of capture antibody microspots. Detection of bound analyte was subsequently achieved using fluorescent tracer antibodies. The pattern of fluorescent complexes was interrogated using a scanning confocal microscope equipped with a 635-nm laser. This microarray system was employed to detect protein and bacterial analytes both individually and in samples containing mixtures of analytes. Assays were completed in 15 min, and detection of cholera toxin, staphylococcal enterotoxin B, ricin, and Bacillus globigii was demonstrated at levels as low as 8 ng/mL, 4 ng/mL, 10 ng/mL, and 6.2 x 10(4) cfu/mL, respectively. The assays presented here are very fast, as compared to previously published methods for measuring antibody-antigen interactions using microarrays (minutes versus hours).

Delrio-Lafreniere, S. A., M. K. Browning, et al. (2004). "Low-density addressable array for the detection and typing of the human papillomavirus." Diagn Microbiol Infect Dis **48**(1): 23-31.

We have developed a low-density DNA array for the detection and typing of human papillomavirus (HPV) DNA. The gene chemistry strategy involves using a combination of the polymerase chain reaction (PCR) with the consensus oligonucleotide primers MY09/MY11 followed by a ligase detection reaction (LDR). Fluorochrome-labeled HPV-specific primers are joined to a common primer modified with a unique anchoring sequence called a zip code on its 3' end. The result is a series of 60-70 base pair and single-stranded ligation products that are then hybridized to their respective zip code complements affixed to glass slide based arrays. Nine separate zip codes were assigned, one for each HPV type (6,11,16,18, 31, 33, 35, and 53) and one for a beta-globin internal control marker. Two additional zip-codes were reserved for a pair of consensus HPV LDR products: the cLDR1 and cLDR2 primers hybridize to a conserved sequence within the HPV L1 open reading frame internal to the MY09/MY11 fragment. These consensus primers were shown to detect over 40 different HPV types. The purpose of this study was to evaluate the analytic performance of this low-density microarray based assay for HPV, as well as to introduce our simplified read-out instrumentation, shown here to be a low cost and highly efficient way to detect and genotype HPV for clinical testing.

Domachowske, J. B., C. A. Bonville, et al. (2002). "Differential expression of proinflammatory cytokine genes in vivo in response to pathogenic and nonpathogenic pneumovirus infections." J Infect Dis **186**(1): 8-14.

Pneumonia virus of mice (PVM; Paramyxoviridae, subfamily Pneumovirinae) is an important pathogen for the study of physiologically relevant acute inflammatory responses in rodent hosts. In contrast to the severe symptomatology observed in response to infection with PVM strain J3666, infection with strain 15 resulted in few clinical symptoms, limited cellular inflammatory response, and no production of macrophage inflammatory protein-1alpha or monocyte chemoattractant peptide (MCP)-1. Microarray analysis of transcripts from lung tissue indicates that PVM J3666 infection promotes up-regulation of specific proinflammatory genes, most notably interferon (IFN)-1beta, IFN response genes, and chemokines MCP-1, MCP-3, RANTES (regulated on activation, normally T cell-expressed and secreted), and eotaxin. Of these, only RANTES expression increased in response to infection with strain 15, with no increased expression of IFN or IFN response genes, despite ongoing viral replication. These results suggest that pneumovirus replication alone is insufficient to promote antiviral inflammation and that evaluation of the more divergent strain-specific pneumovirus proteins may provide some intriguing leads toward the molecular basis of this differential response.

Dorrell, N., J. A. Mangan, et al. (2001). "Whole genome comparison of Campylobacter jejuni human isolates using a low-cost microarray reveals extensive genetic diversity." Genome Res **11**(10): 1706-15.

Campylobacter jejuni is the leading cause of bacterial food-borne diarrhoeal disease throughout the world, and yet is still a poorly understood pathogen. Whole genome microarray comparisons of 11 C.

jejuni strains of diverse origin identified genes in up to 30 NCTC 11168 loci ranging from 0.7 to 18.7 kb that are either absent or highly divergent in these isolates. Many of these regions are associated with the biosynthesis of surface structures including flagella, lipo-oligosaccharide, and the newly identified capsule. Other strain-variable genes of known function include those responsible for iron acquisition, DNA restriction/modification, and sialylation. In fact, at least 21% of genes in the sequenced strain appear dispensable as they are absent or highly divergent in one or more of the isolates tested, thus defining 1300 C. jejuni core genes. Such core genes contribute mainly to metabolic, biosynthetic, cellular, and regulatory processes, but many virulence determinants are also conserved. Comparison of the capsule biosynthesis locus revealed conservation of all the genes in this region in strains with the same Penner serotype as strain NCTC 11168. By contrast, between 5 and 17 NCTC 11168 genes in this region are either absent or highly divergent in strains of a different serotype from the sequenced strain, providing further evidence that the capsule accounts for Penner serotype specificity. These studies reveal extensive genetic diversity among C. jejuni strains and pave the way toward identifying correlates of pathogenicity and developing improved epidemiological tools for this problematic pathogen.

Dourmishev, L. A., A. L. Dourmishev, et al. (2003). "Molecular genetics of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) epidemiology and pathogenesis." Microbiol Mol Biol Rev **67**(2): 175-212, table of contents.

Kaposi's sarcoma had been recognized as unique human cancer for a century before it manifested as an AIDS-defining illness with a suspected infectious etiology. The discovery of Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus-8, in 1994 by using representational difference analysis, a subtractive method previously employed for cloning differences in human genomic DNA, was a fitting harbinger for the powerful bioinformatic approaches since employed to understand its pathogenesis in KS. Indeed, the discovery of KSHV was rapidly followed by publication of its complete sequence, which revealed that the virus had coopted a wide armamentarium of human genes; in the short time since then, the functions of many of these viral gene variants in cell growth control, signaling apoptosis, angiogenesis, and immunomodulation have been characterized. This critical literature review explores the pathogenic potential of these genes within the framework of current knowledge of the basic herpesvirology of KSHV, including the relationships between viral genotypic variation and the four clinicoepidemiologic forms of Kaposi's sarcoma, current viral detection methods and their utility, primary infection by KSHV, tissue culture and animal models of latent- and lytic-cycle gene expression and pathogenesis, and viral reactivation from latency. Recent advances in models of de novo endothelial infection, microarray analyses of the host response to infection, receptor identification, and cloning of full-length, infectious KSHV genomic DNA promise to reveal key molecular mechanisms of the candidate pathogeneic genes when expressed in the context of viral infection.

Dunman, P. M., E. Murphy, et al. (2001). "Transcription profiling-based identification of Staphylococcus aureus genes regulated by the agr and/or sarA loci." J Bacteriol **183**(24): 7341-53.

The advent of transcription profiling technologies has provided researchers with an unprecedented ability to study biological processes. Accordingly, a custom-made Affymetrix GeneChip, constituting >86% of the Staphylococcus aureus genome, was used to identify open reading frames that are regulated by agr and/or SarA, the two best-studied regulators of the organism's virulence response. RNA extracted from wild-type cells and agr, sarA, and agr sarA mutant cells in the early-, mid-, and late-log and stationary phases of growth was analyzed. Open reading frames with transcription patterns expected of genes either up- or downregulated in an agr- and/or SarA-dependent manner were identified. Oligonucleotide microarray and Northern blot analyses confirmed that the transcription of several known virulence genes, including hla (alpha-toxin) and spa (protein A), is regulated by each effector and provided insights about the regulatory cascades involved in both alpha-hemolysin and protein A expression. Several putative virulence factors were also identified as regulated by agr and/or SarA. In addition, genes that are involved in several biological processes but which are difficult to reconcile as

playing a direct role in the organism's pathogenesis also appeared to be regulated by each effector, suggesting that products of both the agr and the sarA locus are more-global transcription regulators than previously realized.

Dziejman, M., E. Balon, et al. (2002). "Comparative genomic analysis of Vibrio cholerae: genes that correlate with cholera endemic and pandemic disease." Proc Natl Acad Sci U S A **99**(3): 1556-61.

Historically, the first six recorded cholera pandemics occurred between 1817 and 1923 and were caused by Vibrio cholerae O1 serogroup strains of the classical biotype. Although strains of the El Tor biotype caused sporadic infections and cholera epidemics as early as 1910, it was not until 1961 that this biotype emerged to cause the 7th pandemic, eventually resulting in the global elimination of classical biotype strains as a cause of disease. The completed genome sequence of 7th pandemic El Tor O1 strain N16961 has provided an important tool to begin addressing questions about the evolution of V. cholerae as a human pathogen and environmental organism. To facilitate such studies, we constructed a V. cholerae genomic microarray that displays over 93% of the predicted genes of strain N16961 as spotted features. Hybridization of labeled genomic DNA from different strains to this microarray allowed us to compare the gene content of N16961 to that of other V. cholerae isolates. Surprisingly, the results reveal a high degree of conservation among the strains tested. However, genes unique to all pandemic strains as well as genes specific to 7th pandemic El Tor and related O139 serogroup strains were identified. These latter genes may encode gain-of-function traits specifically associated with displacement of the preexisting classical strains in South Asia and may also promote the establishment of endemic disease in previously cholera-free locations.

Edman, C. F., P. Mehta, et al. (2000). "Pathogen analysis and genetic predisposition testing using microelectronic arrays and isothermal amplification." J Investig Med **48**(2): 93-101.

BACKGROUND: A simple yet powerful tool for providing for rapid gene identification in the clinic would be the combination of isothermal gene amplification with electronic microchip analysis. This is a first report of such a union of these technologies. METHODS: The first assay demonstrates discrimination between four bacterial pathogens. For this, one portion of the bacterial 16S rRNA gene encompassing a microheterogeneous region was isothermally amplified using Strand Displacement Amplification (SDA). Type identification was then made by "sandwich" assay format either using selective electronic hybridization of amplicons to sequence-specific capture oligonucleotides and a universal, fluorescently labeled reporter oligonucleotide, or, alternatively, sequence-specific reporters and a universal capture oligonucleotide. The second assay tested for the presence or absence of the Factor V Leiden point mutation using DNA obtained from 18 patients in a blind assay. For this, allele-specific SDA was developed. Following amplification using a sense-biotinylated primer and either the corresponding antisense wild type or mutant primer, multiple patient amplicons were targeted to specified locations on the microarray and visualized using a fluorescently labeled reporter oligonucleotide. Positive signals were scored as greater than or equal to two times the background. RESULTS: Bacterial type-specific signals were between 3- to 10-fold greater than nonspecific in both assay formats. Using allele-specific SDA, 100% agreement was observed between PAGE analysis, microarray results, and clinical diagnosis in Factor V mutation analysis. CONCLUSIONS: We demonstrated two model clinical assays combining amplified materials and microelectronic arrays, one potentially suitable for pathogen screening and the other for a deleterious genetic mutation.

Ernst, R. K., D. A. D'Argenio, et al. (2003). "Genome mosaicism is conserved but not unique in Pseudomonas aeruginosa isolates from the airways of young children with cystic fibrosis." Environ Microbiol **5**(12): 1341-9.

Pseudomonas aeruginosa strains from the chronic lung infections of cystic fibrosis (CF) patients are phenotypically and genotypically diverse. Using strain PAO1 whole genome DNA microarrays, we assessed the genomic variation in P. aeruginosa strains isolated from young children with CF (6 months to

8 years of age) as well as from the environment. Eighty-nine to 97% of the PAO1 open reading frames were detected in 20 strains by microarray analysis, while subsets of 38 gene islands were absent or divergent. No specific pattern of genome mosaicism defined strains associated with CF. Many mosaic regions were distinguished by their low G + C content; their inclusion of phage related or pyocin genes; or by their linkage to a vgr gene or a tRNA gene. Microarray and phenotypic analysis of sequential isolates from individual patients revealed two deletions of greater than 100 kbp formed during evolution in the lung. The gene loss in these sequential isolates raises the possibility that acquisition of pyomelanin production and loss of pyoverdin uptake each may be of adaptive significance. Further characterization of P. aeruginosa diversity within the airways of individual CF patients may reveal common adaptations, perhaps mediated by gene loss, that suggest new opportunities for therapy.

Firoved, A. M. and V. Deretic (2003). "Microarray analysis of global gene expression in mucoid Pseudomonas aeruginosa." J Bacteriol **185**(3): 1071-81.

Pseudomonas aeruginosa is the dominant pathogen causing chronic respiratory infections in cystic fibrosis (CF). After an initial phase characterized by intermittent infections, a chronic colonization is established in CF upon the conversion of P. aeruginosa to the mucoid, exopolysaccharide alginate-overproducing phenotype. The emergence of mucoid P. aeruginosa in CF is associated with respiratory decline and poor prognosis. The switch to mucoidy in most CF isolates is caused by mutations in the mucA gene encoding an anti-sigma factor. The mutations in mucA result in the activation of the alternative sigma factor AlgU, the P. aeruginosa ortholog of Escherichia coli extreme stress sigma factor sigma(E). Because of the global nature of the regulators of mucoidy, we have hypothesized that other genes, in addition to those specific for alginate production, must be induced upon conversion to mucoidy, and their production may contribute to the pathogenesis in CF. Here we applied microarray analysis to identify on the whole-genome scale those genes that are coinduced with the AlgU sigmulon upon conversion to mucoidy. Gene expression profiles of AlgU-dependent conversion to mucoidy revealed coinduction of a specific subset of known virulence determinants (the major protease elastase gene, alkaline metalloproteinase gene aprA, and the protease secretion factor genes aprE and aprF) or toxic factors (cyanide synthase) that may have implications for disease in CF. Analysis of promoter regions of the most highly induced genes (>40-fold, P < or = 10(-4)) revealed a previously unrecognized, putative AlgU promoter upstream of the osmotically inducible gene osmE. This newly identified AlgU-dependent promoter of osmE was confirmed by mapping the mRNA 5' end by primer extension. The recognition of genes induced in mucoid P. aeruginosa, other than those associated with alginate biosynthesis, reported here revealed the identity of previously unappreciated factors potentially contributing to the morbidity and mortality caused by mucoid P. aeruginosa in CF.

Firoved, A. M., W. Ornatowski, et al. (2004). "Microarray analysis reveals induction of lipoprotein genes in mucoid Pseudomonas aeruginosa: implications for inflammation in cystic fibrosis." Infect Immun **72**(9): 5012-8.

The main cause of the high morbidity and mortality of cystic fibrosis (CF) is the progressive lung inflammation associated with Pseudomonas aeruginosa colonization. During the course of chronic CF infections, P. aeruginosa undergoes a conversion to a mucoid phenotype. The emergence of mucoid P. aeruginosa in CF is associated with increased inflammation, respiratory decline, and a poor prognosis. Here we show, by the use of microarray analysis, that upon P. aeruginosa conversion to mucoidy, there is a massive and preferential induction of genes encoding bacterial lipoproteins. Bacterial lipoproteins are potent agonists of Toll-like receptor 2 (TLR2) signaling. The expression of TLR2 in human respiratory epithelial cells was ascertained by Western blot analysis. Human respiratory epithelial cells responded in a TLR2-dependent manner to bacterial lipopeptides derived from Pseudomonas lipoproteins induced in mucoid strains. The TLR2 proinflammatory response was further augmented in CF cells. Thus, the excessive inflammation in CF is the result of a global induction in mucoid P. aeruginosa of lipoproteins that act as proinflammatory toxins (here termed lipotoxins) superimposed on the hyperexcitability of CF

cells. Blocking the signaling cascade responding to bacterial lipotoxins may provide therapeutic benefits for CF patients.

Fischer, T. K. and J. R. Gentsch (2004). "Rotavirus typing methods and algorithms." Rev Med Virol **14**(2): 71-82.

Vaccination is the current strategy for control and prevention of severe rotavirus infections, a major cause of acute, dehydrating diarrhoea in young children worldwide. Public health interventions aimed at improving water, food and sanitation are unlikely adequately to control the disease. The development of vaccines against severe rotavirus diarrhoea is based upon homotypic or heterotypic protection provided against either a single common G serotype (monovalent vaccines) or against multiple serotypes (multivalent vaccines). Rotavirus strain surveillance has a high priority in disease control programmes worldwide. The continued identification of the most common G and P serotypes for inclusion in vaccines is an important priority. And subsequent to the introduction of a vaccine candidate, not only monitoring of circulating strains is recommended, but also surveillance of potential reassortment of animal rotavirus genes from the vaccine into human rotavirus strains is critical. Conventional methods used in the characterisation of rotavirus strains, such as enzyme immunoassay serotyping and reverse-transcription PCR-based genotyping often fail to identify uncommon and newly appearing strains. The application of newer molecular approaches, including sequencing and oligonucleotide microarray hybridisation, may be required to characterise such strains. The present paper presents a brief overview of the variety of standard methods available, followed by suggestions for a systematic approach for routine rotavirus strain surveillance as well as for characterisation of incompletely typed rotavirus strains. Improved detection and characterisation of incompletely typed strains will help to develop a comprehensive strain surveillance that may be required for tailoring effective rotavirus vaccines.

Fitzgerald, J. R. and J. M. Musser (2001). "Evolutionary genomics of pathogenic bacteria." Trends Microbiol **9**(11): 547-53.

Complete genome sequences are now available for multiple strains of several bacterial pathogens and comparative analysis of these sequences is providing important insights into the evolution of bacterial virulence. Recently, DNA microarray analysis of many strains of several pathogenic species has contributed to our understanding of bacterial diversity, evolution and pathogenesis. Comparative genomics has shown that pathogens such as Escherichia coli, Helicobacter pylori and Staphylococcus aureus contain extensive variation in gene content whereas Mycobacterium tuberculosis nucleotide divergence is very limited. Overall, these approaches are proving to be a powerful means of exploring bacterial diversity, and are providing an important framework for the analysis of the evolution of pathogenesis and the development of novel antimicrobial agents.

Fitzgerald, J. R., D. E. Sturdevant, et al. (2001). "Evolutionary genomics of Staphylococcus aureus: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic." Proc Natl Acad Sci U S A **98**(15): 8821-6.

An emerging theme in medical microbiology is that extensive variation exists in gene content among strains of many pathogenic bacterial species. However, this topic has not been investigated on a genome scale with strains recovered from patients with well-defined clinical conditions. Staphylococcus aureus is a major human pathogen and also causes economically important infections in cows and sheep. A DNA microarray representing >90% of the S. aureus genome was used to characterize genomic diversity, evolutionary relationships, and virulence gene distribution among 36 strains of divergent clonal lineages, including methicillin-resistant strains and organisms causing toxic shock syndrome. Genetic variation in S. aureus is very extensive, with approximately 22% of the genome comprised of dispensable genetic material. Eighteen large regions of difference were identified, and 10 of these regions have genes that encode putative virulence factors or proteins mediating antibiotic resistance. We find that lateral gene transfer has played a fundamental role in the evolution of S. aureus. The mec gene has been horizontally

transferred into distinct S. aureus chromosomal backgrounds at least five times, demonstrating that methicillin-resistant strains have evolved multiple independent times, rather than from a single ancestral strain. This finding resolves a long-standing controversy in S. aureus research. The epidemic of toxic shock syndrome that occurred in the 1970s was caused by a change in the host environment, rather than rapid geographic dissemination of a new hypervirulent strain. DNA microarray analysis of large samples of clinically characterized strains provides broad insights into evolution, pathogenesis, and disease emergence.

Fohlman, J., J. Blomberg, et al. (2004). "[Microbial diagnosis with PCR will become clinically beneficial with a faster analysis]." Lakartidningen **101**(17): 1488-92.

PCR was introduced in 1985 by Mullis and was immediately recognized as a valuable tool in biomedical research and was awarded the Nobel Prize in 1993. Two culture-negative meningitis cases are described where Haemophilus influenzae and Neisseria meningitidis were found by 16SRNA-PCR. The modern real time PCR technology using fluorescent probes (hybridization probes, lightup probes, molecular beacons etc) for detection of the PCR-product or on DNA microarray chips, is under development for routine use. Multiplex technology can be used to simultaneously detect multiple microorganisms as well as resistance genes. Using super-convection with ultracentrifugation high-speed PCR, results can be obtained in 10 minutes and the amplificate can also be analyzed by DNA-sequencing to achieve species identification as well as detection of resistance gene mutations. The technique has mainly been applied to viruses, but is now slowly adapted to bacteria, fungi, protozoa and helminths. PCR is especially well suited for slow growing bacteria like Mycobacteria, fastidious organisms like Bartonella and contagious agents like tularemia, but also for malaria and fungi, where the advantages in sensitivity and speed can be exploited. The limit for application to routine analysis will depend on the development of simple and fast procedures for nucleic acid extraction, as well as interpretation of the PCR analysis per se, since highly efficient thermocyclers now are on the markets.

Fraunholz, M. J. and D. S. Roos (2003). "PlasmoDB: exploring genomics and post-genomics data of the malaria parasite, Plasmodium falciparum." Redox Rep **8**(5): 317-20.

The recent completion of the genome sequence of Plasmodium falciparum 3D7 provides the foundation for genome-wide analysis of the parasite. In addition to DNA and gene sequence data, postgenomic methods including microarray-based transcript profiling and high-throughput proteomics are now accessible to Plasmodium researchers. The Plasmodium Genome database (<http://PlasmoDB.org>) was developed to provide rapid and convenient access to the terabytes of genomic-scale data now being generated around the world. All data are available in a relational framework, permitting convenient downloading, browsing, and analysis. Combinatorial use of data analysis tools enables powerful data mining queries, such as combining gene and protein expression data to monitor changes through various life-cycle stages. Functional predictions can be used to explore potential targets for antimalarial drug development. This report outlines the use of PlasmoDB to examine redox-active functions in Plasmodium.

Fredericksen, B. L., M. Smith, et al. (2004). "The host response to West Nile Virus infection limits viral spread through the activation of the interferon regulatory factor 3 pathway." J Virol **78**(14): 7737-47.

Recent outbreaks of West Nile Virus (WNV) have been associated with an increase in morbidity and mortality in humans, birds, and many other species. We have initiated studies to define the molecular mechanisms by which a recent pathogenic isolate of WNV evades the host cell innate antiviral response. Biochemical and microarray analyses demonstrated that WNV induced the expression of beta interferon (IFN-beta) and several IFN-stimulated genes late in infection of cultured human cells. The late expression of these antiviral genes was due to the delayed activation of the transcription factor IFN regulatory factor 3 (IRF-3). Despite this host response, WNV was still able to replicate efficiently. The effect of the IRF-3 pathway on WNV replication was assessed by examining virus replication and spread in cultures of wild-

type or IRF-3-null mouse embryo fibroblasts. The absence of IRF-3 was marked by a significant increase in plaque size and a sustained production of infectious particles. Although the activation of the IRF-3 pathway was not sufficient to block virus replication, our results suggest that IRF-3 target genes function to constrain WNV infection and limit cell-to-cell virus spread.

Fukushima, M., K. Kakinuma, et al. (2003). "Detection and identification of Mycobacterium species isolates by DNA microarray." J Clin Microbiol **41**(6): 2605-15.

Rapid identification of Mycobacterium species isolates is necessary for the effective management of tuberculosis. Recently, analysis of DNA gyrase B subunit (gyrB) genes has been identified as a suitable means for the identification of bacterial species. We describe a microarray assay based on gyrB gene sequences that can be used for the identification of Mycobacteria species. Primers specific for a gyrB gene region common to all mycobacteria were synthesized and used for PCR amplification of DNA purified from clinical samples. A set of oligonucleotide probes for specific gyrB gene regions was developed for the identification of 14 Mycobacterium species. Each probe was spotted onto a silylated glass slide with an arrayer and used for hybridization with fluorescently labeled RNA derived from amplified sample DNA to yield a pattern of positive spots. This microarray produced unique hybridization patterns for each species of mycobacteria and could differentiate closely related bacterial species. Moreover, the results corresponded well with those obtained by the conventional culture method for the detection of mycobacteria. We conclude that a gyrB-based microarray can rapidly detect and identify closely related mycobacterial species and may be useful in the diagnosis and effective management of tuberculosis.

Galey, D., K. Becker, et al. (2003). "Differential transcriptional regulation by human immunodeficiency virus type 1 and gp120 in human astrocytes." J Neurovirol **9**(3): 358-71.

Astrocytes may be infected with the human immunodeficiency virus type 1 (HIV-1) or exposed to the HIV protein gp120, yet their role in the pathogenesis of HIV dementia is largely unknown. To characterize the effects of HIV on astrocytic transcription, microarray analysis and ribonuclease protection assays (RPA) were performed. Infection of astrocytes by HIV or treatment with gp120 had differential and profound effects on gene transcription. Of the 1153 oligonucleotides on the immune-based array, the expression of 108 genes (53 up; 55 down) and 82 genes (32 up; 50 down) were significantly modulated by gp120 and HIV infection respectively. Of the 1153 oligonucleotides on the neuro-based array, 58 genes (25 up; 33 down) and 47 genes (17 up; 30 down) were significantly modulated by gp120 and HIV infection respectively. Chemokine and cytokine induction occurred predominantly by HIV infection, whereas gp120 had no significant effect. These results were confirmed by RPA. The authors conclude that profound alterations of astrocytic function occur in response to HIV infection or interaction with viral proteins, suggesting that astrocytes may play an important role in the pathogenesis of HIV dementia.

Galindo, C. L., J. Sha, et al. (2003). "Identification of Aeromonas hydrophila cytotoxic enterotoxin-induced genes in macrophages using microarrays." J Biol Chem **278**(41): 40198-212.

A cytotoxic enterotoxin (Act) of Aeromonas hydrophila possesses several biological activities, and it induces an inflammatory response in the host. In this study, we used microarrays to gain a global and molecular view of the cellular transcriptional responses to Act and to identify important genes up-regulated by this toxin. Total RNA was isolated at 0, 2, and 12 h from Act-treated macrophages and applied to Affymetrix MGU74 arrays, and the data were processed using a multi-analysis approach to identify genes that might be critical in the inflammatory process evoked by Act. Seventy-six genes were significantly and consistently up-regulated. Many of these genes were immune-related, and several were transcription factors, adhesion molecules, and cytokines. Additionally, we identified several apoptosis-associated genes that were significantly up-regulated in Act-treated macrophages. Act-induced apoptosis of macrophages was confirmed by annexin V staining and DNA laddering. Quantitative reverse

transcriptase-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay were used to verify increased expression of some inflammatory and apoptosis-associated genes identified by the microarray analysis. To further confirm Act-induced increases in gene expression, real-time RT-PCR was also used for selected genes. Taken together, the array data provided for the first time a global view of Act-mediated signal transduction and clearly demonstrated an inflammatory response and apoptosis mediated by this toxin in host cells at the molecular level.

Garaizar, J., S. Porwollik, et al. (2002). "DNA microarray-based typing of an atypical monophasic Salmonella enterica serovar." J Clin Microbiol **40**(6): 2074-8.

A multidrug-resistant fljB-lacking Salmonella enterica serovar [4,5,12:i:-] emerged in Spain in 1997. We analyzed the genome from four strains of this serovar using a microarray containing almost all the predicted protein coding regions of serovar Typhimurium strain LT2, including the pSLT plasmid. Only a few differences from serovar Typhimurium LT2 were observed, suggesting the serovar to be Typhimurium as well. Six regions of interest were identified from the microarray data. Cluster I was a deletion of 13 genes, corresponding to part of the regulon responsible for the anaerobic assimilation of allantoin. Clusters II and IV were associated with the absence of the Fels-1 and Fels-2 prophage. Cluster III was a small group of Gifsy-1 prophage-related genes that appeared to be deleted or replaced. Cluster V was a deletion of 16 genes, including iroB and the operon fljAB, which is reflected in the serovar designation. Region VI was the gene STM2240, which appears to have an additional homologue in these strains. The regions spanning the deletions involving the allantoin operon and the fljAB operon were PCR amplified and sequenced. PCR across these regions may be an effective marker for this particular emergent serovar. While the microarray data for all isolates of the new serovar were essentially identical for all LT2 chromosomal genes, the isolates differed in their similarity to pSLT, consistent with the heterogeneity in plasmid content among isolates of the new serovar. Recent isolates have acquired a more-complete subset of homologues to this virulence plasmid. In general, microarrays can provide useful complementary data to other typing methods.

Garcia, J. F., F. I. Camacho, et al. (2003). "Hodgkin and Reed-Sternberg cells harbor alterations in the major tumor suppressor pathways and cell-cycle checkpoints: analyses using tissue microarrays." Blood **101**(2): 681-9.

Tumoral cells in Hodgkin lymphoma (HL) display an increased growth fraction and diminished apoptosis, implying a profound disturbance of the cell cycle and apoptosis regulation. However, limitations of molecular techniques have prevented the analysis of the tumor suppressor pathways and cell-cycle checkpoints. Tissue microarray (TMA) is a powerful tool for analyzing a large number of molecular variables in a large series of tumors, although the feasibility of this technique has not yet been demonstrated in heterogeneous tumors. The expression of 29 genes regulating the cell cycle and apoptosis were analyzed by immunohistochemistry and in situ hybridization in 288 HL biopsies using TMA. The sensitivity of the technique was validated by comparing the results with those obtained in standard tissue sections. The results revealed multiple alterations in different pathways and checkpoints, including G1/S and G2/M transition and apoptosis. Striking findings were the overexpression of cyclin E, CDK2, CDK6, STAT3, Hdm2, Bcl2, Bcl-X(L), survivin, and NF-kappaB proteins. A multiparametric analysis identified proteins associated with increased growth fraction (Hdm2, p53, p21, Rb, cyclins A, B1, D3, and E, CDK2, CDK6, SKP2, Bcl-X(L), survivin, STAT1, and STAT3), and proteins associated with apoptosis (NF-kappaB, STAT1, and RB). The analysis also demonstrated that Epstein-Barr virus (EBV)-positive cases displayed a characteristic profile, confirming the pathogenic role of EBV in HL. Survival probability depends on multiple biologic factors, including overexpression of Bcl2, p53, Bax, Bcl-X(L), MIB1, and apoptotic index. In conclusion, Hodgkin and Reed-Sternberg cells harbor concurrent and overlapping alterations in the major tumor suppressor pathways and cell-cycle checkpoints. This appears to determine the viability of the tumoral cells and the clinical outcome.

Ge, H. (2000). "UPA, a universal protein array system for quantitative detection of protein-protein, protein-DNA, protein-RNA and protein-ligand interactions." Nucleic Acids Res **28**(2): e3.

Protein-protein interactions have been widely used to study gene expression pathways and may be considered as a new approach to drug discovery. Here I report the development of a universal protein array (UPA) system that provides a sensitive, quantitative, multi-purpose, effective and easy technology to determine not only specific protein-protein interactions, but also specific interactions of proteins with DNA, RNA, ligands and other small chemicals. (i) Since purified proteins are used, the results can be easily interpreted. (ii) UPA can be used multiple times for different targets, making it economically affordable for most laboratories, hospitals and biotechnology companies. (iii) Unlike DNA chips or DNA microarrays, no additional instrumentation is required. (iv) Since the UPA uses active proteins (without denaturation and renaturation), it is more sensitive compared with most existing methods. (v) Because the UPA can analyze hundreds (even thousands on a protein microarray) of proteins in a single experiment, it is a very effective method to screen proteins as drug targets in cancer and other human diseases.

Ge, H., Y. Y. Chuang, et al. (2004). "Comparative genomics of Rickettsia prowazekii Madrid E and Breinl strains." J Bacteriol **186**(2): 556-65.

Rickettsia prowazekii, the causative agent of epidemic typhus, has been responsible for millions of human deaths. Madrid E is an attenuated strain of R. prowazekii, while Breinl is a virulent strain. The genomic DNA sequence of Madrid E has recently been published. To study the genomic variations between Madrid E (reference) and Breinl (test) DNAs, cohybridization experiments were performed on a DNA microarray containing all 834 protein-coding genes of Madrid E. Of the 834 genes assessed, 24 genes showed 1.5- to 2.0-fold increases in hybridization signals in Breinl DNA compared to Madrid E DNA, indicating the presence of genomic variations in approximately 3% of the total genes. Eighteen of these 24 genes are predicted to be involved in different functions. Southern blot analysis of five genes, virB4, ftsK, rfbE, lpxA, and rpoH, suggested the presence of an additional paralog(s) in Breinl, which might be related to the observed increase in hybridization signals. Studies by real-time reverse transcription-PCR revealed an increase in expression of the above-mentioned five genes and five other genes. In addition to the elevated hybridization signals of 24 genes observed in the Breinl strain, one gene (rp084) showed only 1/10 the hybridization signal of Madrid E. Further analysis of this gene by PCR and sequencing revealed a large deletion flanking the whole rp084 gene and part of the rp083 gene in the virulent Breinl strain. The results of this first rickettsial DNA microarray may provide some important information for the elucidation of pathogenic mechanisms of R. prowazekii.

George, M. D., S. Sankaran, et al. (2003). "High-throughput gene expression profiling indicates dysregulation of intestinal cell cycle mediators and growth factors during primary simian immunodeficiency virus infection." Virology **312**(1): 84-94.

During primary simian immunodeficiency virus (SIV) infection, CD4+ T cells are severely depleted in gut-associated lymphoid tissue (GALT), while CD8+ T-cell numbers dramatically increase. To gain an understanding of the molecular basis of this disruption in T-cell homeostasis, host gene expression was monitored in longitudinal jejunum tissue biopsies from SIV-infected rhesus macaques by DNA microarray analysis. Transcription of cyclin E1, CDC2, retinoblastoma, transforming growth factor (TGF), fibroblast growth factor (FGF), and interleukin-2 was repressed while cyclins B1 and D2 and transcription factor E2F were upregulated, indicating a complex dysregulation of growth and proliferation within the intestinal mucosa. Innate, cell-mediated, and humoral immune responses were markedly upregulated in animals that significantly reduced their viral loads and retained more intestinal CD4+ T cells. We conclude that the alterations in intestinal gene expression during primary SIV infection were characteristic of a broad-range immune response, and reflective of the efficacy of viral suppression.

Gerion, D., F. Chen, et al. (2003). "Room-temperature single-nucleotide polymorphism and multiallele DNA detection using fluorescent nanocrystals and microarrays." Anal Chem **75**(18): 4766-72.

We report two cDNA microarray-based applications of DNA-nanocrystal conjugates, single-nucleotide polymorphism (SNP) and multiallele detections, using a commercial scanner and two sets of nanocrystals with orthogonal emissions. We focus on SNP mutation detection in the human p53 tumor suppressor gene, which has been found to be mutated in more than 50% of the known human cancers. DNA-nanocrystal conjugates are able to detect both SNP and single-base deletion at room temperature within minutes, with true-to-false signal ratios above 10. We also demonstrate microarray-based multiallele detection, using hybridization of multicolor nanocrystals conjugated to two sequences specific for the hepatitis B and hepatitis C virus, two common viral pathogens that inflict more than 10% of the population in the developing countries worldwide. The simultaneous detection of multiple genetic markers with microarrays and DNA-nanocrystal conjugates has no precedent and suggests the possibility of detecting an even greater number of bacterial or viral pathogens simultaneously.

Gharizadeh, B., M. Kaller, et al. (2003). "Viral and microbial genotyping by a combination of multiplex competitive hybridization and specific extension followed by hybridization to generic tag arrays." Nucleic Acids Res **31**(22): e146.

Detection and identification of microbial pathogens are important for disease diagnosis, treatment and prophylaxis measurements. By introducing an innovative technique, we show a robust, reliable and accurate microarray-based method for identification of microbial pathogens. The technique utilizes a unique combination of multiplex competitive hybridization, which enhances hybridization accuracy of oligonucleotides to the specific target, and apyrase-mediated allele-specific extension, which improves specific extension. As a model system, different clinically relevant human papillomaviruses were selected for this study. The method generated accurate results and proves to be promising for specific and correct microbial and viral typing.

Girard, S., E. Vossman, et al. (2004). "Hepatitis C virus NS5A-regulated gene expression and signaling revealed via microarray and comparative promoter analyses." Hepatology **40**(3): 708-18.

Most individuals exposed to hepatitis C virus (HCV) become chronically infected and are predisposed to liver disease. The mechanisms underlying viral persistence and disease progression are unknown. A role for the HCV NS5A protein in viral replication and interferon resistance has been demonstrated. To identify mechanisms affected by NS5A, we analyzed the gene expression of Huh7 cells expressing NS5A and control cells using oligonucleotide microarrays. A set of 103 genes (43 up-regulated, 60 down-regulated) whose expression was modified by at least twofold was selected. These included genes involved in cell adhesion and motility, calcium homeostasis, lipid transport and metabolism, and genes regulating immune responses. The finding of modulated expression of genes related to the TGF-beta superfamily and liver fibrosis was observed. Interestingly, both the tumor necrosis factor and lymphotoxin beta receptors were down-regulated by NS5A. Similar data were obtained following expression of four NS5A mutants obtained from patients who were not responsive or were sensitive to interferon therapy. Through computational analysis, we determined that 39 of the 43 genes up-regulated by NS5A contained one or more nuclear factor kappaB (NF-kappaB) binding sites within their promoter region. Using the Gibbs sampling method, we also detected enrichment of NF-kappaB consensus binding sites in the upstream regions of the 43 coexpressed genes. Activation of NF-kappaB by NS5A was subsequently demonstrated in luciferase reporter assays. Adenovirus-mediated expression of IkappaBalpha reverted NS5A mediated up-regulation of gene expression. In conclusion, this study suggests a role of NS5A and NF-kappaB in HCV pathogenesis and related liver disease. Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).

Glaunsinger, B. and D. Ganem (2004). "Highly selective escape from KSHV-mediated host mRNA shutoff and its implications for viral pathogenesis." J Exp Med **200**(3): 391-8.

During Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) lytic infection, many virus-

encoded signaling molecules (e.g., viral G protein-coupled receptor [vGPCR]) are produced that can induce host gene expression in transiently transfected cells, and roles for such induced host genes have been posited in KS pathogenesis. However, we have recently found that host gene expression is strongly inhibited by 10-12 h after lytic reactivation of KSHV, raising the question of whether and to what extent de novo host gene expression induced by viral signaling molecules can proceed during the lytic cycle. Here, we show by microarray analysis that expression of most vGPCR target genes is drastically curtailed by this host shutoff. However, rare cellular genes can escape the host shutoff and are potently up-regulated during lytic KSHV growth. Prominent among these is human interleukin-6, whose striking induction may contribute to the overexpression of this cytokine in several disease states linked to KSHV infection.

Goguet de la Salmoniere, Y. O., C. C. Kim, et al. (2004). "High-throughput method for detecting genomic-deletion polymorphisms." J Clin Microbiol **42**(7): 2913-8.

DNA microarrays have been successfully used with different microorganisms, including Mycobacterium tuberculosis, to detect genomic deletions relative to a reference strain. However, the cost and complexity of the microarray system are obstacles to its widespread use in large-scale studies. In order to evaluate the extent and role of large sequence polymorphisms (LSPs) or insertion-deletion events in bacterial populations, we developed a technique, termed deligotyping, which hybridizes multiplex-PCR products to membrane-bound, highly specific oligonucleotide probes. The approach has the benefits of being low cost and capable of simultaneously interrogating more than 40 bacterial strains for the presence of 43 genomic regions. The deletions represented on the membrane were selected from previous comparative genomic studies and ongoing microarray experiments. Highly specific probes for these deletions were designed and attached to a membrane for hybridization with strain-derived targets. The targets were generated by multiplex PCR, allowing simultaneous amplifications of 43 different genomic loci in a single reaction. To validate our approach, 100 strains that had been analyzed with a high-density microarray were analyzed. The membrane accurately detected the deletions identified by the microarray approach, with a sensitivity of 99.9% and a specificity of 98.0%. The deligotyping technique allows the rapid and reliable screening of large numbers of M. tuberculosis isolates for LSPs. This technique can be used to provide insights into the epidemiology, genomic evolution, and population structure of M. tuberculosis and can be adapted for the study of other organisms.

Gonzalez, S. F., M. J. Krug, et al. (2004). "Simultaneous detection of marine fish pathogens by using multiplex PCR and a DNA microarray." J Clin Microbiol **42**(4): 1414-9.

We coupled multiplex PCR and a DNA microarray to construct an assay suitable for the simultaneous detection of five important marine fish pathogens (Vibrio vulnificus, Listonella anguillarum, Photobacterium damselae subsp. damselae, Aeromonas salmonicida subsp. salmonicida, and Vibrio parahaemolyticus). The array was composed of nine short oligonucleotide probes (25-mer) complementary to seven chromosomal loci (cyt, rpoN, gyrB, toxR, ureC, dly, and vapA) and two plasmid-borne loci (fatA and A.sal). Nine primer sets were designed to amplify short fragments of these loci (100 to 177 bp) in a multiplex PCR. PCR products were subsequently labeled by nick translation and hybridized to the microarray. All strains of the five target species (n = 1 to 21) hybridized to at least one species-specific probe. Assay sensitivities ranged from 100% for seven probes to 83 and 67% for the two remaining probes. Multiplex PCR did not produce any nonspecific amplification products when tested against 23 related species of bacteria (n = 40 strains; 100% specificity). Using purified genomic DNA, we were able to detect PCR products with < 20 fg of genomic DNA per reaction (equivalent to four or five cells), and the array was at least fourfold more sensitive than agarose gel electrophoresis for detecting PCR products. In addition, our method allowed the tentative identification of virulent strains of L. anguillarum serotype O1 based on the presence of the fatA gene (67% sensitivity and 100% specificity). This assay is a sensitive and specific tool for the simultaneous detection of multiple pathogenic bacteria that cause disease in fish and humans.

Grandvaux, N., M. J. Servant, et al. (2002). "Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes." J Virol **76**(11): 5532-9.

Ubiquitously expressed interferon regulatory factor 3 (IRF-3) is directly activated after virus infection and functions as a key activator of the immediate-early alpha/beta interferon (IFN) genes, as well as the RANTES chemokine gene. In the present study, a tetracycline-inducible expression system expressing a constitutively active form of IRF-3 (IRF-3 5D) was combined with DNA microarray analysis to identify target genes regulated by IRF-3. Changes in mRNA expression profiles of 8,556 genes were monitored after Tet-inducible expression of IRF-3 5D. Among the genes upregulated by IRF-3 were transcripts for several known IFN-stimulated genes (ISGs). Subsequent analysis revealed that IRF-3 directly induced the expression of ISG56 in an IFN-independent manner through the IFN-stimulated responsive elements (ISREs) of the ISG56 promoter. These results demonstrate that, in addition to its role in the formation of a functional immediate-early IFN-beta enhanceosome, IRF-3 is able to discriminate among ISRE-containing genes involved in the establishment of the antiviral state as a direct response to virus infection.

Grifantini, R., E. Bartolini, et al. (2002). "Gene expression profile in Neisseria meningitidis and Neisseria lactamica upon host-cell contact: from basic research to vaccine development." Ann N Y Acad Sci **975**: 202-16.

Differential gene regulation in the human pathogen Neisseria meningitidis group B (MenB) and in Neisseria lactamica, a human commensal species, was studied by whole genome microarray after bacterial interaction with epithelial cells. Host-cell contact induced changes in the expression of 347 and 285 genes in MenB and N. lactamica, respectively. Of these, only 167 were common to MenB and N. lactamica, suggesting that a different subset of genes is activated by pathogens and commensals. Change in gene expression was stable over time in N. lactamica, but short-lived in MenB. A large part (greater than 30%) of the regulated genes encoded proteins with unknown function. Among the known genes, those coding for pili, capsule, protein synthesis, nucleotide synthesis, cell wall metabolism, ATP synthesis, and protein folding were down-regulated in MenB. Transporters for iron, chloride and sulfate, some known virulence factors, GAPDH and the entire pathway of selenocysteine biosynthesis were upregulated. Gene expression profiling indicates that approximately 40% of the regulated genes encode putative surface-associated proteins, suggesting that upon cell contact Neisseria undergoes substantial surface remodeling. This was confirmed by FACS analysis of adhering bacteria using mouse sera against a subset of recombinant proteins. Finally, a few surface-located, adhesion-activated antigens were capable of inducing bactericidal antibodies, indicating that microarray technology can be exploited for the identification of new vaccine candidates.

Grifantini, R., E. Bartolini, et al. (2002). "Previously unrecognized vaccine candidates against group B meningococcus identified by DNA microarrays." Nat Biotechnol **20**(9): 914-21.

We have used DNA microarrays to follow Neisseria meningitidis serogroup B (MenB) gene regulation during interaction with human epithelial cells. Host-cell contact induced changes in the expression of 347 genes, more than 30% of which encode proteins with unknown function. The upregulated genes included transporters of iron, chloride, amino acids, and sulfate, many virulence factors, and the entire pathway of sulfur-containing amino acids. Approximately 40% of the 189 upregulated genes coded for peripherally located proteins, suggesting that cell contact promoted a substantial reorganization of the cell membrane. This was confirmed by fluorescence activated cell sorting (FACS) analysis on adhering bacteria using mouse sera against twelve adhesion-induced proteins. Of the 12 adhesion-induced surface antigens, 5 were able to induce bactericidal antibodies in mice, demonstrating that microarray technology is a valid approach for identifying new vaccine candidates and nicely complements other genome mining strategies used for vaccine discovery.

Grifantini, R., S. Sebastian, et al. (2003). "Identification of iron-activated and -repressed Fur-dependent

genes by transcriptome analysis of Neisseria meningitidis group B." Proc Natl Acad Sci U S A **100**(16): 9542-7.

Iron is limiting in the human host, and bacterial pathogens respond to this environment by activating genes required for bacterial virulence. Transcriptional regulation in response to iron in Gram-negative bacteria is largely mediated by the ferric uptake regulator protein Fur, which in the presence of iron binds to a specific sequence in the promoter regions of genes under its control and acts as a repressor. Here we describe DNA microarray, computational and in vitro studies to define the Fur regulon in the human pathogen Neisseria meningitidis group B (strain MC58). After iron addition to an iron-depleted bacterial culture, 153 genes were up-regulated and 80 were down-regulated. Only 50% of the iron-regulated genes were found to contain Fur-binding consensus sequences in their promoter regions. Forty-two promoter regions were amplified and 32 of these were shown to bind Fur by gel-shift analysis. Among these genes, many of which had never been described before to be Fur-regulated, 10 were up-regulated on iron addition, demonstrating that Fur can also act as a transcriptional activator. Sequence alignment of the Fur-binding regions revealed that the N. meningitidis Fur-box encompasses the highly conserved (NATWAT)3 motif. Cluster analysis was effective in predicting Fur-regulated genes even if computer prediction failed to identify Fur-box-like sequences in their promoter regions. Microarray-generated gene expression profiling appears to be a very effective approach to define new regulons and regulatory pathways in pathogenic bacteria.

Grimm, V., S. Ezaki, et al. (2004). "Use of DNA microarrays for rapid genotyping of TEM beta-lactamases that confer resistance." J Clin Microbiol **42**(8): 3766-74.

Standard clinical procedures for pathogen resistance identification are laborious and usually require 2 days of cultivation before the resistance can be determined unequivocally. In contrast, clinicians and patients face increasing threats from antibiotic-resistant pathogenic bacteria in terms of their frequencies and levels of resistance. A major class of microbial resistance stems from the occurrence of beta-lactamases, which, if mutated, can cause the severe extended-spectrum beta-lactamase (ESBL) or inhibitor-resistant TEM (IRT) phenotype, which cause resistance to extended-spectrum cephalosporins, monobactams, and beta-lactamase inhibitors. We describe an oligonucleotide microarray for identification of the single nucleotide polymorphisms (SNPs) of 96% of the TEM beta-lactamase variants described to date which are related to the ESBL and/or IRT phenotype. The target DNA, originating from Escherichia coli, Enterobacter cloacae, and Klebsiella pneumoniae cells isolated from clinical samples, was amplified and fluorescently labeled by PCR with consensus primers in the presence of cyanine 5-labeled nucleotides. The total assay, including PCR, hybridization, and image analysis, could be performed in 3.5 h. The microarray results were validated by standard clinical procedures. The microarray outperformed the standard procedures in terms of assay time and the depth of information provided. In conclusion, this array offers an attractive option for the identification and epidemiologic monitoring of TEM beta-lactamases in the routine clinical diagnostic laboratory.

Guadalupe, M., E. Reay, et al. (2003). "Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy." J Virol **77**(21): 11708-17.

Gut-associated lymphoid tissue (GALT) harbors the majority of T lymphocytes in the body and is an important target for human immunodeficiency virus type 1 (HIV-1). We analyzed longitudinal jejunal biopsy samples from HIV-1-infected patients, during both primary and chronic stages of HIV-1 infection, prior to and following the initiation of highly active antiretroviral therapy (HAART) to determine the onset of CD4(+) T-cell depletion and the effect of HAART on the restoration of CD4(+) T cells in GALT. Severe depletion of intestinal CD4(+) T cells occurred during primary HIV-1 infection. Our results showed that the restoration of intestinal CD4(+) T cells following HAART in chronically HIV-1-infected patients was substantially delayed and incomplete. In contrast, initiation of HAART during early stages of infection resulted in near-complete restoration of intestinal CD4(+) T cells, despite the delay in

comparison to peripheral blood CD4(+) T-cell recovery. DNA microarray analysis of gene expression profiles and flow-cytometric analysis of lymphocyte homing and cell proliferation markers demonstrated that cell trafficking to GALT and not local proliferation contributed to CD4(+) T-cell restoration. Evaluation of jejunal biopsy samples from long-term HIV-1-infected nonprogressors showed maintenance of normal CD4(+) T-cell levels in both GALT and peripheral blood. Our results demonstrate that near-complete restoration of mucosal immune system can be achieved by initiating HAART early in HIV-1 infection. Monitoring of the restoration and/or maintenance of CD4(+) T cells in GALT provides a more accurate assessment of the efficacy of antiviral host immune responses as well as HAART.

Guan, H., D. A. Smirnov, et al. (2003). "Identification of genes associated with adenovirus 12 tumorigenesis by microarray." Virology **309**(1): 114-24.

A total of 242 genes were shown to be differentially expressed between haplotypically matched tumorigenic adenovirus 12 (Ad12) and nontumorigenic Ad5-transformed cells using a microarray containing 8734 cDNAs. Eighty-seven of the differentially expressed genes have known roles that include signal transduction, cell growth and proliferation, transcription regulation, protease, and immune functions. The remaining differentially expressed genes are represented by EST cDNAs which have functions that are either completely unknown or proposed, based on sequence similarity to known genes. A subset of 22 differentially expressed genes from the microarray was further examined by Northern blot analyses to verify the identification of new genes associated with Ad12 tumorigenesis. Growth factor receptor binding protein 10 (Grb10) and protease nexin 1 (PN-1) were overexpressed in all of the tumorigenic Ad12-transformed cells examined, whereas expression of these genes was negligible in all of the nontumorigenic Ad5-transformed cells. By contrast, other genes including B cell translocation gene 2 (BTG2) were shown to be significantly up-regulated in Ad5-transformed cells as compared to Ad12-transformed cells.

Guerra, S., L. A. Lopez-Fernandez, et al. (2004). "Microarray analysis reveals characteristic changes of host cell gene expression in response to attenuated modified vaccinia virus Ankara infection of human HeLa cells." J Virol **78**(11): 5820-34.

The potential use of the modified vaccinia virus Ankara (MVA) strain as a live recombinant vector to deliver antigens and elicit protective immune responses against infectious diseases demands a comprehensive understanding of the effect of MVA infection on human host gene expression. We used microarrays containing more than 15,000 human cDNAs to identify gene expression changes in human HeLa cell cultures at 2, 6, and 16 h postinfection. Clustering of the 410 differentially regulated genes identified 11 discrete gene clusters with altered expression patterns after MVA infection. Clusters 1 and 2 (accounting for 16.59% [68 of 410] of the genes) contained 68 transcripts showing a robust induction pattern that was maintained during the course of infection. Changes in cellular gene transcription detected by microarrays after MVA infection were confirmed for selected genes by Northern blot analysis and by real-time reverse transcription-PCR. Upregulated transcripts in clusters 1 and 2 included 20 genes implicated in immune responses, including interleukin 1A (IL-1A), IL-6, IL-7, IL-8, and IL-15 genes. MVA infection also stimulated the expression of NF-kappaB and components of the NF-kappaB signal transduction pathway, including p50 and TRAF-interacting protein. A marked increase in the expression of histone family members was also induced during MVA infection. Expression of the Wiskott-Aldrich syndrome family members WAS, WASF1, and the small GTP-binding protein RAC-1, which are involved in actin cytoskeleton reorganization, was enhanced after MVA infection. This study demonstrates that MVA infection triggered the induction of groups of genes, some of which may be involved in host resistance and immune modulation during virus infection.

Hakenbeck, R., N. Balmelle, et al. (2001). "Mosaic genes and mosaic chromosomes: intra- and interspecies genomic variation of Streptococcus pneumoniae." Infect Immun **69**(4): 2477-86.

Streptococcus pneumoniae remains a major causative agent of serious human diseases. The

worldwide increase of antibiotic resistant strains revealed the importance of horizontal gene transfer in this pathogen, a scenario that results in the modulation of the species-specific gene pool. We investigated genomic variation in 20 S. pneumoniae isolates representing major antibiotic-resistant clones and 10 different capsular serotypes. Variation was scored as decreased hybridization signals visualized on a high-density oligonucleotide array representing 1,968 genes of the type 4 reference strain KNR.7/87. Up to 10% of the genes appeared altered between individual isolates and the reference strain; variability within clones was below 2.1%. Ten gene clusters covering 160 kb account for half of the variable genes. Most of them are associated with transposases and are assumed to be part of a flexible gene pool within the bacterial population; other variable loci include mosaic genes encoding antibiotic resistance determinants and gene clusters related to bacteriocin production. Genomic comparison between S. pneumoniae and commensal Streptococcus mitis and Streptococcus oralis strains indicates distinct antigenic profiles and suggests a smooth transition between these species, supporting the validity of the microarray system as an epidemiological and diagnostic tool.

Hampshire, T., S. Soneji, et al. (2004). "Stationary phase gene expression of Mycobacterium tuberculosis following a progressive nutrient depletion: a model for persistent organisms?" Tuberculosis (Edinb) **84**(3-4): 228-38.

The majority of individuals infected with TB develop a latent infection, in which organisms survive within the body while evading the host immune system. Such persistent bacilli are capable of surviving several months of combinatorial antibiotic treatment. Evidence suggests that stationary phase bacteria adapt to increase their tolerance to environmental stresses. We have developed a unique in vitro model of dormancy based on the characterization of a single, large volume fermenter culture of M. tuberculosis, as it adapts to stationary phase. Cells are maintained in controlled and defined aerobic conditions (50% dissolved oxygen tension), using probes that measure dissolved oxygen tension, temperature, and pH. Microarray analysis has been used in conjunction with viability and nutrient depletion assays to dissect differential gene expression. Following exponential phase growth the gradual depletion of glucose/glycerol resulted in a small population of survivors that were characterized for periods in excess of 100 days. Bacilli adapting to nutrient depletion displayed characteristics associated with persistence in vivo, including entry into a non-replicative state and the up-regulation of genes involved in beta-oxidation of fatty acids and virulence. A reduced population of non-replicating bacilli went on to adapt sufficiently to re-initiate cellular division.

He, J. and L. J. Chang (2004). "Functional characterization of hepatoma-specific stem cell antigen-2." Mol Carcinog **40**(2): 90-103.

Identification of tumor-specific antigens and genetic pathways may lead to potential diagnostic and therapeutic applications in cancer treatment. cDNA microarray has been used in cancer gene profiling, but the broad spectrum of data accruing and narrow signal-to-noise range of this technology have limited its use in rapid identification of highly differentially expressed tumor genes. Here, we used a modified suppression subtractive hybridization (SSH) method to isolate a small number of highly differentially expressed genes from murine hepatoma cells. For functional analysis of these hepatoma-specific genes, we employed the small interference RNA (siRNA)-mediated gene silencing method with lentiviral vectors, which have the advantages of high delivery efficiency and long lasting effect. Stem cell antigen-2 (Sca-2) was identified as one of the highest differentially expressed tumor antigens. Lentiviral siRNA successfully suppressed >90% of Sca-2 expression and the suppression lasted longer than 3 mo. Interestingly, inhibition of Sca-2 induced rapid hepatoma cell apoptosis, and the survival Sca-2-negative hepatoma cells exhibited high sensitivity to extrinsic tumor necrosis factor alpha (TNF-alpha) apoptosis signal but not intrinsic apoptosis signal. Analysis of TNF receptor 1 (TNFR1) by flow cytometry and Western blotting indicated that Sca-2 expression downregulated cell surface but not de novo synthesis of TNFR1 in the hepatoma cells. Together, our results suggested that Sca-2 was a signal transducer situated at the nexus of surface molecules regulating death receptor-mediated apoptosis. The technology

illustrated that this method can deduce a small number of highly differentially expressed tumor genes that may have diagnostic and therapeutic potential.

Hemsley, C., E. Joyce, et al. (2003). "MgrA, an orthologue of Mga, Acts as a transcriptional repressor of the genes within the rlrA pathogenicity islet in Streptococcus pneumoniae." J Bacteriol **185**(22): 6640-7.

Streptococcus pneumoniae normally resides in the human nasopharynx in a nondisease state. In response to unknown triggers this organism can descend to the lower respiratory tract and/or invade the bloodstream. Regulation and activation of virulence genes play essential roles in this process of disease development. Characterization of S. pneumoniae regulatory networks has been a recent area of interest, but despite inroads little is known about regulation of virulence genes in this pathogen. A putative transcriptional regulator in S. pneumoniae, mgrA, which exhibits homology to the virulence gene activator mga of group A streptococcus, was previously identified as a regulator that is required for development of pneumonia in a murine model. In this study we confirmed that mgrA plays a role in both nasopharyngeal carriage and pneumonia. Transcriptional profiling by microarray technology was used to show that mgrA acts as a repressor of the previously characterized rlrA pathogenicity islet. This is manifested phenotypically by a decrease in adherence to epithelial cells in tissue culture since the rlrA pathogenicity islet contains genes mediating adherence.

Hendricks, K. B., F. Shanahan, et al. (2004). "Role for BRG1 in cell cycle control and tumor suppression." Mol Cell Biol **24**(1): 362-76.

Human BRG1, a subunit of the Swi/Snf chromatin remodeling apparatus, has been implicated in regulation of cellular proliferation and is a candidate tumor suppressor. Reintroduction of BRG1 into a breast tumor cell line, ALAB, carrying a defined mutation in the BRG1 gene, induced growth arrest. Gene expression data revealed that the arrest may in part be accounted for by down-regulation of select E2F target genes such as cyclin E, but more dramatically, by up-regulation of mRNAs for the cyclin-dependent kinase inhibitors p21 and p15. Protein levels of both p15 and p21 were induced, and p21 protein was recruited to a complex with cyclin-dependent kinase, CDK2, to inhibit its activity. BRG1 can associate with the p21 promoter in a p53-independent manner, suggesting that the induction of p21 by BRG1 may be direct. Further, using microarray and real-time PCR analysis we identified several novel BRG1-regulated genes. Our work provides further evidence for a role for BRG1 in the regulation of several genes involved in key steps in tumorigenesis and has revealed a potential mechanism for BRG1-induced growth arrest.

Higaki, S., B. M. Gebhardt, et al. (2002). "Effect of immunosuppression on gene expression in the HSV-1 latently infected mouse trigeminal ganglion." Invest Ophthalmol Vis Sci **43**(6): 1862-9.

PURPOSE: To determine alterations in expression of genes in herpes simples virus (HSV)-1 latently infected mouse trigeminal ganglia (TGs), after treatment with cyclophosphamide and dexamethasone. METHODS: Scarified corneas of female BALB/c mice were inoculated with HSV-1 strain McKrae. Four weeks after inoculation, cyclophosphamide and dexamethasone were intravenously injected to induce HSV-1 reactivation. Uninfected mice were also treated with the immunosuppressants. Four groups of animals were studied: uninfected, not treated; uninfected, drug treated; latently infected, not treated; and latently infected, drug treated. PolyA+ mRNA from the TGs of each group was reverse transcribed, labeled with 32P, incubated on a 1185-gene array membrane, and analyzed by phosphorimaging. As a comparison and to confirm microarray results, semiquantitative RT-PCR was also performed for selected genes. RESULTS: The immunosuppressive drugs significantly increased expression of two genes (calpactin 1 light chain and guanine nucleotide-binding protein alpha-stimulating polypeptide [GNAS]) in the ganglia of uninfected mice compared with those in untreated uninfected mice. Ten genes were shown to be significantly increased in the latent TGs of mice treated with immunosuppressants compared with latently infected untreated mice. These genes were prostaglandin E2 receptor EP4 subtype (PTGER4), insulin promoter factor 1 (IPF1), glutathione S-transferase mu2, cyclin

D2, peripherin, plasma glutathione peroxidase, methyl CpG-binding protein 2, retinal S-antigen, ErbB2 proto-oncogene, and GNAS. Eight genes were shown to be significantly decreased in the HSV-1 latent TGs treated with the drugs, compared with untreated latently infected mice. These genes were peripheral myelin protein 22, decorin, transcription factor AP-1, dystroglycan 1, myelin protein zero, mitogen-activated protein kinase 3, prothymosin beta 4, and brain lipid-binding protein. The results obtained by semiquantitative RT-PCR were similar to those obtained by microarray analysis. CONCLUSIONS: Those genes with expression altered by immunosuppressive drug treatment may play an important role in ocular HSV-1 recurrence. Changes in expression of genes in the prostaglandin pathway, a transcription factor, and an enzyme in the cell cycle are considered especially important in HSV-1 reactivation by immunosuppression and are reviewed.

Hinchliffe, S. J., K. E. Isherwood, et al. (2003). "Application of DNA microarrays to study the evolutionary genomics of Yersinia pestis and Yersinia pseudotuberculosis." Genome Res **13**(9): 2018-29.

Yersinia pestis, the causative agent of plague, diverged from Yersinia pseudotuberculosis, an enteric pathogen, an estimated 1500-20,000 years ago. Genetic characterization of these closely related organisms represents a useful model to study the rapid emergence of bacterial pathogens that threaten mankind. To this end, we undertook genome-wide DNA microarray analysis of 22 strains of Y. pestis and 10 strains of Y. pseudotuberculosis of diverse origin. Eleven Y. pestis DNA loci were deemed absent or highly divergent in all strains of Y. pseudotuberculosis. Four were regions of phage origin, whereas the other seven included genes encoding a vitamin B12 receptor and the insect toxin sepC. Sixteen differences were identified between Y. pestis strains, with biovar Antiqua and Mediaevalis strains showing most divergence from the arrayed CO92 Orientalis strain. Fifty-eight Y. pestis regions were specific to a limited number of Y. pseudotuberculosis strains, including the high pathogenicity island, three putative autotransporters, and several possible insecticidal toxins and hemolysins. The O-antigen gene cluster and one of two possible flagellar operons had high levels of divergence between Y. pseudotuberculosis strains. This study reports chromosomal differences between species, biovars, serotypes, and strains of Y. pestis and Y. pseudotuberculosis that may relate to the evolution of these species in their respective niches.

Hinton, J. C., I. Hautefort, et al. (2004). "Benefits and pitfalls of using microarrays to monitor bacterial gene expression during infection." Curr Opin Microbiol **7**(3): 277-82.

The understanding of bacterial pathogenesis is dependent on techniques that elucidate the underlying genetic and biochemical mechanisms. To study the mechanism of bacterial survival and proliferation within host cells we need accurate tools that tell us what is occurring within the infecting organism. It has now become possible to determine the transcriptional status of in vivo-derived bacteria at the level of the whole genome. Such expression profiles serve as a monitor of the host cell environment as well as an indicator of the bacterial adaptation to its intracellular niche. Here, we review the methods used to produce microarray data for defining the bacterial intracellular transcriptome, and examine the pitfalls in extracting bacterial RNA from the infected host compartment.

Hirsh, A. E., A. G. Tsolaki, et al. (2004). "Stable association between strains of Mycobacterium tuberculosis and their human host populations." Proc Natl Acad Sci U S A **101**(14): 4871-6.

Mycobacterium tuberculosis is an important human pathogen in virtually every part of the world. Here we investigate whether distinct strains of M. tuberculosis infect different human populations and whether associations between host and pathogen populations are stable despite global traffic and the convergence of diverse strains of the pathogen in cosmopolitan urban centers. The recent global movement and transmission history of 100 M. tuberculosis isolates was inferred from a molecular epidemiologic study of tuberculosis that spans 12 years. Genetic relationships among these isolates were deduced from the distribution of large genomic deletions, which were identified by DNA microarray and confirmed by PCR and sequence analysis. Phylogenetic analysis of these deletions indicates that they are

unique event polymorphisms and that horizontal gene transfer is extremely rare in M. tuberculosis. In conjunction with the epidemiological data, phylogenies reveal three large phylogeographic regions. A host's region of origin is predictive of the strain of tuberculosis he or she carries, and this association remains strong even when transmission takes place in a cosmopolitan urban center outside of the region of origin. Approximate dating of the time since divergence of East Asian and Philippine clades of M. tuberculosis suggests that these lineages diverged centuries ago. Thus, associations between host and pathogen populations appear to be highly stable.

Hoffman, E. P. and D. Dressman (2001). "Molecular pathophysiology and targeted therapeutics for muscular dystrophy." Trends Pharmacol Sci **22**(9): 465-70.

Experimental therapeutics of the muscular dystrophies has made impressive advances on several fronts. Adeno-associated virus has emerged as the clear 'vector of choice' for muscle gene delivery, with successful functional rescue of dystrophic muscle in rodent models. Correction of the dystrophin gene mutation in a dog model has been reported, and several reports of progress on myogenic stem cell characterization are resurrecting cell transplantation as a possible therapeutic approach. The downstream consequences of dystrophin deficiency are being defined quickly using microarray experiments, and drugs targeting specific biochemical pathways are being tested rapidly in animal models. Such targeted drug discoveries, which are discussed in this article, have begun to be implemented in human clinical trials.

Hoffmann, K. F., T. C. McCarty, et al. (2001). "Disease fingerprinting with cDNA microarrays reveals distinct gene expression profiles in lethal type 1 and type 2 cytokine-mediated inflammatory reactions." Faseb J **15**(13): 2545-7.

Development of polarized immune responses controls resistance and susceptibility to many microorganisms. However, studies of several infectious, allergic, and autoimmune diseases have shown that chronic type-1 and type-2 cytokine responses can also cause significant morbidity and mortality if left unchecked. We used mouse cDNA microarrays to molecularly phenotype the gene expression patterns that characterize two disparate but equally lethal forms of liver pathology that develop in Schistosoma mansoni infected mice polarized for type-1 and type-2 cytokine responses. Hierarchical clustering analysis identified at least three groups of genes associated with a polarized type-2 response and two linked with an extreme type-1 cytokine phenotype. Predictions about liver fibrosis, apoptosis, and granulocyte recruitment and activation generated by the microarray studies were confirmed later by traditional biological assays. The data show that cDNA microarrays are useful not only for determining coordinated gene expression profiles but are also highly effective for molecularly "fingerprinting" diseased tissues. Moreover, they illustrate the potential of genome-wide approaches for generating comprehensive views on the molecular and biochemical mechanisms regulating infectious disease pathogenesis.

Honda, M., S. Kaneko, et al. (2001). "Differential gene expression between chronic hepatitis B and C hepatic lesion." Gastroenterology **120**(4): 955-66.

BACKGROUND & AIMS: Complementary DNA (cDNA) microarray technology allows simultaneous expression analysis of hundreds to thousands of genes. We applied the cDNA microarray technique to clarify gene expression profiles in chronic viral hepatitis tissue lesions. METHODS: We made cDNA microarrays consisting of 1080 human cDNAs and analyzed gene expression using labeled cDNAs prepared from 6 normal, 12 chronic hepatitis B, and 14 chronic hepatitis C liver tissues. Relative expression ratios of individual genes were obtained by comparing hybridization of Cy5-labeled cDNAs from chronic hepatitis lesions and Cy3-labeled cDNA from normal liver tissue. RESULTS: Hierarchical clustering analysis of the gene expression profiles in 26 patients showed that the patients were clustered into 2 groups with respect to similarities in differentially expressed genes. Hepatitis B and C virus infection, but not age, sex, or histology of hepatitis, were significant factors determining clustering (P <

0.05). In hepatitis B tissue lesions, genes involved in inflammation were predominant, whereas in hepatitis C, expression of anti-inflammatory response genes was relatively dominant. CONCLUSIONS: These findings shed new light on the possible differential molecular mechanisms in the pathogenesis of hepatitis caused by hepatitis B virus and hepatitis C virus infection, from which hepatocellular carcinoma frequently develops.

Hu, C. H., J. P. Xie, et al. (2004). "[Differential expression of apoptosis-related gene induced by clinical and laboratory Mycobacterium tuberculosis strain in macrophages U937 revealed by oligonucleotide microarray]." Yi Chuan Xue Bao **31**(3): 231-5.

Tuberculosis(TB) remains one of the major problems in global health. Macrophage (MPhi) apoptosis, induced by Mycobacterium tuberculosis (Mtb), is a cornerstone of effective innate microbial defense mechanism. Elucidation of the complex apoptosis-related gene expression may facilitate understanding the mechanism and regulation of macrophage apoptosis in response to Mtb, and contribute to developing novel measures to counter TB. DNA microarray containing 19,200 gene or gene fragments was used to compare the macrophage cell line U937 gene expression response to the clinical and laboratory Mtb infection. Northern blotting and RT-PCR were used to confirm the microarray results. Mtb H37Rv infection were found to downregulate the bcl-2, vitamin D receptor, interferon regulatory factor 3, cytochrome c oxidase, gene expression by 2-, 3-, 3-, 2.5-fold, respectively, while the clinical strain infection leads to upregulate the SOD2, SOD3, serine protease, toll-like receptor 2, signal transducer and activator (STAT1), hypoxia-inducible factor 22, 2.9-, 2.5-, 2.5-, 2.2-, 2.4-, 5.9-fold respectively. The findings suggest that the clinical strain infection tends to override the macrophage apoptosis by which the host attempt to limit the growth of the invader. The research on the complex factors network involved in the interaction will benefit the vaccine and novel drug target development.

Hu, H., R. Lan, et al. (2002). "Fluorescent amplified fragment length polymorphism analysis of Salmonella enterica serovar typhimurium reveals phage-type- specific markers and potential for microarray typing." J Clin Microbiol **40**(9): 3406-15.

Fluorescent amplified fragment length polymorphism (AFLP) was applied to 46 Salmonella enterica serovar Typhimurium isolates of Australian origin comprising nine phage types, by using the restriction enzymes MseI and EcoRI and all 16 possible MseI +1-EcoRI +1 primer pair combinations. AFLP in the present study showed a very good discrimination power with a Simpson index of diversity of 0.98, and 35 different AFLP patterns were observed in the 46 isolates. AFLP grouped most serovar Typhimurium isolates by phage type and enabled differentiation of phage types. Furthermore, 84 phage-type-specific polymorphic AFLP fragments, for which presence or absence correlated with phage type (including 25 with one exception to phage type specificity) were observed in the 46 strains studied. Eighteen phage-type-specific AFLP fragments were cloned and sequenced. Fifteen are of known genes or have a homologue in the databases. Three sequences are plasmid related, eight are phage related, and four relate to chromosomal genes. Twelve of the 18 fragments are polymorphic because the DNA is present or absent as indicated by Southern hybridization, and we see good potential to use sequences of these fragments as the basis for multiplex PCR and development of a microarray-based molecular phage-typing method for serovar Typhimurium.

Huang, H., W. L. Ma, et al. (2004). "[DNA microarray for the detection of Yersinia pesits]." Di Yi Jun Yi Da Xue Xue Bao **24**(1): 47-9.

OBJECTIVE: To develop a DNA microarray technique for fast diagnosis of plague. METHODS: Restriction display polymerase chain reaction (RD-PCR) and hybridization with fluorescently labeled cy5 were employed for detecting the sample DNA of Yersinia pestis, Yersinia pseudotuberculosis and Yersinia enterocolitica. RESULTS: The prepared microarray is capable of distinguishing Yersinia pestis from Yersinia pseudotuberculosis and Yersinia enterocolitica in the same genus. CONCLUSION: The constructed DNA microarray is an effective diagnostic tool for the detection of Yersinia pestis.

Huang, J., C. J. Lih, et al. (2001). "Global analysis of growth phase responsive gene expression and regulation of antibiotic biosynthetic pathways in Streptomyces coelicolor using DNA microarrays." Genes Dev **15**(23): 3183-92.

The eubacterial species Streptomyces coelicolor proceeds through a complex growth cycle in which morphological differentiation/development is associated with a transition from primary to secondary metabolism and the production of antibiotics. We used DNA microarrays and mutational analysis to investigate the expression of individual genes and multigene antibiotic biosynthetic pathways during these events. We identified expression patterns in biosynthetic, regulatory, and ribosomal protein genes that were associated highly specifically with particular stages of development. A knowledge-based algorithm that correlates temporal changes in expression with chromosomal position identified groups of contiguous genes expressed at discrete stages of morphological development, inferred the boundaries of known antibiotic synthesis gene loci, and revealed novel physical clusters of coordinately regulated genes. Microarray analysis of RNA from cells mutated in genes regulating synthesis of the antibiotics actinorhodin (Act) and undecylprodigiosin (Red) identified proximate and distant sites that contain putative ABC transporter and two-component system genes expressed coordinately with genes of specific biosynthetic pathways and indicated the existence of two functionally and physically discrete regulons in the Red pathway.

Hwang, T. S., J. K. Jeong, et al. (2003). "Detection and typing of HPV genotypes in various cervical lesions by HPV oligonucleotide microarray." Gynecol Oncol **90**(1): 51-6.

OBJECTIVES: This study was conducted to evaluate a clinical efficacy of human papillomavirus (HPV) oligonucleotide microarray (Biomedlab Co., Seoul, South Korea) for the detection of HPVs in various cervical lesions. RESULTS: HPV DNAs from 234 patients were analysed by two methods. Among those, 212 patients were classified into 5 groups according to the histologic diagnosis: chronic cervicitis, cervical intraepithelial neoplasia (CIN) I, CIN II, CIN III, and invasive cervical carcinoma. PCR-RFLP could detect 7 types of high-risk HPVs (HPV 16/18/31/33/35/52/58) and HPV microarray could detect 15 types of high-risk HPVs (HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68/69) and 7 types of low-risk HPVs (HPV 6/11/34/40/42/43/44).HPV genotyping by HPV oligonucleotide microarray revealed that HPV16 was the most frequent type (30.2%) in all specimens tested and was significantly more frequent in CIN III and invasive carcinomas than other lesions. METHODS: HPV DNAs were detected in 158 and 174 of the 234 cervical samples by PCR-RFLP and HPV microarray, respectively. The correlation between the two methods was good in detecting HPVs in general (kappa index = 0.69) and HPVs 31 and 52 (kappa index = 0.70 and 0.70, respectively) and excellent in detecting HPVs 16, 18, 33, 35, and 58 (kappa index = 0.90, 0.88, 0.92, 0.77, and 0.84, respectively). Double HPV infection was detected in 10 cases and one triple infection was detected. By combining cytology and HPV testing, the sensitivity was improved to 87.5, 95.5, 96.1, and 97.2% in CIN I, CIN II, CIN III, and carcinoma, respectively. CONCLUSIONS: This results suggest that HPV oligonucleotide microarray is a highly comparable method to the previously used PCR-RFLP method for the detection of HPVs in cervical specimens. Genetic informations for HPV infection in cervical specimens may offer new strategies in manipulating the patients harboring cervical intraepithelial neoplasia and cervical carcinoma.

Clinical Applications

I-P

Ichikawa, J. K., A. Norris, et al. (2000). "Interaction of pseudomonas aeruginosa with epithelial cells: identification of differentially regulated genes by expression microarray analysis of human cDNAs." Proc Natl Acad Sci U S A **97**(17): 9659-64.

Pseudomonas aeruginosa is an opportunistic pathogen that plays a major role in lung function deterioration in cystic fibrosis patients. To identify critical host responses during infection, we have used

high-density DNA microarrays, consisting of 1,506 human cDNA clones, to monitor gene expression in the A549 lung pneumocyte cell line during exposure to P. aeruginosa. We have identified host genes that are differentially expressed upon infection, several of which require interaction with P. aeruginosa and the expression of the major subunit of type IV pili, PilA. Differential expression of genes involved in various cellular functions was identified, and we selected the gene encoding the transcription factor interferon regulatory factor 1 (IRF-1) for further analysis. The levels of the IRF-1 transcript increased 3- to 4-fold in A549 cells after adherence by P. aeruginosa. A similar increase of IRF-1 mRNA was observed in A549 cells exposed to wild-type P. aeruginosa when compared to an isogenic, nonpiliated strain. However, this difference was abolished when serum was present during the incubation of bacteria. Exposure of A549 cells to purified P. aeruginosa lipopolysaccharide did not result in a significant increase in IRF-1 mRNA. Although the P. aeruginosa-induced increased IRF-1 expression depends on the presence of bacterial adhesin, our findings do not preclude the possibility that other bacterial products are responsible for IRF-1 activation, which is enhanced by bacterial adherence to cells. These data show that microarray technology can be an important tool for studying the complex interplay between bacterial pathogens and host.

Iizuka, N., M. Oka, et al. (2002). "Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method." Cancer Res **62**(14): 3939-44.

Gene expression profiles of hepatocellular carcinomas (HCCs) associated with hepatitis B virus (HBV) and hepatitis C virus (HCV) were analyzed and compared. Oligonucleotide microarrays containing >6000 genes and subsequent gene selection by a supervised learning method yielded 83 genes for which expression differed between the two types of HCCs. Expression levels of 31 of these 83 genes were increased in HBV-associated HCCs, and expression levels of the remaining 52 genes were increased in HCV-associated HCCs. The 31 genes up-regulated in HBV-associated HCC included imprinted genes (H19 and IGF2) and genes relating to signal transduction, transcription, and metastasis. The 52 genes up-regulated in HCV-associated HCC included a number of genes responsible for detoxification and immune response. These results suggest that HBV and HCV cause hepatocarcinogenesis by different mechanisms and provide novel tools for diagnosis and treatment of HBV- and HCV-associated HCCs.

Israel, D. A., N. Salama, et al. (2001). "Helicobacter pylori strain-specific differences in genetic content, identified by microarray, influence host inflammatory responses." J Clin Invest **107**(5): 611-20.

Helicobacter pylori enhances the risk for ulcer disease and gastric cancer, yet only a minority of H. pylori-colonized individuals develop disease. We examined the ability of two H. pylori isolates to induce differential host responses in vivo or in vitro, and then used an H. pylori whole genome microarray to identify bacterial determinants related to pathogenesis. Gastric ulcer strain B128 induced more severe gastritis, proliferation, and apoptosis in gerbil mucosa than did duodenal ulcer strain G1.1, and gastric ulceration and atrophy occurred only in B128+ gerbils. In vitro, gerbil-passaged B128 derivatives significantly increased IL-8 secretion and apoptosis compared with G1.1 strains. DNA hybridization to the microarray identified several strain-specific differences in gene composition including a large deletion of the cag pathogenicity island in strain G1.1. Partial and complete disruption of the cag island in strain B128 attenuated induction of IL-8 in vitro and significantly decreased gastric inflammation in vivo. These results indicate that the ability of H. pylori to regulate epithelial cell responses related to inflammation depends on the presence of an intact cag pathogenicity island. Use of an H pylori whole genome microarray is an effective method to identify differences in gene content between H. pylori strains that induce distinct pathological outcomes in a rodent model of H. pylori infection.

Ivanov, I., C. Schaab, et al. (2000). "DNA microarray technology and antimicrobial drug discovery." Pharmacogenomics **1**(2): 169-78.

The genomics era is providing us with vast amounts of information derived from whole-genome

sequencing. This will doubtlessly revolutionise biology and the way novel medicines will be discovered. To leverage this information efficiently, however, technologies in addition to high-throughput sequencing are required. DNA microarray technology is one technology that has already shown great potential for both basic research and drug discovery. With particular emphasis on antibacterial research we will summarise in this review the key technological aspects and most important applications of DNA microarrays demonstrated so far.

Izmailova, E., F. M. Bertley, et al. (2003). "HIV-1 Tat reprograms immature dendritic cells to express chemoattractants for activated T cells and macrophages." Nat Med **9**(2): 191-7.

Immature dendritic cells are among the first cells infected by retroviruses after mucosal exposure. We explored the effects of human immunodeficiency virus-1 (HIV-1) and its Tat transactivator on these primary antigen-presenting cells using DNA microarray analysis and functional assays. We found that HIV-1 infection or Tat expression induces interferon (IFN)-responsive gene expression in immature human dendritic cells without inducing maturation. Among the induced gene products are chemokines that recruit activated T cells and macrophages, the ultimate target cells for the virus. Dendritic cells in the lymph nodes of macaques infected with simian immunodeficiency virus (SIV) have elevated levels of monocyte chemoattractant protein 2 (MCP-2), demonstrating that chemokine induction also occurs during retroviral infection in vivo. These results show that HIV-1 Tat reprograms host dendritic cell gene expression to facilitate expansion of HIV-1 infection.

Jacobs, S., E. I. Grussendorf-Conen, et al. (2004). "Molecular analysis of the effect of topical imiquimod treatment of HPV 2/27/57-induced common warts." Skin Pharmacol Physiol **17**(5): 258-66.

Imiquimod is effective in the treatment of genital warts and clinical studies suggest activity against common warts as well. We have analyzed the effect of topical imiquimod on gene expression and virus load in human papilloma virus (HPV) 2/27/57-induced common warts. mRNA was extracted from keratinocyte culture, from normal skin, from three untreated common warts and from three common warts treated topically with 5% imiquimod cream twice daily. Differential gene expression was demonstrated by RT-PCR and by cDNA microarray hybridization. We further analyzed viral DNA content in scales from three superficially pared imiquimod-treated warts by real-time PCR. Comparison of normal skin with wart tissue revealed that HPV 2/27/57 infection led to an induction of IL-6, IL-10 and interferon-gamma inducible protein (IP10) and to an up-regulation of TGF-beta. We could further detect expression of PCTAIRE-3, WNT2B, frizzled-3, notch-2, notch-4 and BRCA2 in normal skin and common warts. Analysis of imiquimod-treated warts demonstrated that imiquimod enhanced IL-6 expression and induced IL-8, GM-CSF, MRP-8 and MRP-14. It could also be shown that imiquimod led to an infiltration of wart tissue with macrophages and to a strong decrease of viral copy number in warts within 3 months of treatment. Our data thus provide molecular proof of principle for imiquimod treatment of cutaneous common warts.

Janket, M. L., P. Manickam, et al. (2004). "Differential regulation of host cellular genes by HIV-1 viral protein R (Vpr): cDNA microarray analysis using isogenic virus." Biochem Biophys Res Commun **314**(4): 1126-32.

HIV-1 Vpr is a protein with multiple functions. It has been suggested that such pleiotropic effects by a viral protein may be mediated by its association with viral and cellular proteins or through modulation of expression of specific cellular genes. To address this, we used cDNA microarray techniques to analyze the regulation of a panel of host cellular genes by HIV-1 Vpr using isogenic HIV-1 either with or without Vpr expression. Results indicate that Vpr downregulated the expression of genes involved in cell cycle/proliferation regulation, DNA repair, tumor antigens, and immune activation factors, and upregulated many ribosomal and structural proteins. These results for the first time reveal the involvement of several potential cellular genes, which may be useful, both for understanding Vpr functions and for the development of therapeutics targeting the Vpr molecule.

Ji, X., R. Cheung, et al. (2003). "Interferon alfa regulated gene expression in patients initiating interferon treatment for chronic hepatitis C." Hepatology **37**(3): 610-21.

Interferon alfa (IFN-alpha) is an approved therapeutic agent for chronic hepatitis C. To directly characterize the effects of IFN-alpha in humans, we used microarrays to profile gene expression in peripheral blood mononuclear cells (PBMCs) from hepatitis C patients treated with IFN-alpha. Seven patients were studied using two strategies: (1) in vivo: PBMCs were collected immediately before the first dose of IFN-alpha, and 3 and 6 hours after the dose; (2) ex vivo: PBMCs that were collected before the first IFN-alpha dose were incubated with IFN-alpha for 3 and 6 hours. The microarray datasets were analyzed with significance analysis of microarrays (SAM) to identify genes regulated by IFN-alpha. We identified 516 named genes up-regulated at least 2-fold, at a false discovery rate (FDR) of less than 1%. In vivo and ex vivo studies generated similar results. No genes were identified as regulated differently between these 2 experimental conditions. The up-regulated genes belonged to a broad range of functional pathways and included multiple genes thought to be involved in the direct antiviral effect of IFN-alpha. Of particular interest, 88 genes directly relating to functions of immune cells were up-regulated, including genes involved in antigen processing and presentation, T-cell activation, lymphocyte trafficking, and effector functions, suggesting that IFN-alpha up-regulates multiple genes involving different aspects of immune responses to enhance immunity against hepatitis C virus. In conclusion, IFN-alpha-inducible genes can be identified in human PBMCs in vivo as well as ex vivo. Signature changes associated with different treatment outcomes may be found among these genes.

Jo, J. T., F. S. Brinkman, et al. (2003). "Aminoglycoside efflux in Pseudomonas aeruginosa: involvement of novel outer membrane proteins." Antimicrob Agents Chemother **47**(3): 1101-11.

The expression of tripartite multidrug efflux pumps such as MexA-MexB-OprM in Pseudomonas aeruginosa contributes to intrinsic resistance to a wide variety of antimicrobials, including beta-lactams, chloramphenicol, macrolides, quinolones, and tetracycline. The MexX-MexY linker-pump combination has been shown to be involved in intrinsic resistance to aminoglycosides, but the identity of the cognate outer membrane channel component remains under debate. Fourteen uncharacterized OprM homologs identified in the genome of P. aeruginosa were examined as candidates for this role by assessing the minimum inhibitory concentrations (MICs) of aminoglycosides in P. aeruginosa strain PAK knockout mutants lacking the corresponding genes. Insertional inactivation of OpmG, OpmI, and OpmH resulted in decreases of various degrees in the MICs of streptomycin, kanamycin, and gentamicin. When reintroduced into P. aeruginosa on multicopy plasmids, OpmG was able to complement the susceptibility of an opmG::miniTn5 mutant; however, cloned opmH, the proposed ortholog of Escherichia coli tolC according to our phylogenetic analysis, was able to only partially complement the opmH::miniTn5 mutant. Mini-microarray hybridization analysis demonstrated that opmG disruption does not affect expression of OpmI or OpmH (ruling out such indirect effects on aminoglycoside resistance); however, opmH disruption did have possible effects on expression of OpmG and OpmI. Based on the data, we propose that OpmG is a major outer membrane efflux channel involved in aminoglycoside efflux in P. aeruginosa PAK and that OpmI, its most related paralog, may share an overlapping function.

Jones, J. O. and A. M. Arvin (2003). "Microarray analysis of host cell gene transcription in response to varicella-zoster virus infection of human T cells and fibroblasts in vitro and SCIDhu skin xenografts in vivo." J Virol **77**(2): 1268-80.

During primary infection, varicella-zoster virus (VZV) is spread via lymphocytes to skin, where it induces a rash and establishes latency in sensory ganglia. A live, attenuated varicella vaccine (vOka) was generated by using the VZV Oka strain (pOka), but the molecular basis for vOka attenuation remains unknown. Little is known concerning the effects of wild-type or attenuated VZV on cellular gene regulation in the host cells that are critical for pathogenesis. In this study, transcriptional profiles of primary human T cells and fibroblasts infected with VZV in cell culture were determined by using 40,000-spot human cDNA microarrays. Cellular gene transcription in human skin xenografts in SCID

mice that were infected with VZV in vivo was also evaluated. The profiles of cellular gene transcripts that were induced or inhibited in infected human foreskin fibroblasts (HFFs), T cells, and skin in response to pOka and vOka infection were similar. However, significant alterations in cellular gene regulation were observed among the three differentiated human cell types that were examined, suggesting specific differences in the biological consequences of VZV infection related to the target cell. Changes in cellular gene transcription detected by microarray analysis were confirmed for selected genes by quantitative real-time reverse transcription-PCR analysis of VZV-infected cells. Interestingly, the transcription of caspase 8 was found to be decreased in infected T cells but not in HFFs or skin, which may signify a tissue-specific antiapoptosis mechanism. The use of microarrays to demonstrate differences in effects on host cell genes in primary, biologically relevant cell types provides background information for experiments to link these various response phenotypes with mechanisms of VZV pathogenesis that are important for the natural course of human infection.

Joyce, E. A., A. Kawale, et al. (2004). "LuxS is required for persistent pneumococcal carriage and expression of virulence and biosynthesis genes." Infect Immun **72**(5): 2964-75.

Streptococcus pneumoniae causes several diseases, including otitis media, pneumonia, and meningitis. Although little is known about the regulation of or how individual pneumococcal factors contribute to these disease states, there is evidence suggesting that some factors are regulated by a cell-density-dependent mechanism (quorum sensing). Quorum sensing allows bacteria to couple transcription with changes in cell density; bacteria achieve this by sensing and responding to small diffusible signaling molecules. We investigated how the LuxS signaling system impacts the biology of S. pneumoniae. An analysis of the transcriptional profiles of a serotype 2 strain and an isogenic luxS deletion strain utilizing an S. pneumoniae-specific microarray indicated that LuxS regulates gene expression involved in discrete cellular processes, including pneumolysin expression. Contrary to the paradigm for quorum sensing, we observed pronounced effects on transcription in early log phase, where gene expression was repressed in the mutant. Assessing the mutant for its ability to infect and cause disease in animals revealed a profound defect in ability to persist in the nasopharyngeal tissues. Our analysis of an S. pneumoniae transcriptome revealed a function for LuxS in gene regulation that is not dependent upon high cell density and is likely involved in the maintenance of pneumococcal load in susceptible hosts.

Kakinuma, K., M. Fukushima, et al. (2003). "Detection and identification of Escherichia coli, Shigella, and Salmonella by microarrays using the gyrB gene." Biotechnol Bioeng **83**(6): 721-8.

Commonly, 16S ribosome RNA (16S rRNA) sequence analysis has been used for identifying enteric bacteria. However, it may not always be applicable for distinguishing closely related bacteria. Therefore, we selected gyrB genes that encode the subunit B protein of DNA gyrase (a topoisomerase type II protein) as target genes. The molecular evolution rate of gyrB genes is higher than that of 16S rRNA, and gyrB genes are distributed universally among bacterial species. Microarray technology includes the methods of arraying cDNA or oligonucleotides on substrates such as glass slides while acquiring a lot of information simultaneously. Thus, it is possible to identify the enteric bacteria easily using microarray technology. We devised a simple method of rapidly identifying bacterial species through the combined use of gyrB genes and microarrays. Closely related bacteria were not identified at the species level using 16S rRNA sequence analysis, whereas they were identified at the species level based on the reaction patterns of oligonucleotides on our microarrays using gyrB genes.

Kapp, U., W. C. Yeh, et al. (1999). "Interleukin 13 is secreted by and stimulates the growth of Hodgkin and Reed-Sternberg cells." J Exp Med **189**(12): 1939-46.

Gene expression patterns can provide vital clues to the pathogenesis of neoplastic diseases. We investigated the expression of 950 genes in Hodgkin's disease (HD) by analyzing differential mRNA expression using microarrays. In two independent microarray experiments, the HD-derived cell lines L428 and KMH2 were compared with an Epstein-Barr virus (EBV)-immortalized lymphoblastoid B cell

line, LCL-GK. Interleukin (IL)-13 and IL-5 were found to be highly expressed in the HD-derived cell lines. Examination of IL-13 and IL-5 expression by Northern blot analysis and enzyme-linked immunosorbent assay confirmed these results and revealed the expression of IL-13 in a third HD-derived cell line, HDLM2. Control LCL and EBV-negative non-Hodgkin lymphoma-derived cell lines did not express IL-13. In situ hybridization of lymph node tissue from HD patients showed that elevated levels of IL-13 were specifically expressed by Hodgkin/Reed-Sternberg (H/RS) tumor cells. Treatment of a HD-derived cell line with a neutralizing antibody to IL-13 resulted in a dose-dependent inhibition of H/RS cell proliferation. These data suggest that H/RS cells produce IL-13 and that IL-13 plays an important role in the stimulation of H/RS cell growth, possibly by an autocrine mechanism. Modulation of the IL-13 signaling pathway may be a logical objective for future therapeutic strategies.

Kash, J. C., C. F. Basler, et al. (2004). "Global host immune response: pathogenesis and transcriptional profiling of type A influenza viruses expressing the hemagglutinin and neuraminidase genes from the 1918 pandemic virus." J Virol **78**(17): 9499-511.

To understand more fully the molecular events associated with highly virulent or attenuated influenza virus infections, we have studied the effects of expression of the 1918 hemagglutinin (HA) and neuraminidase (NA) genes during viral infection in mice under biosafety level 3 (agricultural) conditions. Using histopathology and cDNA microarrays, we examined the consequences of expression of the HA and NA genes of the 1918 pandemic virus in a recombinant influenza A/WSN/33 virus compared to parental A/WSN/33 virus and to an attenuated virus expressing the HA and NA genes from A/New Caledonia/20/99. The 1918 HA/NA:WSN and WSN recombinant viruses were highly lethal for mice and displayed severe lung pathology in comparison to the nonlethal New Caledonia HA/NA:WSN recombinant virus. Expression microarray analysis performed on lung tissues isolated from the infected animals showed activation of many genes involved in the inflammatory response, including cytokine, apoptosis, and lymphocyte genes that were common to all three infection groups. However, consistent with the histopathology studies, the WSN and 1918 HA/NA:WSN recombinant viruses showed increased up-regulation of genes associated with activated T cells and macrophages, as well as genes involved in apoptosis, tissue injury, and oxidative damage that were not observed in the New Caledonia HA/NA:WSN recombinant virus-infected mice. These studies document clear differences in gene expression profiles that were correlated with pulmonary disease pathology induced by virulent and attenuated influenza virus infections.

Kato, T., S. Satoh, et al. (2001). "Isolation of a novel human gene, MARKL1, homologous to MARK3 and its involvement in hepatocellular carcinogenesis." Neoplasia **3**(1): 4-9.

Activation of the Wnt-signaling pathway is known to play a crucial role in carcinogenesis of various human organs including the colon, liver, prostate, and endometrium. To investigate the mechanisms underlying hepatocellular carcinogenesis, we attempted to identify genes regulated by beta-catenin/Tcf complex in a human hepatoma cell line, HepG2, in which an activated form of beta-catenin is expressed. By means of cDNA microarray, we isolated a novel human gene, termed MARKL1 (MAP/microtubule affinity-regulating kinase-like 1), whose expression was downregulated in response to decreased Tcf/LEF1 activity. The transcript expressed in liver consisted of 3529 nucleotides that contained an open reading frame of 2256 nucleotides, encoding 752 amino acids homologous to human MARK3 (MAP/microtubule affinity-regulating kinase 3). Expression levels of MARKL1 were markedly elevated in eight of nine HCCs in which nuclear accumulation of beta-catenin were observed, which may suggest that MARKL1 plays some role in hepatocellular carcinogenesis.

Kato-Maeda, M., Q. Gao, et al. (2001). "Microarray analysis of pathogens and their interaction with hosts." Cell Microbiol **3**(11): 713-9.

Microarrays are a promising technique for elucidating and interpreting the mechanistic roles of genes in the pathogenesis of infectious disease. Microarrays have been used to analyse the genetic

polymorphisms of specific loci associated with resistance to antimicrobial agents, to explore the distribution of genes among isolates from the same and similar species, to understand the evolutionary relationship between closely related species and to integrate the clinical and genomic data. This technique has also been used to study host-pathogen interactions, mainly by identifying genes from pathogens that may be involved in pathogenicity and by surveying the scope of the host response to infection. The RNA expression profile of pathogens has been used to identify regulatory mechanisms that ensure gene expression in the appropriate environment, to hypothesize functions of hundreds of uncharacterized genes and to identify virulence genes that promote colonization or tissue damage. This information also has the potential to identify targets for drug design. Furthermore, microarrays have been used to investigate the mechanism of drug action and to delineate and predict adverse effects of new drugs. In this paper, we review the use of spotted and high-density oligonucleotide arrays to study the genetic polymorphisms of pathogens, host-pathogen interactions and whole-genome expression profiles of pathogens, as well as their use for drug discovery.

Kato-Maeda, M., J. T. Rhee, et al. (2001). "Comparing genomes within the species Mycobacterium tuberculosis." Genome Res **11**(4): 547-54.

The study of genetic variability within natural populations of pathogens may provide insight into their evolution and pathogenesis. We used a Mycobacterium tuberculosis high-density oligonucleotide microarray to detect small-scale genomic deletions among 19 clinically and epidemiologically well-characterized isolates of M. tuberculosis. The pattern of deletions detected was identical within mycobacterial clones but differed between different clones, suggesting that this is a suitable genotyping system for epidemiologic studies. An analysis of genomic deletions among an extant population of pathogenic bacteria provided a novel perspective on genomic organization and evolution. Deletions are likely to contain ancestral genes whose functions are no longer essential for the organism's survival, whereas genes that are never deleted constitute the minimal mycobacterial genome. As the amount of genomic deletion increased, the likelihood that the bacteria will cause pulmonary cavitation decreased, suggesting that the accumulation of mutations tends to diminish their pathogenicity. Array-based comparative genomics is a promising approach to exploring molecular epidemiology, microbial evolution, and pathogenesis.

Kaushal, D., B. G. Schroeder, et al. (2002). "Reduced immunopathology and mortality despite tissue persistence in a Mycobacterium tuberculosis mutant lacking alternative sigma factor, SigH." Proc Natl Acad Sci U S A **99**(12): 8330-5.

The pathogenesis of tuberculosis involves multiple phases and is believed to involve both a carefully deployed series of adaptive bacterial virulence factors and inappropriate host immune responses that lead to tissue damage. A defined Mycobacterium tuberculosis mutant strain lacking the sigH-encoded transcription factor showed a distinctive infection phenotype. In resistant C57BL/6 mice, the mutant achieved high bacterial counts in lung and spleen that persisted in tissues in a pattern identical to those of wild-type bacteria. Despite a high bacterial burden, the mutant produced a blunted, delayed pulmonary inflammatory response, and recruited fewer CD4(+) and CD8(+) T cells to the lung in the early stages of infection. In susceptible C3H mice, the mutant again showed diminished immunopathology and was nonlethal at over 170 days after intravenous infection, in contrast to isogenic wild-type bacilli, which killed with a median time to death of 52 days. Complete genomic microarray analysis revealed that M. tuberculosis sigH may mediate the transcription of at least 31 genes directly and that it modulates the expression of about 150 others; the SigH regulon governs thioredoxin recycling and may be involved in the maintenance of intrabacterial reducing capacity. These data show that the M. tuberculosis sigH gene is dispensable for bacterial growth and survival within the host, but is required for the production of immunopathology and lethality. This phenotype demonstrates that beyond an ability to grow and persist within the host, M. tuberculosis has distinct virulence mechanisms that elicit deleterious host responses and progressive pulmonary disease.

Kawaguchi, K., S. Kaneko, et al. (2003). "Detection of hepatitis B virus DNA in sera from patients with chronic hepatitis B virus infection by DNA microarray method." J Clin Microbiol **41**(4): 1701-4.

We have developed a sensitive and quantitative assay using a DNA microarray for the detection of hepatitis B virus (HBV) DNA in serum. Fluorescently labeled target cDNA prepared from cloned HBV DNA or serum HBV DNA was hybridized to capture DNA on a slide. A linear relationship was obtained between the intensities of the array spot and the amount of the cloned or serum HBV DNA, indicating the quantitative accuracy of this assay system. In addition, there was a significant correlation between the number of molecules of serum HBV DNA determined by the DNA microarray and that determined by a branched-DNA assay (n = 21, r = 0.89). Given these results, we conclude that the DNA microarray assay system may be useful as a diagnostic technique in the clinical laboratory.

Kazmierczak, M. J., S. C. Mithoe, et al. (2003). "Listeria monocytogenes sigma B regulates stress response and virulence functions." J Bacteriol **185**(19): 5722-34.

While the stress-responsive alternative sigma factor sigma(B) has been identified in different species of Bacillus, Listeria, and Staphylococcus, the sigma(B) regulon has been extensively characterized only in B. subtilis. We combined biocomputing and microarray-based strategies to identify sigma(B)-dependent genes in the facultative intracellular pathogen Listeria monocytogenes. Hidden Markov model (HMM)-based searches identified 170 candidate sigma(B)-dependent promoter sequences in the strain EGD-e genome sequence. These data were used to develop a specialized, 208-gene microarray, which included 166 genes downstream of HMM-predicted sigma(B)-dependent promoters as well as selected virulence and stress response genes. RNA for the microarray experiments was isolated from both wild-type and Delta sigB null mutant L. monocytogenes cells grown to stationary phase or exposed to osmotic stress (0.5 M KCl). Microarray analyses identified a total of 55 genes with statistically significant sigma(B)-dependent expression under the conditions used in these experiments, with at least 1.5-fold-higher expression in the wild type over the sigB mutant under either stress condition (51 genes showed at least 2.0-fold-higher expression in the wild type). Of the 55 genes exhibiting sigma(B)-dependent expression, 54 were preceded by a sequence resembling the sigma(B) promoter consensus sequence. Rapid amplification of cDNA ends-PCR was used to confirm the sigma(B)-dependent nature of a subset of eight selected promoter regions. Notably, the sigma(B)-dependent L. monocytogenes genes identified through this HMM/microarray strategy included both stress response genes (e.g., gadB, ctc, and the glutathione reductase gene lmo1433) and virulence genes (e.g., inlA, inlB, and bsh). Our data demonstrate that, in addition to regulating expression of genes important for survival under environmental stress conditions, sigma(B) also contributes to regulation of virulence gene expression in L. monocytogenes. These findings strongly suggest that sigma(B) contributes to L. monocytogenes gene expression during infection.

Kelly, A., M. D. Goldberg, et al. (2004). "A global role for Fis in the transcriptional control of metabolism and type III secretion in Salmonella enterica serovar Typhimurium." Microbiology **150**(Pt 7): 2037-53.

Fis is a key DNA-binding protein involved in nucleoid organization and modulation of many DNA transactions, including transcription in enteric bacteria. The regulon of genes whose expression is influenced by Fis in Salmonella enterica serovar Typhimurium (S. typhimurium) has been defined by DNA microarray analysis. These data suggest that Fis plays a central role in coordinating the expression of both metabolic and type III secretion factors. The genes that were most strongly up-regulated by Fis were those involved in virulence and located in the pathogenicity islands SPI-1, SPI-2, SPI-3 and SPI-5. Similarly, motility and flagellar genes required Fis for full expression. This was shown to be a direct effect as purified Fis protein bound to the promoter regions of representative flagella and SPI-2 genes. Genes contributing to aspects of metabolism known to assist the bacterium during survival in the mammalian gut were also Fis-regulated, usually negatively. This category included components of metabolic pathways for propanediol utilization, biotin synthesis, vitamin B(12) transport, fatty acids and

acetate metabolism, as well as genes for the glyoxylate bypass of the tricarboxylic acid cycle. Genes found to be positively regulated by Fis included those for ethanolamine utilization. The data reported reveal the central role played by Fis in coordinating the expression of both housekeeping and virulence factors required by S. typhimurium during life in the gut lumen or during systemic infection of host cells.

Keramas, G., G. Perozziello, et al. (2004). "Development of a multiplex microarray microsystem." Lab Chip **4**(2): 152-8.

A hybrid multiplex microarray microsystem has been developed that consists of 32 individually addressable array reaction chambers, supporting the use of multichannel pipettes for addition of up to 8 samples simultaneously. Discrimination between Campylobacter jejuni and Campylobacter coli bacteria was observed in DNA samples containing Campylobacter spp., with the same specificity and sensitivity as when compared to a full-size microarray. The spinloaded multiplex microarray microsystem described provides a novel and convenient test format for simultaneous low-density microarray analysis and is universally adaptable to other DNA, protein or small molecule microarray based applications.

Kessler, N., O. Ferraris, et al. (2004). "Use of the DNA flow-thru chip, a three-dimensional biochip, for typing and subtyping of influenza viruses." J Clin Microbiol **42**(5): 2173-85.

Influenza A viruses, which are further subtyped on the basis of antigenic differences in external hemagglutinin and neuraminidase glycoproteins, and influenza B viruses are prominent among the viral causes of respiratory diseases and can cause a wide spectrum of illness. Each year these viruses are responsible for recurrent epidemics, frequently in association with genetic variation. There is a requirement for sensitive and rapid diagnostic techniques in order to improve both the diagnosis of infections and the quality of surveillance systems. A new three-dimensional biochip platform (Flow-Thru Chip; MetriGenix) was used to develop a rapid and reliable molecular method for the typing and subtyping of influenza viruses. Oligonucleotide probes immobilized in microchannels of a silicon wafer were selected to recognize multiple fragments of the influenza A virus matrix protein gene; the influenza B virus NS gene; the H1, H3, and H5 hemagglutinin genes; and the N1 and N2 neuraminidase genes. Biotinylated amplicons resulting from either multiplex or random reverse transcription-PCR were hybridized to arrayed oligonucleotides on the influenza virus chip before they were stained with horseradish peroxidase-streptavidin and were imaged by use of a chemiluminescent substrate. The chip analysis procedure, from the time of pipetting of the sample into the chip cartridge to the time of analysis of the results, was performed in less than 5 h. The random PCR exhibited a higher level of performance than the multiplex PCR in terms of the specificity of product hybridization to the influenza virus chip. Analysis of influenza A viruses (H1N1, H3N2, H1N2, and H5N1) and influenza B viruses showed that this microarray-based method is capable of the rapid and unambiguous identification of all types and subtypes of viruses by use of random PCR products. The redundancy of the probes designed for each gene selected yielded an additional criterion of confidence for the subtyping of viruses which are known for antigenic variations in some of their components.

Kim, C. J., J. K. Jeong, et al. (2003). "HPV oligonucleotide microarray-based detection of HPV genotypes in cervical neoplastic lesions." Gynecol Oncol **89**(2): 210-7.

BACKGROUND: In this study we examined the use of a new-human papillomavirus (HPV) detection method, the HPV oligonucleotide microarray system (Biomedlab Co., Korea), which we compared with the well-established HPV DNA detection system (Hybrid Capture II; HC-II, Digene Co.). This new method prompted us to develop a new HPV genotyping technique, using the oligonucleotide microarray, to detect the generic and type-specific sequence of HPV types. In particular, we undertook the evaluation of the clinical efficacy of the HPV oligonucleotide microarray for detecting HPV in cervical neoplastic lesions. METHODS: One hundred forty patients were involved and classified into three groups according to their histopathologic diagnoses: Group I (nonspecific chronic cervicitis; n = 61), Group II (low-grade squamous intraepithelial lesion (SIL); koilocytosis, and mild dysplasia; n = 39),

and Group III (high-grade SIL; moderate, severe dysplasia and in situ carcinoma; n = 40). Cytological diagnoses were based on the Bethesda System and cervical samples were analyzed by the two methods. The HPV oligonucleotide microarray detected 15 types of high-risk HPV (HPV-16/-18/-31/-33/-35/-39/-45/-51/-52/-56/-58/-59/-66/-68/-69) and 7 types of low-risk HPV (HPV-6/-11/-34/-40/-42/-43/-44). RESULTS: In 105 of the 140 cervical samples (75%), HPV DNAs were examined using the HC-II method. HPV detection rates using the HPV microarray agreed with those of HC-II. One HC-II-positive, but HPV microarray-negative, case occurred in the low-grade SIL (Group II) and was later confirmed negative for HPV. The other HPV microarray-positive but HC-II-negative case was found to be HPV-18 by PCR. Low-risk types of HPV were detected in 3 of 39 low-grade SIL cases (Group II) using the HPV microarray. HPV-16 was the most frequent type (32.1%) in all specimens tested, and was significantly more frequent in low-grade or high-grade intraepithelial lesions (Groups II or III) than in normal controls (Group I) ($P < 0.05$). HPV-58 was the second most common type (17.5%) in Group III. The HPV microarray was found to have advantages in terms of identifying the HPV genotypes and cases of multiple HPV infection. Double HPV infections were detected in 12 cases and triple HPV infections in 7 cases. Two cases were positive for four types of HPV (HPV-16/18/33/35, HPV-16/18/58/68). The sensitivity of HPV testing (HC-II; 94.9%, HPV microarray; 93.7%) for identifying patients with squamous intraepithelial lesion was significantly better than the sensitivity of cytology (77.1%, $P < 0.05$). On using multiple logistic regression analysis to estimate the relative risk of SIL versus HPV type, HPV-16-positive cases were found to have a 7.5-fold risk of SIL (95% CI = 3.28-16.51; $P < 0.01$). HPV-33 and HPV-58 were found to be significantly related to high-grade SILs ($P < 0.01$). CONCLUSIONS: Our results suggest that the HPV oligonucleotide microarray is highly comparable to HC-II for detecting HPV in cervical specimens. The HPV oligonucleotide microarray provides useful information on viral genotype and multiple HPV infections in HPV-related cervical lesions. Genetic information on HPV in cervical specimens might be a particular benefit of the new procedure in the management of cervical neoplastic lesions.

Kim, J. W., Q. Ye, et al. (2004). "Cancer-associated molecular signature in the tissue samples of patients with cirrhosis." Hepatology **39**(2): 518-27.

Several types of aggressive cancers, including hepatocellular carcinoma (HCC), often arise as a multifocal primary tumor. This suggests a high rate of premalignant changes in noncancerous tissue before the formation of a solitary tumor. Examination of the messenger RNA expression profiles of tissue samples derived from patients with cirrhosis of various etiologies by complementary DNA (cDNA) microarray indicated that they can be grossly separated into two main groups. One group included hepatitis B and C virus infections, hemochromatosis, and Wilson's disease. The other group contained mainly alcoholic liver disease, autoimmune hepatitis, and primary biliary cirrhosis. Analysis of these two groups by the cross-validated leave-one-out machine-learning algorithms revealed a molecular signature containing 556 discriminative genes ($P <.001$). It is noteworthy that 273 genes in this signature (49%) were also significantly altered in HCC ($P <.001$). Many genes were previously known to be related to HCC. The 273-gene signature was validated as cancer-associated genes by matching this set to additional independent tumor tissue samples from 163 patients with HCC, 56 patients with lung carcinoma, and 38 patients with breast carcinoma. From this signature, 30 genes were altered most significantly in tissue samples from high-risk individuals with cirrhosis and from patients with HCC. Among them, 12 genes encoded secretory proteins found in sera. In conclusion, we identified a unique gene signature in the tissue samples of patients with cirrhosis, which may be used as candidate markers for diagnosing the early onset of HCC in high-risk populations and may guide new strategies for chemoprevention. Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).

Kim, W. H. (2001). "[Tissue array technology for translational research. From gene discovery to application]." Exp Mol Med **33**(1 Suppl): 135-48.

Large scale scanning of the human genome has become possible with the introduction of DNA microarray. The ability to survey the expression of up to 5000 to 50,000 genes in a single experiment provides significant new opportunities, as well as new challenge. It will be important to translate genomic scale information on cancer biology to functional or clinical application. This requires prioritization of hundreds of targets discovered, functional validation of these targets, as well as a thorough knowledge of the involvement of the candidate target genes in vivo in human tissue. We have developed a tissue array technology for genome scale expressional and clinical cancer research. This technology enables high-throughput molecular analysis of large number of specimens. Our tissue arrays are constructed by arranging the cylindrical biopsies of 2.0 mm diameter from 60 individual tumor tissues into a tissue array block, which is then sliced into 200 or more identical slides for probing RNA or protein targets. A single immunohistochemistry or in situ hybridization experiment provides information on all 60 specimens on the slides, while subsequent sections can be analyzed with other probes or antibodies. We produced gastric cancer tissue array slides with various kinds of subsets, including 600 subsequent cancer cases, 100 preneoplastic lesions, 60 metastatic lesions, 60 synchronous cancers, 60 metachronous cancers, 60 young age patients, and 120 familial cases. We searched the presence of Epstein-Barr virus in those cancer specimens. We also applied 10 antibodies in those samples and stratify the prognostic significance of these antibodies. Tissue array technology expand the scope of high-throughput molecular analysis of archival tissue specimens with multiple probes for specific genes or proteins for functional or clinical application.

Kingsley, M. T., T. M. Straub, et al. (2002). "Fingerprinting closely related xanthomonas pathovars with random nonamer oligonucleotide microarrays." Appl Environ Microbiol **68**(12): 6361-70.

Current bacterial DNA-typing methods are typically based on gel-based fingerprinting methods. As such, they access a limited complement of genetic information and many independent restriction enzymes or probes are required to achieve statistical rigor and confidence in the resulting pattern of DNA fragments. Furthermore, statistical comparison of gel-based fingerprints is complex and nonstandardized. To overcome these limitations of gel-based microbial DNA fingerprinting, we developed a prototype, 47-probe microarray consisting of randomly selected nonamer oligonucleotides. Custom image analysis algorithms and statistical tools were developed to automatically extract fingerprint profiles from microarray images. The prototype array and new image analysis algorithms were used to analyze 14 closely related Xanthomonas pathovars. Of the 47 probes on the prototype array, 10 had diagnostic value (based on a chi-squared test) and were used to construct statistically robust microarray fingerprints. Analysis of the microarray fingerprints showed clear differences between the 14 test organisms, including the separation of X. oryzae strains 43836 and 49072, which could not be resolved by traditional gel electrophoresis of REP-PCR amplification products. The proof-of-application study described here represents an important first step to high-resolution bacterial DNA fingerprinting with microarrays. The universal nature of the nonamer fingerprinting microarray and data analysis methods developed here also forms a basis for method standardization and application to the forensic identification of other closely related bacteria.

Kitadai, Y., A. Sasaki, et al. (2003). "Helicobacter pylori infection influences expression of genes related to angiogenesis and invasion in human gastric carcinoma cells." Biochem Biophys Res Commun **311**(4): 809-14.

Infection with Helicobacter pylori (H. pylori) is considered a risk factor for gastric carcinoma. The purpose of this study was to clarify whether H. pylori infection plays a role in progression of gastric carcinoma. We examined the expression of genes encoding angiogenic factors and proteases by human gastric carcinoma cell lines (MKN-1 and TMK-1) co-cultured with or without H. pylori by cDNA microarray analysis. Co-culture with H. pylori increased expression of mRNAs encoding interleukin (IL)-8, vascular endothelial growth factor (VEGF), angiogenin, urokinase-type plasminogen activator (uPA), and metalloproteinase (MMP)-9 by gastric carcinoma cells. Up-regulation of these genes at the mRNA

and protein levels was confirmed by Northern blot analysis, semi-quantitative RT-PCR analysis, and ELISA. In vitro angiogenic and collagenase activities of conditioned medium from the gastric carcinoma cells were also stimulated by co-culture with H. pylori. These results indicate that H. pylori infection may regulate angiogenesis and invasion of human gastric carcinoma.

Klaassen, C. H., C. F. Prinsen, et al. (2004). "DNA microarray format for detection and subtyping of human papillomavirus." J Clin Microbiol **42**(5): 2152-60.

A new human papillomavirus (HPV) assay using high-density DNA microarrays is described. An HPV DNA fragment from the 3' end of the E1 gene was amplified and digoxigenin labeled by PCR, and the resulting amplicons were hybridized onto type-specific oligonucleotides immobilized on high-density DNA microarrays. For detection, a simple immunohistochemical staining procedure was used with a substrate that has both colorimetric and fluorescent properties. This detection chemistry enables the rapid identification of reactive spots by regular light microscopy and semiquantification by laser scanning. Both single and multiple HPV infections are recognized by this assay, and the corresponding HPV types are easily identified. With this assay, 53 mucosal HPV types were detected and identified. A total of 45 HPV types were identified by a single type-specific probe, whereas the remaining 8 mucosal HPV types could be identified by a specific combination of probes. The simple assay format allows usage of this assay without expensive equipment, making it accessible to all diagnostic laboratories with PCR facilities.

Knox, D. P., D. L. Redmond, et al. (2001). "The contribution of molecular biology to the development of vaccines against nematode and trematode parasites of domestic ruminants." Vet Parasitol **101**(3-4): 311-35.

Rapid developments in molecular biology have had an enormous impact on the prospects for the development of vaccines to control the major nematode and trematode infestations of livestock. Vaccine candidates are purified using conventional protein chemistry techniques but the limitations imposed by the scarcity of parasite material provide an insurmountable barrier for commercial vaccine production by this means. The ability to purify mRNA from different parasite life-cycle stages and to prepare cDNA expression libraries from it has proven central to the identification of immunogenic parasite proteins. Potentially, protective parasite antigens can now be produced in recombinant form in a variety of vectors and this represents a key breakthrough on the road to commercial vaccine production. The contribution of molecular biology to this process is discussed using several examples, particularly in vaccine development against the pathogenic abomasal nematode of sheep and goats, Haemonchus contortus, and the liver fluke of sheep and cattle, Fasciola hepatica. The difficulties of producing recombinant proteins in the correct form, with appropriate post-translational modification and conformation, are discussed as well as emerging means of antigen delivery including DNA vaccination. The opportunities offered by genome and expressed sequence tag analyses programmes for antigen targeting are discussed in association with developing microarray and proteomics technologies which offer the prospect of large scale, rapid antigen screening and identification.

Kobayashi, S. D. and F. R. DeLeo (2003). "Apoptosis in human polymorphonuclear leukocytes: searching for a genetic roadmap." Arch Immunol Ther Exp (Warsz) **51**(1): 1-8.

Polymorphonuclear leukocytes (PMNs or neutrophils) are essential components of the innate immune system in humans and function primarily to eliminate invading microorganisms. Neutrophil influx to sites of infection is desirable because it also initiates an inflammatory response. Paradoxically, PMNs are also intimately associated with inflammatory disease. As part of normal neutrophil turnover in humans and to limit inflammatory potential, PMNs undergo programmed cell death, or apoptosis. Several host factors, including cytokines and growth factors, are capable of extending neutrophil survival and, thus, capacity to fight infection. On the other hand, phagocytosis of bacterial pathogens generally accelerates PMN apoptosis. Due in part to the extensive complexity of programmed cell death, relatively little is known about the signaling pathways that govern these processes in PMNs. Recently, microarray

strategies have been employed to gain an understanding of these processes in activated PMNs, and new evidence indicates that gene transcription is important in the regulation of neutrophil apoptosis and, thus, inflammation. A series of provocative discoveries led to the hypothesis that neutrophil programmed cell death is the result of an apoptosis-differentiation program, a final stage of transcriptionally regulated PMN maturation or hematopoietic differentiation. Further characterization of the apoptosis-differentiation program and associated biochemical pathways in mature PMNs will likely yield important insights into the resolution of inflammation and infection.

Koga, H., K. Imada, et al. (2004). "Identification of differentially expressed molecules in adult T-cell leukemia cells proliferating in vivo." Cancer Sci **95**(5): 411-7.

HTLV-I is the causative agent of adult T-cell leukemia (ATL). However, the precise mechanism underlying the neoplastic cell growth of ATL remains unclear. In this study, we established a leukemic cell line, termed SYK-11L(+), from tumor cells (S-YU) in an in vivo cell proliferation model of ATL using severe combined immunodeficiency (SCID) mice. Unexpectedly, SYK-11L(+) was found to have no tumorigenicity in SCID mice. Flow cytometric analysis showed that S-YU expressed cell adhesion molecules including CD44, ICAM-1 and OX40, whereas SYK-11L(+) had lost the expression of these molecules. The administration of anti-OX40 monoclonal antibody inhibited the engraftment of S-YU cells into SCID mice, suggesting that OX40 is a potential target for immunotherapy. Significant differences in responsiveness to IL-2 and IL-15 were observed between the two cell types. To better understand the molecular basis of tumorigenicity, cDNA microarray analysis was performed using tumorigenic S-YU and non-tumorigenic SYK-11L(+) cells. We obtained several candidate genes differentially overexpressed in S-YU compared with SYK-11L(+). Interestingly, one such gene, regulator of G protein signaling 1 (RGS1), was shown to be overexpressed in most ATL patients. Further characterization of the differentially expressed molecules, such as OX40 and RGS1, would provide useful information not only to elucidate the mechanism of ATL cell growth in vivo, but also to develop novel molecularly targeted therapies.

Koide, T., P. A. Zaini, et al. (2004). "DNA microarray-based genome comparison of a pathogenic and a nonpathogenic strain of Xylella fastidiosa delineates genes important for bacterial virulence." J Bacteriol **186**(16): 5442-9.

Xylella fastidiosa is a phytopathogenic bacterium that causes serious diseases in a wide range of economically important crops. Despite extensive comparative analyses of genome sequences of Xylella pathogenic strains from different plant hosts, nonpathogenic strains have not been studied. In this report, we show that X. fastidiosa strain J1a12, associated with citrus variegated chlorosis (CVC), is nonpathogenic when injected into citrus and tobacco plants. Furthermore, a DNA microarray-based comparison of J1a12 with 9a5c, a CVC strain that is highly pathogenic and had its genome completely sequenced, revealed that 14 coding sequences of strain 9a5c are absent or highly divergent in strain J1a12. Among them, we found an arginase and a fimbrial adhesin precursor of type III pilus, which were confirmed to be absent in the nonpathogenic strain by PCR and DNA sequencing. The absence of arginase can be correlated to the inability of J1a12 to multiply in host plants. This enzyme has been recently shown to act as a bacterial survival mechanism by down-regulating host nitric oxide production. The lack of the adhesin precursor gene is in accordance with the less aggregated phenotype observed for J1a12 cells growing in vitro. Thus, the absence of both genes can be associated with the failure of the J1a12 strain to establish and spread in citrus and tobacco plants. These results provide the first detailed comparison between a nonpathogenic strain and a pathogenic strain of X. fastidiosa, constituting an important step towards understanding the molecular basis of the disease.

Kong, X., H. San Juan, et al. (2003). "Respiratory syncytial virus infection activates STAT signaling in human epithelial cells." Biochem Biophys Res Commun **306**(2): 616-22.

Acute respiratory syncytial virus (RSV) infection causes airway inflammation and exacerbates

asthma, but the mechanism of inflammation is poorly understood. The role of the STAT-signaling pathway in RSV infection in epithelial cells was examined in this study. DNA microarray analyses of RSV-infected human alveolar type II (A549) epithelial cells identified several genes whose expression was altered from -5.5 to +56.4-fold. Four of the highly expressed genes contained STAT-binding elements. In A549 and normal human bronchial epithelial cells (NHBE), RSV induced phosphorylation and nuclear translocation of STAT-1alpha that was abrogated when RSV attachment was blocked. Treatment with a JAK-2 inhibitor or transfection with dominant-negative STAT-1alpha blocked STAT-1alpha activation and RSV infection. RSV also activated STAT-3 and IL-6 specific antibodies blocked this activation. Thus, activation of the STAT-1alpha and STAT-3 pathways play a role in RSV infection.

Koreen, L., S. V. Ramaswamy, et al. (2004). "spa typing method for discriminating among Staphylococcus aureus isolates: implications for use of a single marker to detect genetic micro- and macrovariation." J Clin Microbiol **42**(2): 792-9.

Strain typing of microbial pathogens has two major aims: (i). to index genetic microvariation for use in outbreak investigations and (ii). to index genetic macrovariation for use in phylogenetic and population-based analyses. Until now, there has been no clear indication that one genetic marker can efficiently be used for both purposes. Previously, we had shown that DNA sequence analysis of the protein A gene variable repeat region (spa typing) provides a rapid and accurate method to discriminate Staphylococcus aureus outbreak isolates from those deemed epidemiologically unrelated. Here, using the hypothesis that the genetic macrovariation within a low-level recombinogenic species would accurately be characterized by a single-locus marker, we tested whether spa typing could congruently index the extensive genetic variation detected by a whole-genome DNA microarray in a collection of 36 isolates, which was recovered from 10 countries on four continents over a period of four decades, that is representative of the breadth of diversity within S. aureus. Using spa and coa typing, pulsed-field gel electrophoresis (PFGE), and microarray and multilocus enzyme electrophoresis (MLEE) data in molecular epidemiologic and evolutionary analyses, we determined that S. aureus likely has a primarily clonal population structure and that spa typing can singly index genetic variation with 88% direct concordance with the microarray and can correctly assign isolates to phylogenetic lineages. spa typing performed better than MLEE, PFGE, and coa typing in discriminatory power and in the degree of agreement with the microarray at various phylogenetic depths. This study showed that genetic analysis of the repeat region of protein A comprehensively characterizes both micro- and macrovariation in the primarily clonal population structure of S. aureus.

Kramer, M. F., W. J. Cook, et al. (2003). "Latent herpes simplex virus infection of sensory neurons alters neuronal gene expression." J Virol **77**(17): 9533-41.

The persistence of herpes simplex virus (HSV) and the diseases that it causes in the human population can be attributed to the maintenance of a latent infection within neurons in sensory ganglia. Little is known about the effects of latent infection on the host neuron. We have addressed the question of whether latent HSV infection affects neuronal gene expression by using microarray transcript profiling of host gene expression in ganglia from latently infected versus mock-infected mouse trigeminal ganglia. (33)P-labeled cDNA probes from pooled ganglia harvested at 30 days postinfection or post-mock infection were hybridized to nylon arrays printed with 2,556 mouse genes. Signal intensities were acquired by phosphorimager. Mean intensities (n = 4 replicates in each of three independent experiments) of signals from mock-infected versus latently infected ganglia were compared by using a variant of Student's t test. We identified significant changes in the expression of mouse neuronal genes, including several with roles in gene expression, such as the Clk2 gene, and neurotransmission, such as genes encoding potassium voltage-gated channels and a muscarinic acetylcholine receptor. We confirmed the neuronal localization of some of these transcripts by using in situ hybridization. To validate the microarray results, we performed real-time reverse transcriptase PCR analyses for a selection of the genes. These studies demonstrate that latent HSV infection can alter neuronal gene expression and might

provide a new mechanism for how persistent viral infection can cause chronic disease.

Krzeminski, Z. (2003). "[Advances in microbiological diagnosis of infectious diseases]." Przegl Epidemiol **57**(2): 377-80.

Development of new methods in microbiological diagnosis of infectious diseases is described. Particularly methods which are based on techniques DNA microarray, protein microarray and nanotechnology.

Laassri, M., V. Chizhikov, et al. (2003). "Detection and discrimination of orthopoxviruses using microarrays of immobilized oligonucleotides." J Virol Methods **112**(1-2): 67-78.

Variola virus (VARV), causing smallpox, is a potential biological weapon. Methods to detect VARV rapidly and to differentiate it from other viruses causing similar clinical syndromes are needed urgently. We have developed a new microarray-based method that detects simultaneously and discriminates four orthopoxvirus (OPV) species pathogenic for humans (variola, monkeypox, cowpox, and vaccinia viruses) and distinguishes them from chickenpox virus (varicella-zoster virus or VZV). The OPV gene C23L/B29R, encoding the CC-chemokine binding protein, was sequenced for 41 strains of seven species of orthopox viruses obtained from different geographical regions. Those C23L/B29R sequences and the ORF 62 sequences from 13 strains of VZV (selected from GenBank) were used to design oligonucleotide probes that were immobilized on an aldehyde-coated glass surface (a total of 57 probes). The microchip contained several unique 13-21 bases long oligonucleotide probes specific to each virus species to ensure redundancy and robustness of the assay. A region approximately 1100 bases long was amplified from samples of viral DNA and fluorescently labeled with Cy5-modified dNTPs, and single-stranded DNA was prepared by strand separation. Hybridization was carried out under plastic coverslips, resulting in a fluorescent pattern that was quantified using a confocal laser scanner. 49 known and blinded samples of OPV DNA, representing different OPV species, and two VZV strains were tested. The oligonucleotide microarray hybridization technique identified reliably and correctly all samples. This new procedure takes only 3 h, and it can be used for parallel testing of multiple samples.

Lakey, D. L., Y. Zhang, et al. (2002). "Priming reverse transcription with oligo(dT) does not yield representative samples of Mycobacterium tuberculosis cDNA." Microbiology **148**(Pt 8): 2567-72.

Several recent publications have suggested that oligo(dT) can prime reverse transcription of several mycobacterial mRNAs. To determine if this is the case for most Mycobacterium tuberculosis mRNA species, reverse transcription reactions of M. tuberculosis RNA were primed with oligo(dT) or with other primers that did not target polyadenylylated sequences, and the resultant cDNA product was evaluated. Priming with oligo(dT) yielded more cDNA than priming with an arbitrary primer for only 1 of 12 unrelated M. tuberculosis genes, as measured by competitive PCR. Priming with oligo(dT) yielded cDNA for only 30% of the genes primed for by 37 M. tuberculosis genome-directed oligonucleotides, as assessed by hybridization of cDNA with an M. tuberculosis microarray. These data demonstrate that priming of reverse transcription of mycobacterial mRNA with oligo(dT) does not yield representative samples of cDNA.

Lam, L. T., C. Ronchini, et al. (2000). "Suppression of erythroid but not megakaryocytic differentiation of human K562 erythroleukemic cells by notch-1." J Biol Chem **275**(26): 19676-84.

The Notch signal transduction pathway is a highly conserved regulatory system that controls multiple developmental processes. We have established an erythroleukemia cell model to study how Notch regulates cell fate and erythroleukemic cell differentiation. K562 and HEL cells expressed the Notch-1 receptor and the Notch ligand Jagged-1. The stable expression of the constitutively active intracellular domain of Notch-1 (NIC-1) in K562 cells inhibited erythroid without affecting megakaryocytic maturation. Expression of antisense Notch-1 induced spontaneous erythroid maturation. Suppression of erythroid maturation by NIC-1 did not result from down-regulation of GATA-1 and TAL-

1, transcription factors necessary for erythroid differentiation. Microarray gene expression analysis identified genes activated during erythroid maturation, and NIC-1 disrupted the maturation-dependent changes in the expression of these genes. These results show that NIC-1 alters the pattern of gene expression in K562 cells leading to a block in erythroid maturation and therefore suggest that Notch signaling may control the developmental potential of normal and malignant erythroid progenitor cells.

Lan, R., A. M. Davison, et al. (2003). "AFLP analysis of Salmonella enterica serovar Typhimurium isolates of phage types DT 9 and DT 135: diversity within phage types and its epidemiological significance." Microbes Infect **5**(10): 841-50.

Amplified fragment length polymorphism (AFLP) was applied to 35 and 34 isolates, respectively, of Salmonella enterica serovar Typhimurium phage types DT 9 and DT 135, using eight primer pair combinations. Eight and 17 AFLP types were observed in DT 9 and DT 135, respectively. DT 9 is rare in the UK and common in Australia, but one AFLP form dominated with 28 isolates, comprising 22 of 25 UK isolates, four of five Australian isolates, one Jamaican and one Spanish isolate. Of the others, two UK isolates are closely related to the major form, two from elsewhere are in the major cluster and three isolates from different countries are in a separate cluster. For DT 135, two closely related AFLP types of seven and 11 isolates form the major cluster, which also includes 11 isolates, mostly in single-isolate AFLP types, while five isolates from different countries form a well-separated minor cluster. For both DTs all isolates are grouped together if only the phage type specific bands identified earlier are used, confirming their value for molecular-based 'phage typing'. Polymorphic markers identified in this study could also be used for subtyping within both phage types. The value of AFLP is in locating DNA fragments useful for typing, but implementation of a replacement typing scheme would probably involve multiplex PCR or microarray technologies.

Langmann, T., C. Moehle, et al. (2004). "Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes." Gastroenterology **127**(1): 26-40.

BACKGROUND & AIMS: Phase 1, phase 2, and cellular efflux transporters are critical components in intestinal barrier function against xenobiotics and bacteria. We therefore performed global gene expression profiling in patients with ulcerative colitis (UC) and Crohn's disease as well as control specimens, with a special emphasis on genes involved in detoxification and epithelial membrane integrity. METHODS: Mucosal biopsy specimens from nonaffected regions of the colon and the terminal ileum were subjected to DNA microarray analysis and pathway-related data mining. Real-time reverse-transcription polymerase chain reaction was used for verification of selected regulated candidate genes in larger inflammatory bowel disease sample numbers and intestinal cell lines. RESULTS: Several dysregulated genes were identified in both disease groups and tissues. A set of genes coordinately down-regulated in the colon of patients with UC was composed of cellular detoxification and defense genes, which are target genes for the transcription factor pregnane X receptor (PXR). Messenger RNA expression of ABCB1 (MDR1) and PXR was significantly reduced in the colon of patients with UC but was unaffected in patients with Crohn's disease. In contrast to some of its target genes, the expression of PXR was not sensitive to tumor necrosis factor alpha stimulation of intestinal cell lines. CONCLUSIONS: A disease- and tissue-specific decrease in the expression of detoxification enzymes and ABC transporters was observed, which may be explained by a loss of PXR expression. Thus, dysregulation of xenobiotic metabolism and PXR activity in the gut is likely to contribute to the pathophysiology of UC.

Leonard, E. E., 2nd, T. Takata, et al. (2003). "Use of an open-reading frame-specific Campylobacter jejuni DNA microarray as a new genotyping tool for studying epidemiologically related isolates." J Infect Dis **187**(4): 691-4.

Findings from use of an open-reading frame-specific Campylobacter jejuni DNA microarray to investigate genetic diversity among clinical isolates associated with 5 independent clusters of infection

were compared with data from random amplified polymeric DNA (RAPD) and Penner serotyping analyses. The DNA microarray provides a highly specific epidemiological typing tool for analysis of C. jejuni isolates and reveals both divergent and highly conserved gene classes among isolates.

Leonard, E. E., 2nd, L. S. Tompkins, et al. (2004). "Comparison of Campylobacter jejuni isolates implicated in Guillain-Barre syndrome and strains that cause enteritis by a DNA microarray." Infect Immun **72**(2): 1199-203.

We asked whether Campylobacter jejuni isolated from patients with Guillain-Barre syndrome (GBS) differ from isolates isolated from patients with uncomplicated gastrointestinal infection using DNA microarray analysis. We found that specific GBS genes or regions were not identified, and microarray analysis confirmed significant genomic heterogeneity among the isolates.

Li, C. H., A. Q. Zhang, et al. (2004). "[Analysis of the early and late gene expression of lipopolysaccharide activated macrophages by cDNA microarray]." Zhongguo Wei Zhong Bing Ji Jiu Yi Xue **16**(6): 338-44.

OBJECTIVE: To study the early and late changes in mRNA expression in macrophages in response to lipopolysaccharide (LPS) with a cDNA microarray approach using the Clontech Atlas microarray. METHODS: mRNA was isolated from unstimulated control and LPS stimulated murine peritoneal macrophages at 2 hours and 24 hours poststimulation, converted to (33)P radiolabeled cDNA, and hybridized to mouse array membranes. RESULTS: In macrophages being stimulated for 2 hours, 69 out of 1 176 genes were found to differ by over 3-fold compared with the control. Among them 44 genes were up-regulated and 25 genes were down-regulated. In macrophages stimulated for 24 hours, 11 genes were up-regulated and 26 genes were down-regulated compared with the control. Only 8 genes were identified both at 2 hours and at 24 hours poststimulation. The expressions of many genes encoding transcription factor, cytokines, cell signaling modulators and apoptosis associated proteins were found to have changed. Some genes that were not previously linked to this model, such as bric-a-brac (BTB) and cap-n-collar(CNC) homology 1(BACH1), early growth response protein 2 (EGR2), E47 interaction protein 1 (EIP1), Ngfi-A binding protein 2 (NAB2), myeloblastosis oncogene-like protein (MYBL2), neurofibromatosis 1 (NF1), ciliarry neurotropic factor (CNTF) and semaphorin 4A (Sema4A). CONCLUSION: This study has allowed us to identify genes that may potentially be regulated by LPS at early and late phase in macrophages. These may contribute to better understanding of the mechanism underlying LPS or bacteria induced inflammatory and immune response following infection and trauma.

Li, H. S., W. J. Doyle, et al. (2002). "Suppression of epithelial ion transport transcripts during pneumococcal acute otitis media in the rat." Acta Otolaryngol **122**(5): 488-94.

Until recently, it was not feasible to conduct genome-wide screening for gene transcript variations that play key roles in the pathogenesis of otitis media. In this study microarray technology was used to profile differential gene expression patterns from rat middle ear mucosa at 12 and 48 h after Streptococcus pneumoniae challenge. Real-time polymerase chain reaction was performed for independent verification of the microarray results. Three ion transport mRNAs were simultaneously suppressed more than 4-fold at 12 h in bacteria-challenged ears, including Na,K-ATPase alpha I subunit (SPATPa1), sodium channel beta 2 subunit (SCNB2) and sodium-hydrogen exchange protein isoform 2 subunit (NHE2). At 48 h after infection, the mRNA levels of SCNB2 and NHE2 had decreased 7- and 10-fold, respectively, whereas the relatively abundant SPATPa1 transcript showed recovery. The downregulation of Na(+)-transporting transcripts suggests a reduced number of epithelial cells and transporting proteins and/or the dysfunction of sodium transporters secondary to the bacterial infection. These changes can disrupt the coupling of the apical Na + entry and basolateral Na + extrusion, deplete the electrochemical Na+ transmembrane gradient, disrupt the intracellular osmotic equilibrium and lead to intracellular acidification and the accumulation of excess sodium, water and other organic and inorganic molecules in the middle ear cavity. Any or all of these changes may contribute to the initiation and

persistence of middle ear mucosa inflammation and effusion during an episode of bacterial acute otitis media.

Li, H. S., W. J. Doyle, et al. (2003). "Mucosal expression of genes encoding possible upstream regulators of Na+ transport during pneumococcal otitis media." Acta Otolaryngol **123**(5): 575-82.

OBJECTIVE: Recently, we reported that gene transcripts encoding 3 Na+ transport proteins (pump, channel and exchanger) in the middle ear mucosa (MEM) were simultaneously suppressed at 12 and 48 h after Streptococcus pneumoniae (SP) challenge of rat middle ears. MATERIAL AND METHODS: From cDNA microarray screening of those specimens, several gene clusters, including Nos2 and the transcription factors Fos, Fosl1, Jun and Nfkb1, were identified as possible upstream regulators of Na+ transport protein expression. The altered expression of those genes in MEM was validated and quantified using real-time polymerase chain reaction and MEM protein expression for Atp1a1, Nos2 and Nfkb1 was studied using Western blot and/or immunohistochemistry assays. RESULTS: At both time-points. Atp1a1 mRNA and protein were decreased and Nos2 mRNA and protein were increased in MEM. While Nfkb1 protein was decreased at those times. the corresponding mRNA was increased at 12 h but decreased at 48 h. Gene expression for Fos was suppressed at both times, while that for Fosl1 and Jun was augmented at 12 h and suppressed at 48 h. Immunohistochemical study of specimens challenged with SP showed a swollen MEM with infiltration of inflammatory cells that stained positive for Nos2. CONCLUSION: Given the known activities of Nos2, these results can be interpreted as evidencing a transcriptional suppression of Na+ transport protein synthesis secondary to upregulated Nos2 expression during SP infection of the rat MEM. This proposed signaling pathway does not require the continuous upregulation of Nfkb1 or the other assayed transcription factors as early as 12 h after middle ear infection.

Li, J., S. Chen, et al. (2001). "Typing and subtyping influenza virus using DNA microarrays and multiplex reverse transcriptase PCR." J Clin Microbiol **39**(2): 696-704.

A model DNA microarray has been prepared and shown to facilitate typing and subtyping of human influenza A and B viruses. Reverse transcriptase PCR was used to prepare cDNAs encoding approximately 500-bp influenza virus gene fragments, which were then cloned, sequenced, reamplified, and spotted to form a glass-bound microarray. These target DNAs included multiple fragments of the hemagglutinin, neuraminidase, and matrix protein genes. Cy3- or Cy5-labeled fluorescent probes were then hybridized to these target DNAs, and the arrays were scanned to determine the probe binding site(s). The hybridization pattern agreed perfectly with the known grid location of each target, and the signal-to-background ratio varied from 5 to 30. No cross-hybridization could be detected beyond that expected from the limited degree of sequence overlap between different probes and targets. At least 100 to 150 bp of homology was required for hybridization under the conditions used in this study. Combinations of Cy3- and Cy5-labeled DNAs can also be hybridized to the same chip, permitting further differentiation of amplified molecules in complex mixtures. In a more realistic test of the technology, several sets of multiplex PCR primers that collectively target influenza A and B virus strains were identified and were used to type and subtype several previously unsequenced influenza virus isolates. The results show that DNA microarray technology provides a useful supplement to PCR-based diagnostic methods.

Li, L., W. L. Ma, et al. (2002). "[Preliminary study of development of gene chips for HIV diagnosis]." Di Yi Jun Yi Da Xue Xue Bao **22**(8): 724-7.

OBJECTIVE: To study the technology for establishing DNA chips for the diagnosis of HIV. METHODS: HIV 1U26942 DNA fragments were isolated by restriction display-PCR (RD-PCR) and printed onto aminosilane-coated glass slides by Pixsys 5500 arrayer as probes to prepare the gene chips. HIV samples, after labeled with Cy3, were hybridized with the microarray followed by scanning for analysis of hybridization kinetics of the RD fragments. RESULTS: The experimental condition for preparing the gene chips was investigated and 12 RD fragments were screened as probes for further study. CONCLUSION: The technique established in this study for preparing DNA chips is specific and

applicable.

Li, Y., J. J. Su, et al. (2003). "[Differentially expressed genes in hepatocellular carcinoma of tree shrew induced by different factors]." Ai Zheng **22**(10): 1018-22.

BACKGROUND & OBJECTIVE: Previous studies on differentially expressed genes in hepatocellular carcinoma (HCC) used to perform with para-cancerous tissues as normal control. However, the para-cancerous tissue of HCC is actually abnormal because they frequently contain hepatitis, cirrhosis, hyperplastic nodules or foci, etc. In order to explore the molecular mechanism and the responsible genes for hepatocarcinogenesis, through applying the HCC model of tree shrew (Tupaia belangeri chinensis), this study was designed to compare gene expression levels between HCC induced by different factors and their corresponding biopsies taken before HCC formation. METHODS: Tree shrews were divided into two groups. Group AFB(1) was fed with aflatoxin B1 (AFB(1)). Group AFB(1)+HBV was infected firstly with human hepatitis B virus (HBV) and then fed with AFB(1) as group AFB(1). Serum tests for HBV markers and liver biopsies were performed periodically during the experiment. After appearance of HCC, 2 HCC samples from each group and their corresponding 30th-week biopsies were tested respectively by cDNA microarray assay. The gene expression levels were compared between each HCC and the corresponding biopsies, and the differentially expressed genes from the two groups of HCC induced by different factors were analyzed. RESULTS: The incidence rates of HCC in group AFB(1) and group AFB(1)+HBV were 73.3% and 77.8%, respectively. A considerable number of genes in both groups showed changes in their expression levels, which were mainly up-regulated in group AFB(1) but down-regulated in group AFB(1)+HBV. On the other hand, among the 588 checked genes (16 functional classifications) that were known related to human cancer, 11 genes were similarly expressed in all of the 4 HCC from the two animal groups. Most of these 11 genes belonged functionally to 3 types, namely "apoptosis-associated protein","DNA synthesis,repair and recombination proteins", and "growth factors, cytokines and chemokines". CONCLUSION: (1)HBV can affect AFB(1)-induced gene expression in certain extent. (2)The gene expression profiles of HCC induced by different factors are different. (3)The common differentially expressed genes in these two HCC groups are worthwhile for further study as the possibly responsible genes for hepatocarcinogenesis.

Li-Korotky, H. S., J. D. Swarts, et al. (2004). "Cathepsin gene expression profile in rat acute pneumococcal otitis media." Laryngoscope **114**(6): 1032-6.

OBJECTIVES/HYPOTHESIS: Acute otitis media, often caused by infection with Streptococcus pneumoniae, is characterized by inflammation of the middle ear mucosa. A prominent feature of the host response to bacterial infection of the middle ear mucosa is an influx of inflammatory cells that contributes to the local pool of inflammatory mediators by releasing additional inflammatory chemicals, which in turn cause further tissue injury. The objective was to identify candidate effector and signaling molecules involved in acute otitis media pathogenesis caused by S pneumoniae infection. STUDY DESIGN: Male Sprague-Dawley rats were randomly assigned to 1 of 5 groups, including 1 control group without treatment, 2 placebo groups (12 and 48 hours) and 2 infected groups (12 and 48 hours). The rat middle ear was bilaterally inoculated with either 25 microL of tryptic soy broth (TSB group) or 25 microL of TSB containing approximately 1.24 x 10(9) cfu/mL of S pneumonias type 6A (SP group). Rats were killed at 12 and 48 hours after inoculation and the middle ear mucosa was collected. Total RNA was extracted and pooled from each group for gene expression assays. METHODS: Gene expression profiles for rat middle ear mucosa at 12 and 48 hours after S pneumoniae or placebo inoculation were constructed using microarray technology (Clontech Atlas Rat 1.2 Array, 1176 cDNAs). Genes of interest were further validated by real-time polymerize chain reaction. RESULTS: Middle ear mucosa expression of a gene cluster encoding the lysosomal cysteine proteases, cathepsins B (Ctsb), L (Ctsl), and K (Ctsk), was modified after S pneumoniae challenge. Specifically, at 12 hours, Ctsk and Ctsl messenger RNA that was abundantly expressed in the normal middle ear mucosa was decreased, whereas Ctsb transcript was induced. The changes in Ctsb and Ctsk gene expression were sustained at 48 hours. CONCLUSION: The

constitutive expression of Ctsk and Ctsl messenger RNA in normal middle ear mucosa supports a function in the maintenance of middle ear mucosa homeostasis, and their downregulation as an early event in acute otitis media may reflect a disruption in that function. The induction of Ctsb messenger RNA in the infected middle ear mucosa suggests a role in early tissue injury; thus, Ctsb may represent a potential target for molecular diagnostics and/or rational intervention during the development of acute otitis media.

Lim, J. W., H. Kim, et al. (2003). "Cell adhesion-related gene expression by Helicobacter pylori in gastric epithelial AGS cells." Int J Biochem Cell Biol **35**(8): 1284-96.

Helicobacter pylori (H. pylori) infection leads to gastroduodenal inflammation, peptic ulceration and gastric carcinoma. H. pylori may induce disease-specific gene expression in gastric epithelial cells. cDNA microarray for 352 cancer-related genes was used to identify the genes altered by H. pylori (cagA positive) in gastric epithelial AGS cells. Expressions of the genes identified on the microarray and other genes closely associated with these genes were determined by reverse transcriptase-polymerase chain reaction (RT-PCR). Western blot analysis and cell adhesion assay were performed to confirm the protein levels of the genes and the role of the genes on cell adhesion in H. pylori-infected AGS cells. As a result, the expression of four genes (galectin 1, aldolase A, integrin alpha5, LIM domain only 7 (LMO7)) were up-regulated by H. pylori in AGS cells, determined by cDNA microarray. RT-PCR analysis showed that the genes up-regulated by H. pylori were the genes regulating cell-cell adhesion and cell-extracellular matrix interaction, such as galectin-1 and galectin-3, integrin alpha5, and LIM domain only 7 (LMO7), and cancer-related glycolytic enzyme aldolase A and C. Cell adhesion to extracellular matrix proteins such as poly-L-lysine and fibronectin was mediated by H. pylori-induced expression of integrin alpha5. RT-PCR and Western blot analysis showed that E-cadherin, regulating cell adhesion and contact cell inhibition, was decreased by H. pylori in AGS cells. In conclusion, the increased expression of cell adhesion molecules and decrease in E-cadherin expression by H. pylori might contribute to cell adhesion, invasion and possibly cell proliferation in gastric epithelial cells.

Lin, B., G. J. Vora, et al. (2004). "Use of oligonucleotide microarrays for rapid detection and serotyping of acute respiratory disease-associated adenoviruses." J Clin Microbiol **42**(7): 3232-9.

The cessation of the adenovirus vaccination program for military trainees has resulted in several recent acute respiratory disease (ARD) outbreaks. In the absence of vaccination, rapid detection methods are necessary for the timely implementation of measures to prevent adenovirus transmission within military training facilities. To this end, we have combined a fluorogenic real-time multiplex PCR assay with four sets of degenerate PCR primers that target the E1A, fiber, and hexon genes with a long oligonucleotide microarray capable of identifying the most common adenovirus serotypes associated with adult respiratory tract infections (serotypes 3, 4, 7, 16, and 21) and a representative member of adenovirus subgroup C (serotype 6) that is a common cause of childhood ARD and that often persists into adulthood. Analyses with prototype strains demonstrated unique hybridization patterns for representative members of adenovirus subgroups B(1), B(2), C, and E, thus allowing serotype determination. Microarray-based sensitivity assessments revealed lower detection limits (between 1 and 100 genomic copies) for adenovirus serotype 4 (Ad4) and Ad7 cell culture lysates, clinical nasal washes, and throat swabs and purified DNA from clinical samples. When adenovirus was detected from coded clinical samples, the results obtained by this approach demonstrated an excellent concordance with those obtained by the more established method of adenovirus identification as well as by cell culture with fluorescent-antibody staining. Finally, the utility of this method was further supported by its ability to detect adenoviral coinfections, contamination, and, potentially, recombination events. Taken together, the results demonstrate the usefulness of the simple and rapid diagnostic method developed for the unequivocal identification of ARD-associated adenoviral serotypes from laboratory or clinical samples that can be completed in 1.5 to 4.0 h.

Liu, C. H., W. L. Ma, et al. (2003). "Possibility of using DNA chip technology for diagnosis of human papillomavirus." J Biochem Mol Biol **36**(4): 349-53.

To explore the application of DNA chip technology for the detection and typing of Human Papillomavirus (HPV), the HPV6, 11, 16 and 18 gene fragments were isolated and printed onto aminosilane-coated glass slides by a PixSys 5500 microarrayer as probes to prepare the HPV gene chips. HPV samples, after being labeled with fluorescent dye by restriction display PCR (RD-PCR) technology, were hybridized with the microarray, which was followed by scanning and analysis. The experimental condition for preparing the HPV gene chips was investigated, and the possibility of HPV genotyping using gene chips was discussed. The technique that was established in this study for preparing HPV gene chips is practical. The results of the present study demonstrated the versatility and inspiring prospect of using this technology to detect and genotype HPV.

Liu, H. C., H. H. Cheng, et al. (2001). "A strategy to identify positional candidate genes conferring Marek's disease resistance by integrating DNA microarrays and genetic mapping." Anim Genet **32**(6): 351-9.

Marker-assisted selection (MAS) to enhance genetic resistance to Marek's disease (MD), a herpesvirus-induced T cell cancer in chicken, is an attractive alternative to augment control with vaccines. Our earlier studies indicate that there are many quantitative trait loci (QTL) containing one or more genes that confer genetic resistance to MD. Unfortunately, it is difficult to sufficiently resolve these QTL to identify the causative gene and generate tightly linked markers. One possible solution is to identify positional candidate genes by virtue of gene expression differences between MD resistant and susceptible chicken using deoxyribonucleic acid (DNA) microarrays followed by genetic mapping of the differentially-expressed genes. In this preliminary study, we show that DNA microarrays containing approximately 1200 genes or expressed sequence tags (ESTs) are able to reproducibly detect differences in gene expression between the inbred ADOL lines 63 (MD resistant) and 72 (MD susceptible) of uninfected and Marek's disease virus (MDV)-infected peripheral blood lymphocytes. Microarray data were validated by quantitative polymerase chain reaction (PCR) and found to be consistent with previous literature on gene induction or immune response. Integration of the microarrays with genetic mapping data was achieved with a sample of 15 genes. Twelve of these genes had mapped human orthologues. Seven genes were located on the chicken linkage map as predicted by the human-chicken comparative map, while two other genes defined a new conserved syntenic group. More importantly, one of the genes with differential expression is known to confer genetic resistance to MD while another gene is a prime positional candidate for a QTL.

Liu, R., A. M. Enstrom, et al. (2003). "Combinatorial peptide library methods for immunobiology research." Exp Hematol **31**(1): 11-30.

The field of combinatorial peptide chemistry has emerged as a powerful tool in the study of many biological systems. This review focuses on combinatorial peptide library methodology, which includes biological library methods, spatially addressable parallel library methods, library methods requiring deconvolution, the "one-bead one-compound" library method, and affinity chromatography selection method. These peptide libraries have successfully been employed to study a vast array of cell surface receptors, as well as have been useful in identifying protein kinase substrates and inhibitors. In recent immunobiological applications, peptide libraries have proven monumental in the definition of MHC anchor residues, in lymphocyte epitope mapping, and in the development of peptide vaccines. Peptides identified from such libraries, when presented in a chemical microarray format, may prove useful in immunodiagnostics. Combinatorial peptide libraries offer a high-throughput approach to study limitless biological targets. Peptides discovered from such studies may be therapeutically and diagnostically useful agents.

Liu, R. H., J. Yang, et al. (2004). "Self-contained, fully integrated biochip for sample preparation,

polymerase chain reaction amplification, and DNA microarray detection." Anal Chem **76**(7): 1824-31.

A fully integrated biochip device that consists of microfluidic mixers, valves, pumps, channels, chambers, heaters, and DNA microarray sensors was developed to perform DNA analysis of complex biological sample solutions. Sample preparation (including magnetic bead-based cell capture, cell preconcentration and purification, and cell lysis), polymerase chain reaction, DNA hybridization, and electrochemical detection were performed in this fully automated and miniature device. Cavitation microstreaming was implemented to enhance target cell capture from whole blood samples using immunomagnetic beads and accelerate DNA hybridization reaction. Thermally actuated paraffin-based microvalves were developed to regulate flows. Electrochemical pumps and thermopneumatic pumps were integrated on the chip to provide pumping of liquid solutions. The device is completely self-contained: no external pressure sources, fluid storage, mechanical pumps, or valves are necessary for fluid manipulation, thus eliminating possible sample contamination and simplifying device operation. Pathogenic bacteria detection from approximately milliliters of whole blood samples and single-nucleotide polymorphism analysis directly from diluted blood were demonstrated. The device provides a cost-effective solution to direct sample-to-answer genetic analysis and thus has a potential impact in the fields of point-of-care genetic analysis, environmental testing, and biological warfare agent detection.

Locht, C., R. Antoine, et al. (2004). "Bordetella pertussis from functional genomics to intranasal vaccination." Int J Med Microbiol **293**(7-8): 583-8.

Whooping cough still represents a major health problem, despite the use of effective vaccines for several decades. Being classically a typical childhood disease, whooping cough in young adults is now more common than it used to be, suggesting that protection after vaccination wanes during adolescence. As an alternative to the current vaccines, we wish to develop live attenuated vaccines to be delivered by the nasal route, such as to mimic the natural route of infection and to induce long lasting immunity. Bordetella pertussis, the etiological agent of whooping cough, produces a number of virulence factors, including toxins. Its recently determined genome sequence makes it now possible to apply functional genomics, such as transcriptomics and systematic knock-out mutagenesis. The expression of most known B. pertussis virulence genes is controlled by the two-component system BvgA/S. DNA microarray analyses have led to the identification of novel genes in the BvgA/S regulon, some of which are activated by BvgA/S and others are repressed by BvgA/S. In addition, some genes appear to be differentially modulated by nicotinic acid and MgSO4, both known to modulate the expression of BvgA/S-regulated genes. Among others, the functional genomics approach has uncovered two strongly BvgA/S-activated genes, named hotA and hotB (for 'homolog of toxin'), the products of which show high sequence similarities to pertussis toxin subunits. The identification of the full array of virulence factors, as well as an integrated understanding of the bacterial physiology should allow us to design attenuated B. pertussis strains useful for intranasal vaccination. A first generation of attenuated strains has already shown full protection in mice after a single intranasal administration. Such strains may also serve as vaccine carriers for heterologous antigens, in order to vaccinate against several different pathogens simultaneously.

Lorenz, M. C. (2002). "Genomic approaches to fungal pathogenicity." Curr Opin Microbiol **5**(4): 372-8.

Within a few years, the genome sequences of a large number of medically and agriculturally important fungi will be known. With this resource come the promises of genomic approaches to study pathogenicity and host-fungus interactions. Genomics is particularly attractive for these questions, as conventional genetic and biochemical approaches are limited in many pathogenic fungi. Recent work has applied signature-tagged mutagenesis and DNA microarray analysis to virulence studies in several fungal species, and novel approaches, such as protein arrays and genomic deletion libraries, are being developed in Saccharomyces cerevisiae and have significant potential in other fungi. High-throughput gene-discovery approaches should greatly increase our understanding of fungal pathogenesis.

Lovmar, L., C. Fock, et al. (2003). "Microarrays for genotyping human group a rotavirus by multiplex

capture and type-specific primer extension." J Clin Microbiol **41**(11): 5153-8.

Human group A rotavirus (HRV) is the major cause of severe gastroenteritis in infants worldwide. HRV shares the feature of a high degree of genetic diversity with many other RNA viruses, and therefore, genotyping of this organism is more complicated than genotyping of more stable DNA viruses. We describe a novel microarray-based method that allows high-throughput genotyping of RNA viruses with a high degree of polymorphism by multiplex capture and type-specific extension on microarrays. Denatured reverse transcription (RT)-PCR products derived from two outer capsid genes of clinical isolates of HRV were hybridized to immobilized capture oligonucleotides representing the most commonly occurring P and G genotypes on a microarray. Specific primer extension of the type-specific capture oligonucleotides was applied to incorporate the fluorescent nucleotide analogue cyanine 5-labeled dUTP as a detectable label. Laser scanning and fluorescence detection of the microarrays was followed by visual or computer-assisted interpretation of the fluorescence patterns generated on the microarrays. Initially, the method detected HRV in all 40 samples and correctly determined both the G and the P genotypes of 35 of the 40 strains analyzed. After modification by inclusion of additional capture oligonucleotides specific for the initially unassigned genotypes, all genotypes could be correctly defined. The results of genotyping with the microarray fully agreed with the results obtained by nucleotide sequence analysis and sequence-specific multiplex RT-PCR. Owing to its robustness, simplicity, and general utility, the microarray-based method may gain wide applicability for the genotyping of microorganisms, including highly variable RNA and DNA viruses.

Loy, A., A. Lehner, et al. (2002). "Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment." Appl Environ Microbiol **68**(10): 5064-81.

For cultivation-independent detection of sulfate-reducing prokaryotes (SRPs) an oligonucleotide microarray consisting of 132 16S rRNA gene-targeted oligonucleotide probes (18-mers) having hierarchical and parallel (identical) specificity for the detection of all known lineages of sulfate-reducing prokaryotes (SRP-PhyloChip) was designed and subsequently evaluated with 41 suitable pure cultures of SRPs. The applicability of SRP-PhyloChip for diversity screening of SRPs in environmental and clinical samples was tested by using samples from periodontal tooth pockets and from the chemocline of a hypersaline cyanobacterial mat from Solar Lake (Sinai, Egypt). Consistent with previous studies, SRP-PhyloChip indicated the occurrence of Desulfomicrobium spp. in the tooth pockets and the presence of Desulfonema- and Desulfomonile-like SRPs (together with other SRPs) in the chemocline of the mat. The SRP-PhyloChip results were confirmed by several DNA microarray-independent techniques, including specific PCR amplification, cloning, and sequencing of SRP 16S rRNA genes and the genes encoding the dissimilatory (bi)sulfite reductase (dsrAB).

Lu, C., W. Liu, et al. (2002). "[Primitive study of the cell lineage and Epstein-Barr virus infection in so called malignant histiocytosis with tissue microarray technique]." Zhonghua Bing Li Xue Za Zhi **31**(6): 497-501.

OBJECTIVE: To investigate the cell lineage and Epstein-Barr virus infection in previously diagnosed cases of malignant histiocytosis (MH) with tissue microarray technique. METHODS: Using tissue-chips, immunohistochemical staining, in situ hybridization and PCR to analyze 5 autopsy cases of MH. RESULTS: (1) In all 5 cases, positive reactions of CD45RO, CD3 epsilon, TIA-1, Granzyme B were detected in the neoplastic cells, whereas negative reactions were found with CD30, CD20, CD56. (2) All cases revealed EBER1/2 positivity in neoplastic cells. CONCLUSION: Previously diagnosed malignant histiocytosis is an EBV-associated aggressive NK/T cell lymphoma.

Lu, L., W. L. Ma, et al. (2003). "Quick preparations of human parvovirus B19 microarray probes using PCR." Di Yi Jun Yi Da Xue Xue Bao **23**(11): 1121-4.

OBJECTIVE: To prepare DNA microarray probes for the detection of human parvovirus B19.

Method Specific PCR primers were designed with the Primer Premier 5.0 to amplify the conserved regions of human parvovirus B19 genome. The PCR products were cloned into the pMD-18 T vector. METHOD: Sequences analysis showed the PCR products conformed to the sequences contained in the genome of human parvovirus B19. RESULT: Sequences analysis showed the PCR products conformed to the sequences contained in the genome of human parvovirus B19. CONCLUSION: PCR amplification of the conserved and specific human parvovirus B19 genes is simple and effective to prepare the desired probes.

Luchtan, M., C. Warade, et al. (2004). "TcruziDB: an integrated Trypanosoma cruzi genome resource." Nucleic Acids Res **32 Database issue**: D344-6.

TcruziDB (http://TcruziDB.org) is an integrated genome database for the parasitic organism Trypanosoma cruzi, the causative agent of Chagas' disease. The database currently incorporates all available sequence data (Genomic, BAC, EST) in a single user-friendly location. The database contains a variety of tools specifically designed for searching unannotated draft sequence via BLAST, keyword searches of pre-computed BLAST results, and protein motif searches. Release 1.0 of the database contains nearly 730 million bp of genome sequence from 1.1 million sequence reads generated by the TIGR-Karolinska-SBRI Trypanosoma cruzi Genome Consortium and 15 million bp of clustered EST and genomic sequence obtained from other sources. As annotation, microarray and proteomic data become available, the database will incorporate and integrate these data using the GUS (http://www.gusdb. org) relational framework.

Lueking, A., M. Horn, et al. (1999). "Protein microarrays for gene expression and antibody screening." Anal Biochem **270**(1): 103-11.

Proteins translate genomic sequence information into function, enabling biological processes. As a complementary approach to gene expression profiling on cDNA microarrays, we have developed a technique for high-throughput gene expression and antibody screening on chip-size protein microarrays. Using a picking/spotting robot equipped with a new transfer stamp, protein solutions were gridded onto polyvinylidene difluoride filters at high density. Specific purified protein was detected on the filters with high sensitivity (250 amol or 10 pg of a test protein). On a microarray made from bacterial lysates of 92 human cDNA clones expressed in a microtiter plate, putative protein expressors could be reliably identified. The rate of false-positive clones, expressing proteins in incorrect reading frames, was low. Product specificity of selected clones was confirmed on identical microarrays using monoclonal antibodies. Cross-reactivities of some antibodies with unrelated proteins imply the use of protein microarrays for antibody specificity screening against whole libraries of proteins. Because this application would not be restricted to antigen-antibody systems, protein microarrays should provide a general resource for high-throughput screens of gene expression and receptor-ligand interactions.

Luo, Y., J. Cai, et al. (2003). "Designing, testing, and validating a focused stem cell microarray for characterization of neural stem cells and progenitor cells." Stem Cells **21**(5): 575-87.

Fetal neural stem cells (NSCs) have received great attention not only for their roles in normal development but also for their potential use in the treatment of neurodegenerative disorders. To develop a robust method of assessing the state of stem cells, we have designed, tested, and validated a rodent NSC array. This array consists of 260 genes that include cell type-specific markers for embryonic stem (ES) cells and neural progenitor cells as well as growth factors, cell cycle-related genes, and extracellular matrix molecules known to regulate NSC biology. The 500-bp polymerase chain reaction products amplified and validated by using gene-specific primers were arrayed along with positive controls. Blanks were included for quality control, and some genes were arrayed in duplicate. No cross-hybridization was detected. The quality of the arrays and their sensitivity were also examined by using probes prepared by conventional reverse transcriptase or by using amplified probes prepared by linear polymerase replication (LPR). Both methods showed good reproducibility, and probes prepared by LPR labeling appeared to

detect expression of a larger proportion of expressed genes. Expression detected by either method could be verified by RT-PCR with high reproducibility. Using these stem cell chips, we have profiled liver, ES, and neural cells. The cell types could be readily distinguished from each other. Nine markers specific to mouse ES cells and 17 markers found in neural cells were verified as robust markers of the stem cell state. Thus, this focused neural stem array provides a convenient and useful tool for detection and assessment of NSCs and progenitor cells and can reliably distinguish them from other cell populations.

Maeda, S., M. Otsuka, et al. (2001). "cDNA microarray analysis of Helicobacter pylori-mediated alteration of gene expression in gastric cancer cells." Biochem Biophys Res Commun **284**(2): 443-9.

Helicobacter pylori infection stimulates several intracellular signaling pathways and is accompanied by increased gene expression in gastric epithelial cells. High-density cDNA microarray was used to characterize the mRNA expression profile of genes in human gastric cancer cells (MKN45, AGS) cocultured with H. pylori. Coculture with cag pathogenicity island (PAI)-positive H. pylori (wild-type) significantly up-regulated mRNA expression in 8 of 2304 genes tested. In 6 (interleukin-8, I(kappaB)alpha, A20, ERF-1, keratin K7, glutathione peroxidase) of the 8 genes, up-regulation was confirmed by RT-PCR. In coculture with isogenic cagE-negative mutant ((Delta)cagE), which encodes a type IV secretion system with other genes in the cag PAI, no significant up-regulation was found. We further analyzed the role of A20. Transfection of expression vector encoding A20 resulted in an inhibition of H. pylori-mediated NF-kappaB activation, indicating that H. pylori-mediated A20 expression could be a negative regulator of NF-kappaB activation. Taken together, these results indicate the importance of microarray technology as a tool for analyzing the complex interplay between H. pylori and the host.

Mahony, J. B. (2002). "Chlamydiae host cell interactions revealed using DNA microarrays." Ann N Y Acad Sci **975**: 192-201.

Chlamydiae are obligate intracellular bacterial parasites that infect eukaryotic cells and live their entire life cycle within a cytoplasmic vacuole or inclusion. We have employed cDNA microarray and conventional biological approaches to study the pathogen-host cell interaction during C. pneumoniae infection of eukaryotic cells. Two host cell signaling pathways, MEK/ERK and PI 3-kinase/Akt, were activated within 5 and 20 minutes, respectively, following infection with chlamydiae. Pharmacological inhibition of these pathways blocked invasion of HEp2 cells indicating that activation of these pathways was required for infection. Rho family GTPase activity was essential for invasion, since the pan-Rho GTPase inhibitor, compactin, blocked infection of HEp2 cells. cDNA microarrays and reverse transcriptase PCR were used to study host cell and chlamydial gene expression during the replication cycle. Analysis of host cell gene expression following infection with C. pneumoniae indicated that genes coding for cytokines, growth factors, and signaling molecules were upregulated, as early as 2 hours postinfection. Analysis of chlamydial gene expression indicated a temporal regulation of transcription with distinct early-, mid-, and late-cycle classes of RNA transcripts. Newly discovered genes encoding three Ser/Thr protein kinases and one protein phosphatase were upregulated 6-12 hours postinfection. One protein kinase, designated CpnPK1, was first detected at 12 hours postinfection, accumulated in the inclusion throughout the replication cycle, and may be a type III effector molecule. An increased understanding of chlamydial host cell interactions, in particular the role of various chlamydial proteins in infection and identification of essential virulence factors should provide novel targets for the development of new antimicrobials.

Maitra, A., Y. Cohen, et al. (2004). "The Human MitoChip: a high-throughput sequencing microarray for mitochondrial mutation detection." Genome Res **14**(5): 812-9.

Somatic mitochondrial mutations are common in human cancers, and can be used as a tool for early detection of cancer. We have developed a mitochondrial Custom Reseq microarray as an array-based sequencing platform for rapid and high-throughput analysis of mitochondrial DNA. The MitoChip contains oligonucleotide probes synthesized using standard photolithography and solid-phase synthesis,

and is able to sequence >29 kb of double-stranded DNA in a single assay. Both strands of the entire human mitochondrial coding sequence (15,451 bp) are arrayed on the MitoChip; both strands of an additional 12,935 bp (84% of coding DNA) are arrayed in duplicate. We used 300 ng of genomic DNA to amplify the mitochondrial coding sequence in three overlapping long PCR fragments. We then sequenced >2 million base pairs of mitochondrial DNA, and successfully assigned base calls at 96.0% of nucleotide positions. Replicate experiments demonstrated >99.99% reproducibility. In matched fluid samples (urine and pancreatic juice, respectively) obtained from five patients with bladder cancer and four with pancreatic cancer, the MitoChip detected at least one cancer-associated mitochondrial mutation in six (66%) of nine samples. The MitoChip is a high-throughput sequencing tool for the reliable identification of mitochondrial DNA mutations from primary tumors in clinical samples.

Makower, D., A. Rozenblit, et al. (2003). "Phase II clinical trial of intralesional administration of the oncolytic adenovirus ONYX-015 in patients with hepatobiliary tumors with correlative p53 studies." Clin Cancer Res **9**(2): 693-702.

PURPOSE: ONYX-015 is a genetically modified adenovirus with a deletion of the E1B early gene and is therefore designed to replicate preferentially in p53-mutated cells. A Phase II trial of intralesional ONYX-015 was conducted in patients with hepatobiliary tumors to determine the safety and efficacy of such a treatment. EXPERIMENTAL DESIGN: All patients had biopsy-proven, measurable tumors of the liver, gall bladder, or bile ducts that were beyond the scope of surgical resection. Patients received intralesional injections of ONYX-015 at either 6 x 10(9) or 1 x 10(10) plaque-forming units/lesion up to a total dose of 3 x 10(10) plaque-forming units, and i.p. injections were allowed in patients with malignant ascites. The status of p53 was assessed by immunohistochemistry or Affymetrix GeneChip microarray analysis. Studies were conducted for viral shedding and for the presence of antiadenoviral antibodies before and after the injection of ONYX-015. Patients were assessed for response and toxicity. RESULTS: Twenty patients were enrolled, and 19 patients were eligible. Half of the patients had primary bile duct carcinomas. Serious toxicities (> grade 2) were uncommon and included hepatic toxicity (three patients), anemia (one patient), infection (one patient), and cardiac toxicity (one patient, atrial fibrillation). Sixteen patients were evaluable for response. Among these evaluable patients, 1 of 16 (6.3%) had a partial response, 1 of 16 (6.3%) had prolonged disease stabilization (49 weeks), and 8 of 16 (50%) had a >50% reduction in tumor markers. Of the 19 eligible patients, 18 (94.7%) had specimens available for p53 analysis. Fifteen of these 18 patients (83.3%) had evidence of p53 mutation by one or both methods, although the methods correlated poorly. Viral shedding was confined to bile (two of two patients) and ascites (four of four patients). Pretreatment adenoviral antibodies were present in 14 of 14 patients and increased by 33.2% after ONYX-015 treatment. CONCLUSIONS: Intralesional treatment with ONYX-015 in patients with hepatobiliary tumors is safe and well tolerated, and some patients had evidence of an anticancer effect. The high incidence of p53 mutations in these tumors makes this a logical population in which to test this therapy but precludes definitive evaluation about the necessity of a p53 mutation for ONYX-015 clinical activity.

Malloff, C. A., R. C. Fernandez, et al. (2001). "Bacterial comparative genomic hybridization: a method for directly identifying lateral gene transfer." J Mol Biol **312**(1): 1-5.

Horizontally transferred DNA is largely responsible for the dissemination of virulence traits amongst bacteria. Rapid identification of acquired DNA remains difficult as whole-genome sequencing of outbreak strains is impractical, and microarray-based approaches, while powerful, are limited to genes present only in the reference strains. Here we present a novel bacterial comparative genomic hybridization method that directly compares the genomes of related strains at sub-kilobase resolution in order to identify acquired DNA. Bacterial comparative genomic hybridization utilizes the concept of metaphase chromosome comparative genomic hybridization, and exploits the resolving power of two-dimensional DNA electrophoresis. Comparison of isogenic variants of the pathogen Pseudomonas aeruginosa detected a single-copy gene insertion responsible for gentamicin resistance.

Malorny, B., B. Guerra, et al. (2003). "[Typing of Salmonella by DNA-microarrays]." Berl Munch Tierarztl Wochenschr **116**(11-12): 482-6.

Microarrays (DNA-Chips) are miniaturized carriers on which many nucleic acid molecule probes such as oligonucleotides or PCR products are immobilized in a high density, and compactness. Homologue DNA hybridises with the immobilized complementary nucleic acid probes. This study gives after a short general introduction in the principle of DNA-microarrays an overview about published data on the field of typing of Salmonella by microarrays. An onset of a DNA-microarray developed by the National Reference Laboratory for Salmonella (NRL-Salm) will be introduced. By this new technique, it is possible to answer epidemiological questions as well as to find genes involved in certain biochemical processes, such as pathogenicity or resistance of salmonellae.

Manabe, Y. C., A. M. Dannenberg, Jr., et al. (2003). "Different strains of Mycobacterium tuberculosis cause various spectrums of disease in the rabbit model of tuberculosis." Infect Immun **71**(10): 6004-11.

The rabbit model of tuberculosis has been used historically to differentiate between Mycobacterium tuberculosis and Mycobacterium bovis based on their relative virulence in this animal host. M. tuberculosis infection in market rabbits is cleared over time, whereas infection with M. bovis results in chronic, progressive, cavitary disease leading to death. Because of the innate resistance of commercial rabbits to M. tuberculosis, 320 to 1,890 log-phase, actively growing inhaled bacilli were required to form one grossly visible pulmonary tubercle at 5 weeks. The range of inhaled doses required to make one tubercle allows us to determine the relative pathogenicities of different strains. Fewer inhaled organisms of the M. tuberculosis Erdman strain were required than of M. tuberculosis H37Rv to produce a visible lesion at 5 weeks. Furthermore, with the Erdman strain, only 7 of 15 rabbits had healed lesions at 16 to 18 weeks; among the other animals, two had chronic, progressive cavitary disease, a phenotype usually seen only with M. bovis infection. Genotypic investigation of the Erdman strain with an H37Rv-based microarray identified gene differences in the RD6 region. Southern blot and PCR structural genetic analysis showed significant differences between M. tuberculosis strains in this region. Correlation of the relative pathogenicity, including disease severity, in the rabbit model with the strain genotype may help identify stage-specific M. tuberculosis genes important in human disease.

Manger, I. D. and D. A. Relman (2000). "How the host 'sees' pathogens: global gene expression responses to infection." Curr Opin Immunol **12**(2): 215-8.

Innate immune responses to pathogens are believed to be patterned and stereotyped. Adaptive responses display variety but in relatively few types of products and with limited numbers of mechanisms. Is this apparent disparity between microbial pathogen diversity and a restricted set of host responses an accurate picture of infection or is it the result of a limited collection of analytic tools? DNA microarray technology permits one to address simple descriptive questions about global gene expression inside cells. In particular, it offers an opportunity to examine the relationship between host and pathogen in much greater detail than has been possible previously. One can now ask, firstly, how a host cell or organism 'sees' a microbial pathogen from the viewpoint of gene expression responses and, secondly, at what level it is able to discriminate between different agents. Other potential insights to be reaped include the identification of microbial determinants of the host response, the temporal features of the 'conversation' between host and pathogen, novel strategies for therapeutic and prophylactic intervention and prognostic markers of outcome.

Marks, S. L. and E. J. Kather (2003). "Bacterial-associated diarrhea in the dog: a critical appraisal." Vet Clin North Am Small Anim Pract **33**(5): 1029-60.

The clinical documentation of enteropathogenic bacteria causing diarrhea in dogs is clouded by the presence of many of these organisms existing as normal constituents of the indigenous intestinal flora. The diagnosis of a putative bacterial enteropathogen(s) in dogs should be made based on a combination of parameters, including signalment and predisposing factors, clinical signs, serologic assays for toxins,

fecal culture, and PCR. Relying on results of fecal culture alone is problematic, because C perfringens, C difficile, Campylobacter spp, and pathogenic and non-pathogenic E coli are commonly isolated from apparently healthy dogs [10,13,33]. Nevertheless, culture may be useful in procuring isolates for the application of molecular techniques, such as PCR, for detection of specific toxin genes or molecular typing of isolated strains to establish clonality in suspected outbreaks. The oversimplistic attempt to characterize bacterially associated diarrhea by anatomic localization of clinical signs should be discouraged, because most of the previously mentioned bacteria have been associated with small and large intestinal diarrhea. Accurate diagnosis of infections may require diagnostic laboratories to incorporate PCR-based assays using genus- and species-specific primers to facilitate detection of toxin genes and differentiation of species that appear phenotypically and biochemically similar. There has been tremendous interest in the application of microarray technology for the simultaneous detection of thousands of genes or target DNA sequences on one glass slide. This powerful tool could be used for detection of specific pathogenic bacterial strains in fecal specimens obtained from dogs in the future.

McClelland, M., K. E. Sanderson, et al. (2001). "Complete genome sequence of Salmonella enterica serovar Typhimurium LT2." Nature **413**(6858): 852-6.

Salmonella enterica subspecies I, serovar Typhimurium (S. typhimurium), is a leading cause of human gastroenteritis, and is used as a mouse model of human typhoid fever. The incidence of non-typhoid salmonellosis is increasing worldwide, causing millions of infections and many deaths in the human population each year. Here we sequenced the 4,857-kilobase (kb) chromosome and 94-kb virulence plasmid of S. typhimurium strain LT2. The distribution of close homologues of S. typhimurium LT2 genes in eight related enterobacteria was determined using previously completed genomes of three related bacteria, sample sequencing of both S. enterica serovar Paratyphi A (S. paratyphi A) and Klebsiella pneumoniae, and hybridization of three unsequenced genomes to a microarray of S. typhimurium LT2 genes. Lateral transfer of genes is frequent, with 11% of the S. typhimurium LT2 genes missing from S. enterica serovar Typhi (S. typhi), and 29% missing from Escherichia coli K12. The 352 gene homologues of S. typhimurium LT2 confined to subspecies I of S. enterica-containing most mammalian and bird pathogens-are useful for studies of epidemiology, host specificity and pathogenesis. Most of these homologues were previously unknown, and 50 may be exported to the periplasm or outer membrane, rendering them accessible as therapeutic or vaccine targets.

McClung, C. A. and E. J. Nestler (2003). "Regulation of gene expression and cocaine reward by CREB and DeltaFosB." Nat Neurosci **6**(11): 1208-15.

DeltaFosB (a truncated form of FosB) and CREB (cAMP response element binding protein) are transcription factors induced in the brain's reward pathways after chronic exposure to drugs of abuse. However, their mechanisms of action and the genes they regulate remain unclear. Using microarray analysis in the nucleus accumbens of inducible transgenic mice, we found that CREB and a dominant-negative CREB have opposite effects on gene expression, as do prolonged expression of DeltaFosB and the activator protein-1 (AP-1) antagonist DeltacJun. However, unlike CREB, short-term and prolonged DeltaFosB induction had opposing effects on gene expression. Gene expression induced by short-term DeltaFosB and by CREB was strikingly similar, and both reduced the rewarding effects of cocaine, whereas prolonged DeltaFosB expression increased drug reward. Gene expression after a short cocaine treatment was more dependent on CREB, whereas gene expression after a longer cocaine treatment became increasingly DeltaFosB dependent. These findings help define the molecular functions of CREB and DeltaFosB and identify clusters of genes that contribute to cocaine addiction.

McGill, G. G., M. Horstmann, et al. (2002). "Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability." Cell **109**(6): 707-18.

Kit/SCF signaling and Mitf-dependent transcription are both essential for melanocyte development and pigmentation. To identify Mitf-dependent Kit transcriptional targets in primary

melanocytes, microarray studies were undertaken. Among identified targets was BCL2, whose germline deletion produces melanocyte loss and which exhibited phenotypic synergy with Mitf in mice. BCL2's regulation by Mitf was verified in melanocytes and melanoma cells and by chromatin immunoprecipitation of the BCL2 promoter. Mitf also regulates BCL2 in osteoclasts, and both Mitf(mi/mi) and Bcl2(-/-) mice exhibit severe osteopetrosis. Disruption of Mitf in melanocytes or melanoma triggered profound apoptosis susceptible to rescue by BCL2 overexpression. Clinically, primary human melanoma expression microarrays revealed tight nearest neighbor linkage for MITF and BCL2. This linkage helps explain the vital roles of both Mitf and Bcl2 in the melanocyte lineage and the well-known treatment resistance of melanoma.

Menssen, A. and H. Hermeking (2002). "Characterization of the c-MYC-regulated transcriptome by SAGE: identification and analysis of c-MYC target genes." Proc Natl Acad Sci U S A **99**(9): 6274-9.

To identify target genes of the oncogenic transcription factor c-MYC, serial analysis of gene expression (SAGE) was performed after adenoviral expression of c-MYC in primary human umbilical vein endothelial cells: 216 different SAGE tags, corresponding to unique mRNAs, were induced, whereas 260 tags were repressed after c-MYC expression ($P < 0.05$). The induction of 53 genes was confirmed by using microarray analysis and quantitative real-time PCR: among these genes was MetAP2/p67, which encodes an activator of translational initiation and represents a validated target for inhibition of neovascularization. Furthermore, c-MYC induced the cell cycle regulatory genes CDC2-L1, Cyclin E binding protein 1, and Cyclin B1. The DNA repair genes BRCA1, MSH2, and APEX were induced by c-MYC, suggesting that c-MYC couples DNA replication to processes preserving the integrity of the genome. MNT, a MAX-binding antagonist of c-MYC function, was up-regulated, implying a negative feedback loop. In vivo promoter occupancy by c-MYC was detected by chromatin immunoprecipitation for CDK4, Prohibitin, MNT, Cyclin B1, and Cyclin E binding protein 1, showing that these genes are direct c-MYC targets. The c-MYC-regulated genes/tags identified here will help to define the set of bona fide c-MYC targets and may have potential therapeutic value for inhibition of cancer cell proliferation, tumor-vascularization, and restenosis.

Mezzasoma, L., T. Bacarese-Hamilton, et al. (2002). "Antigen microarrays for serodiagnosis of infectious diseases." Clin Chem **48**(1): 121-30.

BACKGROUND: Progress in robotic printing technology has allowed the development of high-density nucleic acid and protein arrays that have increased the throughput of a variety of assays. We generated protein microarrays by printing microbial antigens to simultaneously determine in human sera antibodies directed against Toxoplasma gondii, rubella virus, cytomegalovirus (CMV), and herpes simplex virus (HSV) types 1 and 2 (ToRCH antigens). METHODS: The antigens were printed on activated glass slides with high-speed robotics. The slides were incubated first with serum samples and subsequently with fluorescently labeled secondary antibodies. Human IgG and IgM bound to the printed antigens were detected by confocal scanning microscopy and quantified with internal calibration curves. Both microarrays and commercial ELISAs were utilized to detect serum antibodies against the ToRCH antigens in a panel of characterized human sera. RESULTS: The detection limit (mean + 2 SD) of the microarray assay was 0.5 pg of IgG or IgM bound to the slides. Within-slide, between-slide, and between-batch precision profiles showed CVs of 1.7-18% for all antigens. Overall, >80% concordance was obtained between microarray assays and ELISAs in the classification of sera; for T. gondii, CMV, and HSV1, concordance exceeded 90%. CONCLUSIONS: The microarray is a suitable assay format for the serodiagnosis of infectious diseases and can be easily optimized for clinical use. The ToRCH assay performs equivalently to ELISA and may have potentially important advantages in throughput, convenience, and cost.

Mikovits, J., F. Ruscetti, et al. (2001). "Potential cellular signatures of viral infections in human hematopoietic cells." Dis Markers **17**(3): 173-8.

Expression profiling of cellular genes was performed using a 10,000 cDNA human gene array in order to identify expression changes following chronic infection of human hematopoietic cells with Kaposi's Sarcoma-associated Virus (KSHV) also known as Human Herpesvirus 8 (HHV8) and Human T cell leukemia virus-1 (HTLV-1). We performed cell-free in vitro infection of primary bone marrow derived CD34+ cells using semi-purified HHV8 and a mature IL-2 dependent T cell line, KIT 225, using highly concentrated viral stocks prepared from an infectious molecular clone of HTLV-1. Thirty days post infection, mRNA was isolated from infected cultures and uninfected controls and submitted for microarray analysis. More than 400 genes were differentially expressed more than two-fold following HHV8 infection of primary bone marrow derived CD34+ cells. Of these 400, interferon regulatory factor 4 (IRF4), cyclin B2, TBP-associated factor, eukaryotic elongation factor and pim 2 were up-regulated more than 3.5 fold. In contrast, less than 100 genes were differentially expressed more than two-fold following chronic infection of a mature T cell line with HTLV-1. Of these, only cdc7 was up-regulated more than 3.5 fold. These data may provide insight into cellular signatures of infection useful for diagnosis of infection as well as potential targets for therapeutic intervention.

Min, W., H. S. Lillehoj, et al. (2003). "Profiling local gene expression changes associated with Eimeria maxima and Eimeria acervulina using cDNA microarray." Appl Microbiol Biotechnol **62**(4): 392-9.

Eimeria parasites show preferential sites of invasion in the avian intestine and produce a species-specific host immune response. Two economically important species, Eimeria acervulina and Eimeria maxima, preferentially invade and develop in the avian duodenum and jejunum/ileum, respectively. To investigate local host immune responses induced by parasite infection, global transcriptional changes in intestinal intraepithelial lymphocytes (IELs) induced by oral inoculation of chickens with E. acervulina or E. maxima were monitored using cDNA microarrays containing 400 unique chicken genes. Multiple gene transcripts were significantly up- or down-regulated following primary or secondary infection with E. acervulina or E. maxima. In general, infection by either parasite resulted in the expression changes of more genes following primary infection than following secondary infection, and E. acervulina caused more changes than did E. maxima. Although different regions of the small intestine were infected, similar changes in the levels of several cytokine mRNAs were observed in both Eimeria species following primary infection. Also identified was a set of transcripts whose expression was commonly enhanced or repressed in intestinal IELs of chickens infected with either parasite. Microarray analysis of chicken genes induced or repressed following Eimeria infection offers a powerful tool to enhance our understanding of host-parasite interactions leading to protective immunity.

Mitterer, G., M. Huber, et al. (2004). "Microarray-based identification of bacteria in clinical samples by solid-phase PCR amplification of 23S ribosomal DNA sequences." J Clin Microbiol **42**(3): 1048-57.

The rapid identification of the bacteria in clinical samples is important for patient management and antimicrobial therapy. We describe a DNA microarray-based PCR approach for the quick detection and identification of bacteria from cervical swab specimens from mares. This on-chip PCR method combines the amplification of a variable region of bacterial 23S ribosomal DNA and the simultaneous sequence-specific detection on a solid phase. The solid phase contains bacterial species-specific primers covalently bound to a glass support. During the solid-phase amplification reaction the polymerase elongates perfectly matched primers and incorporates biotin-labeled nucleotides. The reaction products are visualized by streptavidin-cyanine 5 staining, followed by fluorescence scanning. This procedure successfully identified from pure cultures 22 bacteria that are common causes of abortion and sterility in mares. Using the on-chip PCR method, we also tested 21 cervical swab specimens from mares for the presence of pathogenic bacteria and compared the results with those of conventional bacteriological culture methods. Our method correctly identified the bacteria in 12 cervical swab samples, 8 of which contained more than one bacterial species. Due to the higher sensitivity of the on-chip PCR, this method identified bacteria in five cervical swab samples which were not detected by the conventional identification procedure. Our results show that this method will have great potential to be incorporated

into the routine microbiology laboratory.

Miyachi, H. (2001). "[The present status and future prospect of the molecular diagnostic tests]." Rinsho Byori **49**(2): 139-49.

Assays for DNA or RNA sequences to diagnose infectious, neoplastic and genetic diseases have been widely used through recent progress in the molecular biology and biotechnology, and are now essential in care of patients under the advanced medicine through earlier and more accurate diagnosis. Automated systems have been developed for amplification and detection of nucleic acid sequence for infectious agents, using various nucleic acid amplification technology such as PCR. A fully automated PCR system and automated extraction of specific sequence for infectious agents such as hepatitis C virus RNA has been developed. These automated systems have provided improvement of not only assay efficiency but also quality control of the tests and have contributed to the standardization of them. Importance of development of systems for quality assessment and laboratory accreditation has been emphasized, particularly in those that still have been performed with manual methods. Based on the information on the genome sequence as the outcome of the human genome project, functions of genes and proteins have been studied by post-genomics such as expression profiling using DNA microarray, proteomics, single nucleotide polymorphisms analysis, coupled with bioinformatics. Along with advances in pharmacogenomics, these studies have raised the prospect of the development of tests for individualized medicine based on genetic information such as those predicting individual susceptibility to diseases for prevention and responsiveness to drugs for choice of treatment. For practice of such medicine, each genetic information and tests for it must be carefully evaluated and determined whether it is appropriate for cost-effective medicine through contributions to efficient process of decision-makings on patient care for prevention or avoidance of diseases and thus to cost savings.

Modrusan, Z., C. Marlowe, et al. (2000). "CPT-EIA assays for the detection of vancomycin resistant vanA and vanB genes in enterococci." Diagn Microbiol Infect Dis **37**(1): 45-50.

Cycling Probe Technology (CPT) was combined with a colorimetric enzyme-immuno assay (EIA) to develop two assays for the detection of vanA and vanB genes in vancomycin resistant enterococci (VRE). The CPT-EIA assay employs a gene-specific fluorescein labeled DNA-RNA-DNA probe that gets cleaved within the probe : target duplex. The cleaved DNA probe fragments dissociate from the target, making it available for further cycling. Following the separation of cleaved probe fragments, anti-fluorescein-horseradish peroxidase antibodies are used for the detection of uncleaved probes. The two CPT-EIA assays were used to screen a collection of 440 clinical isolates (Modrusan et al., 1999). All of the 154 VanA and 131 VanB isolates were correctly identified in the vanA and vanB CPT-EIA, respectively. The VanA and VanB isolates were differentiated from vancomycin sensitive enterococci (VSE) and also from the VanC isolates. In addition, an accurate VRE detection in the CPT-EIA assay was shown with cultures grown on eight different media.

Moir, S., A. Malaspina, et al. (2004). "Decreased survival of B cells of HIV-viremic patients mediated by altered expression of receptors of the TNF superfamily." J Exp Med **200**(5): 587-99.

Human immunodeficiency virus (HIV) infection leads to numerous perturbations of B cells through mechanisms that remain elusive. We performed DNA microarray, phenotypic, and functional analyses in an effort to elucidate mechanisms of B cell perturbation associated with ongoing HIV replication. 42 genes were up-regulated in B cells of HIV-viremic patients when compared with HIV-aviremic and HIV-negative patients, the majority of which were interferon (IFN)-stimulated or associated with terminal differentiation. Flow cytometry confirmed these increases and indicated that CD21(low) B cells, enhanced in HIV-viremic patients, were largely responsible for the changes. Increased expression of the tumor necrosis factor (TNF) superfamily (TNFSF) receptor CD95 correlated with increased susceptibility to CD95-mediated apoptosis of CD21(low) B cells, which, in turn, correlated with HIV plasma viremia. Increased expression of BCMA, a weak TNFSF receptor for B lymphocyte stimulator

(BLyS), on CD21(low) B cells was associated with a concomitant reduction in the expression of the more potent BLyS receptor, BAFF-R, that resulted in reduced BLyS binding and BLyS-mediated survival. These findings demonstrate that altered expression of genes associated with IFN stimulation and terminal differentiation in B cells of HIV-viremic patients lead to an increased propensity to cell death, which may have substantial deleterious effects on B cell responsiveness to antigenic stimulation.

Mongodin, E., J. Finan, et al. (2003). "Microarray transcription analysis of clinical Staphylococcus aureus isolates resistant to vancomycin." J Bacteriol **185**(15): 4638-43.

The transcriptomes of vancomycin intermediate-resistance Staphylococcus aureus (VISA) clinical isolates HIP5827 and Mu50 (MIC = 8 micro g/ml) were compared to those of highly vancomycin-resistant S. aureus (VRSA; MIC = 32 micro g/ml) passage derivatives by microarray. There were 35 genes with increased transcription and 16 genes with decreased transcription in common between the two VRSAs compared to those of their VISA parents. Of the 35 genes with increased transcription, 15 involved purine biosynthesis or transport, and the regulator (purR) of the major purine biosynthetic operon (purE-purD) was mutant. We hypothesize that increased energy (ATP) is required to generate the thicker cell walls that characterize resistant mutants.

Moses, A. V., M. A. Jarvis, et al. (2002). "A functional genomics approach to Kaposi's sarcoma." Ann N Y Acad Sci **975**: 180-91.

Kaposi's sarcoma (KS) is the most frequent malignancy afflicting acquired immune-deficiency syndrome (AIDS) patients. Tumor lesions are characterized by spindle cells of vascular origin and vascularization. Kaposi's sarcoma-associated herpes virus (KSHV) is consistently found in all forms of KS. Infection of dermal microvascular endothelial cells (DMVEC) with KSHV recapitulates spindle cell formation in vitro. We studied this transformation process by DNA microarray analysis comparing the RNA expression profiles of KSHV-infected and mock-infected DMVEC. Genes involved in tumorigenesis, angiogenesis, host defense, cell growth and differentiation, transcription, and metabolism were observed to change significantly upon infection with KSHV. One of the most consistently KSHV-induced genes was the receptor tyrosine kinase and proto-oncogene c-Kit. Inhibition of c-Kit activity with the pharmacological inhibitor of c-Kit signaling STI571 reversed the KSHV-induced morphological transformation of DMVEC. Moreover, overexpression studies showed that c-Kit was sufficient to induce spindle cell formation (Moses et al. J. Virol. 76(16): 8383-8399). These data demonstrate that microarrays are useful for the identification of pharmacological targets essential for KS tumorigenesis.

Munir, S. and V. Kapur (2003). "Transcriptional analysis of the response of poultry species to respiratory pathogens." Poult Sci **82**(6): 885-92.

Respiratory tract diseases are the single most important cause of economic loss due to infections among poultry populations worldwide. However, the molecular mechanisms of the host response to infections remain unknown. Here, we review the literature and describe the adoption of a conceptually simple approach to understand the genetic and biochemical responses of host cells during infection with respiratory pathogens, such as avian pneumovirus (APV). The strategy that we have adopted integrates the powerful techniques of cDNA subtraction hybridization and microarray analysis for global transcriptional profiling. The results of our investigations identify the specific transcriptional alterations in host-cell gene expression that result from an attempt by the host to combat and limit the spread of the pathogen or by the pathogen to enhance its own survival and ability to reproduce. Our studies suggest that a molecular description of host-pathogen interactions in terms of differential gene expression will provide key insights on the molecular basis of disease pathogenesis, pathogen virulence, and host immunity. In addition, the results suggest that the identification of genes and pathways with a role in host response to infection has considerable practical implications for the future design and development of effective immunomodulators and vaccines.

Nagasako, T., T. Sugiyama, et al. (2003). "Up-regulated Smad5 mediates apoptosis of gastric epithelial cells induced by Helicobacter pylori infection." J Biol Chem **278**(7): 4821-5.

The gastric pathogen Helicobacter pylori activates epithelial cell signaling pathways, and its infection induces changes in the expression of several genes in infected human gastric tissues. Recent studies have indicated that the ability of H. pylori to regulate epithelial cell responses depends on the presence of an intact cag pathogenicity island (cagPAI). We investigated altered mRNA expression of gastric epithelial cells after infection with H. pylori, both cagPAI-positive and cagPAI-negative strains, by cDNA microarray, reverse transcription PCR, and Northern blot analysis. Our results indicated that cagPAI-positive H. pylori strains (ATCC 43504 and clinical isolated strains) significantly activated Smad5 mRNA expression of human gastric epithelial cells (AGS, KATOIII, MKN28, and MKN45). We further examined whether the up-regulated Smad5 was related to apoptosis of gastric epithelial cells induced by H. pylori. Smad5 RNA interference completely inhibited H. pylori-induced apoptosis. These results suggest that Smad5 is up-regulated in gastric epithelial cells through the presence of cagPAI of H. pylori and that Smad5 mediates apoptosis of gastric epithelial cells induced by H. pylori infection.

Nam, J. H., K. A. Hwang, et al. (2003). "Expression of interferon inducible genes following Hantaan virus infection as a mechanism of resistance in A549 cells." Virus Genes **26**(1): 31-8.

Hantaan virus (HTN) is a causative agent of hemorrhagic fever with renal syndrome (HFRS). Little is known of its pathogenesis or the molecular mechanisms underlying resistance to HTN infection. In the present study, DNA microarray technology was used to monitor changes in mRNA levels after HTN infection, to elucidate resistance mechanisms to viral infection by understanding virus-host interactions. We found that several interferon (IFN)-inducible genes were up-regulated in host cells infected with HTN. According to previous available data, IFNs have been reported to be inhibitory, but their mode of action has not been yet clear. In this study, the 2',5'-oligoadenylated synthetase (OAS) and Mx1 genes, not a double-stranded RNA-dependent protein kinase R (PKR), of the IFN response pathways are associated with antiviral activity during HTN infection. Furthermore, A549 cells treated with IFN-alpha were protected against HTN infection. Taken together, these results confirmed that IFN plays a role in cellular defenses against HTN infection at an early stage of the infection and revealed the resistance mechanism for HTN infection.

Naranatt, P. P., H. H. Krishnan, et al. (2004). "Host gene induction and transcriptional reprogramming in Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8)-infected endothelial, fibroblast, and B cells: insights into modulation events early during infection." Cancer Res **64**(1): 72-84.

Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) is etiologically linked to the endothelial tumor Kaposi's sarcoma and with two lymphoproliferatve disorders, primary effusion lymphoma and multicentric Castleman's disease. HHV-8 infects a variety of target cells both in vivo and in vitro, binds to the in vitro target cells via cell surface heparan sulfate, and uses the alpha(3)beta(1) integrin as one of the entry receptors. Within minutes of infection, HHV-8 induced the integrin-mediated signaling pathways and morphological changes in the target cells (S. M. Akula et al., Cell, 108: 407-419, 2002; P. P. Naranatt et al., J. Virol., 77: 1524-1539, 2003). As an initial step toward understanding the role of host genes in HHV-8 infection and pathogenesis, modulation of host cell gene expression immediately after infection was examined. To reflect HHV-8's broad cellular tropism, mRNAs collected at 2 and 4 h after infection of primary human endothelial [human adult dermal microvascular endothelial cells (HMVECd)] and foreskin fibroblast [human foreskin fibroblast (HFF)] cells and human B cell line (BJAB) were analyzed by oligonucleotide array with approximately 22,000 human transcripts. With a criteria of >2-fold gene induction as significant, approximately 1.72% of the genes were differentially expressed, of which, 154 genes were shared by at least two cells and 33 genes shared by all three cells. HHV-8-induced transcriptional profiles in the endothelial and fibroblast cells were closely similar, with substantial differences in the B cells. In contrast to the antiapoptotic regulators induced in HMVECd and HFF cells, proapoptotic regulators were induced in the B cells. A robust increase in the expression of

IFN-induced genes suggestive of innate immune response induction was observed in HMVECd and HFF cells, whereas there was a total lack of immunity related protein inductions in B cells. These striking cell type-specific behaviors suggest that HHV-8-induced host cell gene modulation events in B cells may be different compared with the adherent endothelial and fibroblast target cells. Functional clustering of modulated genes identified several host molecules hitherto unknown to HHV-8 infection. These results indicate that early during infection, HHV-8 reprograms the host transcriptional machinery regulating a variety of cellular processes including apoptosis, transcription, cell cycle regulation, signaling, inflammatory response, and angiogenesis, all of which may play important roles in the biology and pathogenesis of HHV-8.

Neuman de Vegvar, H. E., R. R. Amara, et al. (2003). "Microarray profiling of antibody responses against simian-human immunodeficiency virus: postchallenge convergence of reactivities independent of host histocompatibility type and vaccine regimen." J Virol **77**(20): 11125-38.

We developed antigen microarrays to profile the breadth, strength, and kinetics of epitope-specific antiviral antibody responses in vaccine trials with a simian-human immunodeficiency virus (SHIV) model for human immunodeficiency virus (HIV) infection. These arrays contained 430 distinct proteins and overlapping peptides spanning the SHIV proteome. In macaques vaccinated with three different DNA and/or recombinant modified vaccinia virus Ankara (rMVA) vaccines encoding Gag-Pol or Gag-Pol-Env, these arrays distinguished vaccinated from challenged macaques, identified three novel viral epitopes, and predicted survival. Following viral challenge, anti-SHIV antibody responses ultimately converged to target eight immunodominant B-cell regions in Env regardless of vaccine regimen, host histocompatibility type, and divergent T-cell specificities. After challenge, responses to nonimmunodominant epitopes were transient, while responses to dominant epitopes were gained. These data suggest that the functional diversity of anti-SHIV B-cell responses is highly limited in the presence of persisting antigen.

Neuman de Vegvar, H. E. and W. H. Robinson (2004). "Microarray profiling of antiviral antibodies for the development of diagnostics, vaccines, and therapeutics." Clin Immunol **111**(2): 196-201.

Multiplex analysis of antiviral antibody (Ab) responses provides a potentially powerful strategy for viral diagnosis, prognostication, and development of vaccines and prophylactic Abs. In the coming years, advancements in proteomic technologies will provide even more robust methods to characterize antiviral Ab responses. Biomedical researchers will be faced with the exciting challenge of identifying antiviral Ab specificities that correlate with improved outcomes and efficacious interventions, and translating the findings into more effective diagnostics, prophylactics, and therapeutics.

Ng, L. K., P. Sawatzky, et al. (2002). "Characterization of ciprofloxacin resistance in Neisseria gonorrhoeae isolates in Canada." Sex Transm Dis **29**(12): 780-8.

BACKGROUND: Ciprofloxacin (500 mg orally, single dose) is one of the recommended therapies for gonorrhea in Canada. In Canada, the first ciprofloxacin-resistant (CipR) Neisseria gonorrhoeae strain was isolated in 1993. Antimicrobial susceptibilities of N gonorrhoeae isolates were monitored as part of a national surveillance program to ensure efficacy of antimicrobial therapies. GOAL: The goal was to determine the characteristics of ciprofloxacin resistance in Canadian gonococcal isolates. STUDY DESIGN: Susceptibility testing was performed on gonococcal strains from different provinces in Canada to determine the prevalence of CipR strains and their distribution. The CipR strains were further differentiated according to auxotype (A), serotype (S), plasmid profile (P), and pulsed-field gel electrophoresis (PFGE) profile. DNA sequencing and DNA microarray technology were used to determine mutations in gyrA and parC. RESULTS: In Canada, between 1997 and 1999, 4.8% of resistant strains (130 of 2687 antibiotic-resistant N gonorrhoeae isolates) were CipR (MICs of 1-32 microg/l) and belonged to 48 A/S/P classes. Sixty-eight of the strains that were not differentiated by A/S/P were subtyped into 47 classes with PFGE. DNA sequencing and DNA microarray showed that the most

common mutations had amino acid substitutions of Ser-->Phe at codon 91 and Asp-->Gly at codon 95 of the gyrA and Ser-->Arg at codon 87 of parC. CONCLUSION: The CipR strains isolated in Canada are phenotypically and genotypically diverse, indicating that they were imported from overseas and not endemic in Canada. Mutations in gyrA and parC previously only identified by DNA sequencing were successfully identified with DNA microarray technology. DNA microarray technology could be an alternative tool for identifying point mutations in resistance genes or other epidemiologic markers when clinical laboratories replace culture methods with rapid and automated molecular methods for diagnosis.

Ng, W. L., K. M. Kazmierczak, et al. (2003). "Transcriptional regulation and signature patterns revealed by microarray analyses of Streptococcus pneumoniae R6 challenged with sublethal concentrations of translation inhibitors." J Bacteriol **185**(1): 359-70.

The effects of sublethal concentrations of four different classes of translation inhibitors (puromycin, tetracycline, chloramphenicol, and erythromycin) on global transcription patterns of Streptococcus pneumoniae R6 were determined by microarray analyses. Consistent with the general mode of action of these inhibitors, relative transcript levels of genes that encode ribosomal proteins and translation factors or that mediate tRNA charging and amino acid biosynthesis increased or decreased, respectively. Transcription of the heat shock regulon was induced only by puromycin or streptomycin treatment, which lead to truncation or mistranslation, respectively, but not by other antibiotics that block translation, transcription, or amino acid charging of tRNA. In contrast, relative transcript amounts of certain genes involved in transport, cellular processes, energy metabolism, and purine nucleotide (pur) biosynthesis were changed by different translation inhibitors. In particular, transcript amounts from a pur gene cluster and from purine uptake and salvage genes were significantly elevated by several translation inhibitors, but not by antibiotics that target other cellular processes. Northern blotting confirmed increased transcript amounts from part of the pur gene cluster in cells challenged by translation inhibitors and revealed the presence of a 10-kb transcript. Purine metabolism genes were negatively regulated by a homologue of the PurR regulatory protein, and full derepression in a DeltapurR mutant depended on optimal translation. Unexpectedly, hierarchical clustering of the microarray data distinguished among the global transcription patterns caused by antibiotics that inhibit different steps in the translation cycle. Together, these results show that there is extensive control of transcript amounts by translation in S. pneumoniae, especially for de novo purine nucleotide biosynthesis. In addition, these global transcription patterns form a signature that can be used to classify the mode of action and potential mechanism of new translation inhibitors.

Nordstrom, H., P. Johansson, et al. (2004). "Microarray technology for identification and distinction of hantaviruses." J Med Virol **72**(4): 646-55.

DNA microarrays combine high-precision technology with advanced molecular biology to achieve high-throughput screening of DNA fragments. In this study, we investigated the potential of the cDNA microarray technique to identify and discriminate PCR derived amplicons from genetically highly similar viruses. The wide range of sequence variation among hantaviruses makes them suitable as a model for this purpose. The hantaviruses, carried by rodents, cause several hundred thousand cases of severe human disease every year in many parts of the world. A hantavirus-specific microarray, including DNA fragments from 12 viral isolates of six different hantaviruses, was designed. The S and M genome segments were represented by 500-nucleotide overlapping and 250-nucleotide non-overlapping fragments. A considerable ability to distinguish between different hantaviruses was demonstrated using a novel analysis method. Even different isolates of a single virus, were identified correctly despite 90% sequence similarity. The distinction ability was accompanied by a tolerance for smaller sequence differences, which makes the microarray suitable for testing samples containing unknown viruses. Viral genetic material found in samples from the lungs of bank voles caught in the wild was identified precisely, which demonstrated further the potential for this technology.

Oh, M. K. and J. C. Liao (2000). "Gene expression profiling by DNA microarrays and metabolic fluxes in Escherichia coli." Biotechnol Prog **16**(2): 278-86.

DNA microarray technology was applied to detect differential transcription profiles of a subset of the Escherichia coli genome. A total of 111 E. coli genes, including those in central metabolism, key biosyntheses, and some regulatory functions, were cloned, amplified, and used as probes for detecting the level of transcripts. An E. coli strain was grown in glucose, acetate, and glycerol media, and the transcript levels of the selected genes were detected. Despite extensive studies on E. coli physiology, many new features were found in the regulation of these genes. For example, several genes were unexpectedly up-regulated, such as pps, ilvG, aroF, secA, and dsbA in acetate and asnA and asnB in glycerol, or down-regulated, such as ackA, pta, and adhE in acetate. These genes were regulated with no apparent reasons by unknown mechanisms. Meanwhile, many genes were regulated for apparent purposes but by unknown mechanisms. For example, the glucose transport genes (ptsHI, ptsG, crr) in both acetate and glycerol media were down-regulated, and the ppc, glycolytic, and biosynthetic genes in acetate were also down-regulated because of the reduced fluxes. However, their molecular mechanisms remain to be elucidated. Furthermore, a group of genes were regulated by known mechanisms, but the physiological roles of such regulation remain unclear. This group includes pckA and aspA, which are up-regulated in glycerol, and gnd and aspA, which are down- and up-regulated, respectively, in acetate. The DNA microarray technology demonstrated here is a powerful yet economical tool for characterizing gene regulation and will prove to be useful for strain improvement and bioprocess development.

Oh, T. J., C. J. Kim, et al. (2004). "Development and clinical evaluation of a highly sensitive DNA microarray for detection and genotyping of human papillomaviruses." J Clin Microbiol **42**(7): 3272-80.

Human papillomavirus (HPV) has been found in cervical cancer, tonsillar cancer, and certain types of head and neck cancers. We report on a DNA microarray-based method for the simultaneous detection and typing of HPVs. The genotype spectrum discriminated by this HPV DNA microarray includes 15 high-risk HPV genotypes and 12 low-risk HPV genotypes. The HPV DNA microarray showed high degrees of specificity and reproducibility. We evaluated the performance of the HPV DNA microarray by application to three HPV-positive cell lines (HeLa, Caski, and SiHa cells) and two HPV-negative cell lines (C33A and A549 cells). The HPV DNA microarray successfully identified the known types of HPV present in the cell lines. The detection limit of the HPV DNA microarray was at least 100-fold higher than that of PCR. To assess the clinical applicability of the HPV DNA microarray, we performed the HPV genotyping assay with 73 nonmalignant and malignant samples from 39 tonsillar cancer patients. Twenty-five of the 39 (64.1%) malignant samples were positive for HPV, whereas 3 of 34 (8.8%) nonmalignant samples were positive for HPV. This result shows a preferential association of HPV with tonsillar carcinomas. The correlations of the presence of HPV with the grade of differentiation and risk factors were not significant. Our data show that the HPV DNA microarray may be useful for the diagnosis and typing of HPV in large-scale epidemiological studies.

Ohmine, K., J. Ota, et al. (2001). "Characterization of stage progression in chronic myeloid leukemia by DNA microarray with purified hematopoietic stem cells." Oncogene **20**(57): 8249-57.

Chronic myeloid leukemia (CML) is characterized by the clonal expansion of hematopoietic stem cells (HSCs). Without effective treatment, individuals in the indolent, chronic phase (CP) of CML undergo blast crisis (BC), the prognosis for which is poor. It is therefore important to clarify the mechanism underlying stage progression in CML. DNA microarray is a versatile tool for such a purpose. However, simple comparison of bone marrow mononuclear cells from individuals at different disease stages is likely to result in the identification of pseudo-positive genes whose change in expression only reflects the different proportions of leukemic blasts in bone marrow. We have therefore compared with DNA microarray the expression profiles of 3456 genes in the purified HSC-like fractions that had been isolated from 13 CML patients and healthy volunteers. Interestingly, expression of the gene for PIASy, a potential inhibitor of STAT (signal transducer and activator of transcription) proteins, was down-

regulated in association with stage progression in CML. Furthermore, forced expression of PIASy has induced apoptosis in a CML cell line. These data suggest that microarray analysis with background-matched samples is an efficient approach to identify molecular events underlying the stage progression in CML.

Okabe, H., S. Satoh, et al. (2001). "Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression." Cancer Res **61**(5): 2129-37.

To disclose detailed genetic mechanisms in hepatocellular carcinoma (HCC) with a view toward development of novel therapeutic targets, we analyzed expression profiles of 20 primary HCCs and their corresponding noncancerous tissues by means of cDNA microarrays consisting of 23,040 genes. Up-regulation of mitosis-promoting genes was observed in the majority of the tumors examined. Some genes showed expression patterns in hepatitis B virus-positive HCCs that were different from those in hepatitis C virus-positive HCCs; most of them encoded enzymes that metabolize carcinogens and/or anticancer agents. Furthermore, we identified a number of genes associated with malignant histological type or invasive phenotype. Accumulation of such data will make it possible to define the nature of individual tumors, to provide clues for identifying new therapeutic targets, and ultimately to optimize treatment of each patient.

Osin, P., J. Shipley, et al. (1998). "Experimental pathology and breast cancer genetics: new technologies." Recent Results Cancer Res **152**: 35-48.

The goal is to understand the critical events in tumour development and to apply this understanding to new approaches to diagnosis, prevention and treatment. It is clear that breast cancer is a heterogeneous disease at the molecular level, raising the possibility of a future functional classification based on mechanisms rather than morphology. These molecular phenotypes will also confer predictive value on the potential of the tumour to invade, metastasise and respond to or resist new therapeutic strategies. Studies of the genome in individuals are predicted also to enable the identification of polymorphisms that are associated with increased susceptibility to environmental factors, in addition to possibly explaining de novo variations in responses to drugs and radiation. The difficulty is how to identify which, of the approximately 30,000 genes expressed by a typical cancer cell alone or in combination, are the ones involved in these processes. The majority of breast cancers have such a multitude of molecular changes that it is difficult to distinguish between those that are critical to tumour progression and those that are epiphenomena of genetic instability and abnormalities in DNA repair. The identification of the earliest events in carcinogenesis must be the best hope, as it will then be possible to target the events that predispose to other secondary changes before they occur. Genomics and proteomics is the current hope to take us forward. This involves the application of a number of new technologies to facilitate the profiling of individual tumours, including laser-guided microdissection of microscopic lesions, comparative genomic hybridisation and loss of heterozygosity analysis of DNA using microarray technology to study DNA and expressed RNAs and protein profiling using 2D gel mass spectroscopy. With over 100,000 mRNAs and proteins to examine in complex tissues and in various combinations, there is obviously going to be a requirement for a large investment in computing power (bioinformatics) to facilitate the analysis of these data in relation to the clinical characteristics of the individual tumour and the patient.

Otake, K., S. Omoto, et al. (2004). "HIV-1 Nef protein in the nucleus influences adipogenesis as well as viral transcription through the peroxisome proliferator-activated receptors." Aids **18**(2): 189-98.

BACKGROUND: Although the HIV-1 Nef protein (27 kDa) localizes primarily in cytoplasm, there is considerable evidence suggesting its occasional localization in the nucleus. Nef is known to play an important role in transcriptional events and viral replication, but the actual target of Nef in the nucleus remains to be identified. OBJECTIVE: To examine the functional roles of Nef in the nucleus and its

possible interactions with other unknown factors in the nucleus. METHODS: High-density microarray analysis was used to screen directly the unique functions of Nef on host gene transcription. The nuclear localization of Nef and its effects on the expression of peroxisome proliferator-activated receptors (PPAR) was examined using PPAR promoter/reporter assay and immunoblotting. A long terminal repeat/reporter assay was used to investigated the effects of Nef and PPAR on viral transcription. RESULTS: Nef in the nucleus suppressed PPAR gamma expression and reduced fatty acid levels in human T and macrophage cell lines. Expression of Nef or PPAR suppressed viral replication; the effect of PPAR gamma or retinoid X receptor-alpha on viral replication were reduced by coexpression of Nef in MT(-)4 T cells. CONCLUSION: Nef may be involved in both viral replication and the wasting syndrome associated with AIDS.

Ozawa, K. (2001). "[Perspectives on postgenome medicine: Hematological diseases]." Nippon Rinsho **59**(1): 59-64.

With advances in DNA chip(DNA microarray) technology, it has become possible to obtain genome-wide gene-expression profiling. This novel technology is now applied to the study of molecular pathogenesis, differential diagnosis, and prediction of prognosis in the field of hematological malignancies. Importantly, the list of informative genes includes new markers of diseases, which will be utilized for further investigation. As for clinical gene therapy, it has been criticized for promising too much and providing too little during the last 10 years. In 1999, however, the success of hematopoietic stem cell gene therapy was reported in the patients with X-SCID(X-linked severe combined immunodeficiency). The gene therapy of hemophilia B using AAV(adeno-associated virus) vector has also successfully started. Genetic manipulation will be applied to the treatment of various diseases with the development of novel technologies.

Park, J. S., D. Y. Noh, et al. (2004). "Gene expression analysis in SV40-immortalized human breast luminal epithelial cells with stem cell characteristics using a cDNA microarray." Int J Oncol **24**(6): 1545-58.

The epithelial compartment of the human breast comprises two distinct cell types. Type I human breast epithelial cells (HBECs) are expressing luminal epithelial cell markers and stem cell characteristics, whereas Type II HBECs show basal epithelial cell phenotypes. When defined in terms of markers for normal cell lineages, most invasive breast cancer cells correspond to the phenotype of the common luminal epithelial cell. We had developed simian virus 40-immortalized cell lines from normal HBECs with luminal and stem cell characteristics. To identify molecular changes involved in immortalization, we analyzed the differential gene expression profiles of normal and non-tumorigenic immortalized Type I HBECs using cDNA microarray with 7,448 sequence-verified clones. Out of the 7,448 genes screened, consistent gene expression changes among biological replicates included 67 in Type I HBECs and 86 in Type II HBECs for 4-fold change criteria. Surprisingly, we identified 148 genes (>2.0-fold) as being either up- or down-regulated related to immortalization: 67 genes (MYBL2, UCHL1 et al) were up-regulated, and 81 genes (IGFBP3, CDKN1A et al) were down-regulated significantly. The altered expression levels of the selected genes were subsequently confirmed by semiquantitative RT-PCR. Our studies suggest that the immortalization of Type I HBECs might be an early step in the initiation of a subset of breast cancer. Furthermore, these results will open up an avenue for more detailed understanding of breast stem cell and tumor biology.

Paulose-Murphy, M., N. K. Ha, et al. (2001). "Transcription program of human herpesvirus 8 (kaposi's sarcoma-associated herpesvirus)." J Virol **75**(10): 4843-53.

Human herpesvirus 8 (HHV-8), a gammaherpesvirus implicated in Kaposi's sarcoma, primary effusion lymphoma, and Castleman's disease, encodes several pathogenically important cellular homologs. To define the HHV-8 transcription program, RNA obtained from latently infected body cavity-based lymphoma 1 cells induced to undergo lytic replication was used to query a custom HHV-8 DNA

microarray containing nearly every known viral open reading frame. The patterns of viral gene expression offer insights into the replication and pathogenic strategies of HHV-8.

Paulsen, I. T., J. Chen, et al. (2001). "Comparative genomics of microbial drug efflux systems." J Mol Microbiol Biotechnol **3**(2): 145-50.

The complete genome sequences of 36 microorganisms have now been published and this wealth of genome data has enabled the development of comparative genomic and functional genomic approaches to investigate the biology of these organisms. Comparative genomic analyses of membrane transport systems have revealed that transporter substrate specificities correlate with an organism's lifestyle. The types and numbers of predicted drug efflux systems vary dramatically amongst sequenced organisms. Microarray and gene knockout studies to date have suggested that predicted drug efflux genes often appear to be a) non-essential and b) expressed at detectable levels under standard laboratory growth conditions.

Perrin, A., D. Duracher, et al. (2003). "A combined oligonucleotide and protein microarray for the codetection of nucleic acids and antibodies associated with human immunodeficiency virus, hepatitis B virus, and hepatitis C virus infections." Anal Biochem **322**(2): 148-55.

A multiplexed assay based on the codetection of nucleic acids and antibodies in human serum infected by human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus was proposed. The combined immuno- and oligosorbent array (CombOLISA) microarray is prepared in 96-well standard microplates by spotting (1). nucleic probes specific for a virus genome, (2). viral proteins for the capture of serum antibodies, and (3). nonspecific proteins for verifying specificity. Experimental assay conditions were optimized so that both DNA hybridization and immunological reactions can be achieved simultaneously in the same well and buffer and all at the same temperature. A generic detection system based on the precipitation of an insoluble colorimetric substrate in the presence of enzyme-labeled antibodies or streptavidin was proposed. The optical density of each spot was correlated to the corresponding analyte concentration. The influence of critical parameters on CombOLISA performance such as serum concentration was studied. Calibration curves and sensitivity thresholds were established for each parameter. Serial dilutions of serum were correlated to results obtained with validated immunoassay platforms such as a microplate enzyme-linked immunosorbent assay or the VIDAS automat. Also, several HIV- and HBV-infected serum samples were tested independently by CombOLISA and VIDAS. Coefficients of variation for genomic and proteomic parameters vs spot density were below 15%.

Petrik, J. (2001). "Microarray technology: the future of blood testing?" Vox Sang **80**(1): 1-11.

The increasing pace of development in molecular biological techniques during the last 10-15 years has had a direct effect on mass testing and diagnostic applications, including blood screening. Nucleic acid amplification techniques (NAT), usually based on the polymerase chain reaction (PCR), have been successfully applied to blood grouping and implemented recently in screening of blood donations for hepatitis C virus (HCV). The majority of microarray technologies involve an amplification step, yet the main benefits of this technology come from simultaneous analysis of thousands of analytes. Microarrays were developed to utilize the huge amount of information provided by genome projects, but they have clear potential in mass screening and diagnostics. The application of microarray technology may revolutionize blood testing, providing for the first time the prospect of an integrated platform for comprehensive donor and donation testing, replacing multiple individual assays. Design features of a blood-testing chip and various technologies with potential application in this field are discussed in this review.

Phadtare, S., I. Kato, et al. (2002). "DNA microarray analysis of the expression profile of Escherichia coli in response to treatment with 4,5-dihydroxy-2-cyclopenten-1-one." J Bacteriol **184**(23): 6725-9.

We carried out DNA microarray-based global transcript profiling of Escherichia coli in response to 4,5-dihydroxy-2-cyclopenten-1-one to explore the manifestation of its antibacterial activity. We show that it has widespread effects in E. coli affecting genes encoding proteins involved in cell metabolism and membrane synthesis and functions. Genes belonging to the regulon involved in synthesis of Cys are upregulated. In addition, rpoS and RpoS-regulated genes responding to various stresses and a number of genes responding to oxidative stress are upregulated.

Porwollik, S., E. F. Boyd, et al. (2004). "Characterization of Salmonella enterica subspecies I genovars by use of microarrays." J Bacteriol **186**(17): 5883-98.

Subspecies 1 of Salmonella enterica is responsible for almost all Salmonella infections of warm-blooded animals. Within subspecies 1 there are over 2,300 known serovars that differ in their prevalence and the diseases that they cause in different hosts. Only a few of these serovars are responsible for most Salmonella infections in humans and domestic animals. The gene contents of 79 strains from the most prevalent serovars were profiled by microarray analysis. Strains within the same serovar often differed by the presence and absence of hundreds of genes. Gene contents sometimes differed more within a serovar than between serovars. Groups of strains that share a distinct profile of gene content can be referred to as "genovars" to distinguish them from serovars. Several misassignments within the Salmonella reference B collection were detected by genovar typing and were subsequently confirmed serologically. Just as serology has proved useful for understanding the host range and pathogenic manifestations of Salmonella, genovars are likely to further define previously unrecognized specific features of Salmonella infections.

Potvin, E., D. E. Lehoux, et al. (2003). "In vivo functional genomics of Pseudomonas aeruginosa for high-throughput screening of new virulence factors and antibacterial targets." Environ Microbiol **5**(12): 1294-308.

Pseudomonas aeruginosa is a model for studying opportunistic pathogens that are highly resistant to most classes of antibiotics and cause chronic pulmonary infections. We have developed and adapted a multiplex polymerase chain reaction-based signature-tagged mutagenesis (STM) for high-throughput screening of a collection of 7968 P. aeruginosa mutants in a rat model of chronic respiratory infection. After three rounds of screening, a total of 214 mutants, representing transposition events into 148 open reading frames, were shown to be attenuated in lung infection and were retained for further analysis. As proof of concept supporting this technology, we identified 11 insertions in typical virulence genes such as those coding for pili implicated in motility, attachment and swarming, alginate synthesis and its expression, a mucus transcription regulator, extracellular enzymes such as alkaline protease, esterase and amino peptidase, a rhamnosyl surfactant transferase and a lipopolysaccharide glycosyl transferase. Detailed analysis of the 148 STM mutants, including seven auxotrophs, revealed insertions in 21 of the 26 known gene classes used to characterize sequenced bacterial genomes. We noted that at least 46% of STM mutants identified had insertions in hypothetical proteins or proteins of unknown function and that approximately 40% of all STM mutants had insertions in surface proteins including the outer membrane, the periplasm and the inner membrane. Interestingly, 11 STM mutants attenuated for lung infection were also identified in microarray and transcriptome for quorum sensing and mucoidy production. The remaining 130 mutants were systematically analysed for their capability to express fully known virulence factors. In addition, testing the ability of these mutants to infect alternative model host Drosophila melanogaster revealed 36 STM mutants defective in protease, twitching motility, swimming and swarming. Finally, we identified many genes, the activity of which in respiratory infection was not fully appreciated.

Prima, V., M. Tennant, et al. (2004). "Differential modulation of energy balance by leptin, ciliary neurotrophic factor, and leukemia inhibitory factor gene delivery: microarray deoxyribonucleic acid-chip analysis of gene expression." Endocrinology **145**(4): 2035-45.

Most obese animal models, whether associated with genetic, diet-induced, or age-related obesity,

display pronounced leptin resistance, rendering leptin supplement therapy ineffective in treating obesity. Ciliary neurotrophic factor (CNTF) has been recently used to invoke leptin-like signaling pathways, thereby circumventing leptin resistance. In the current study, we characterize immediate and long-term molecular events in the hypothalamus of rats exposed to the sustained ectopic expression of leptin, CNTF, or leukemia inhibitory factor, another neurocytokine of IL-6 family, all delivered centrally via a viral vector. The respective transgene-encoded ligands induced similar but not identical metabolic responses as assessed by the reduction in body weight gain and changes in food intake. To define molecular mechanisms of weight-reducing and anorexigenic action of cytokines, we have analyzed the gene expression profiles of 1300 brain-specific genes in the hypothalami of normal rats subjected to the prolonged cytokine action for 10 wk. We present evidence that constitutive expression of cytokines in the brain induces changes in gene expression characteristic of chronic inflammation leading to either temporal weight reduction (CNTF) or severe cachexia (leukemia inhibitory factor). Our results convey a cautionary note regarding potential use of the tested cytokines in therapeutic applications.

Clinical Applications

Q-Z

Rajakumar, K., J. Shafi, et al. (2004). "Use of genome level-informed PCR as a new investigational approach for analysis of outbreak-associated Mycobacterium tuberculosis isolates." J Clin Microbiol **42**(5): 1890-6.

Mycobacterium tuberculosis strain CH, the index isolate linked to a major tuberculosis outbreak associated with high levels of transmissibility and virulence, was characterized by microarray analysis by use of a PCR product array representative of the genome of M. tuberculosis strain H37Rv. Seven potential genomic deletions were identified in CH, five of which were confirmed by PCR analysis across the predicted deletion points. The panel of five PCRs required to individually interrogate these loci was collectively referred to as the genome level-informed PCR (GLIP) assay. GLIP analysis was performed with CH, 12 other epidemiologically linked isolates, and 43 recent, non-outbreak-associated isolates derived from patients within the local area. All 13 outbreak-linked isolates showed a profile corresponding to the presence of all five deletions. These 13 isolates were also found to share common variable-number tandem repeat and mycobacterial interspersed repetitive unit profiles. None of the 43 non-outbreak-associated isolates exhibited the five-deletion profile. Although three individual deletions were present in upwards of 44% of the non-outbreak-associated isolates, no single-deletion isolates were detected. Interestingly, none of these deletions had been previously recognized, and sequence analysis of the immediate flanking regions in CH failed to identify a likely mechanism of deletion for four of the five loci. The GLIP assay also proved valuable in ongoing surveillance of the outbreak, rapidly identifying a further two outbreak-associated cases months after the initial cluster and, importantly, dismissing a further 12 epidemiologically suspect cases, which allowed the optimum deployment of public health resources.

Rathod, P. K., K. Ganesan, et al. (2002). "DNA microarrays for malaria." Trends Parasitol **18**(1): 39-45.

DNA microarrays are a powerful tool for the analysis of RNA and DNA composition on a whole-genome scale. The first applications of this technology in parasitology are in place. This review examines the various approaches to Plasmodium transcript-profiling that are being adopted using DNA microarray analysis and discusses additional strategies for obtaining and collating information relevant to the search for drug and vaccine candidates in malaria.

Resor, L., T. J. Bowen, et al. (2001). "Unraveling human cancer in the mouse: recent refinements to modeling and analysis." Hum Mol Genet **10**(7): 669-75.

The ability to manipulate the mouse genome has made the mouse the primary mammalian genetic

model organism. It has been possible to model human cancer in the mouse by overexpressing oncogenes or inactivating tumor suppressor genes, and these experiments have provided much of our in vivo understanding of cancer. However, these transgenic approaches do not always completely and accurately model human carcinogenesis. Recent developments in transgenic and knockout approaches have improved the accuracy of modeling somatic cancer in the mouse and analyzing the genomic instability that occurs in murine tumors. It is possible to use retroviral gene delivery, chromosome engineering and inducible transgenes to selectively manipulate the genome in a more precise spatial and temporal pattern. In addition, the development of powerful cytogenetic tools such as spectral karyotyping, fluorescence in situ hybridization and comparative genome hybridization have improved our ability to detect chromosomal rearrangements. Finally, global patterns of gene expression can be determined by microarray analysis to decipher complex gene patterns which occur in cancers. Several of these advances in mouse modeling of human cancer are discussed in this review.

Rice, A. M., M. A. Currier, et al. (2002). "Ewing sarcoma family of tumors express adenovirus receptors and are susceptible to adenovirus-mediated oncolysis." J Pediatr Hematol Oncol **24**(7): 527-33.

PURPOSE: Attenuated viruses derived from adenoviruses (Ad) that kill tumor cells (oncolysis) are currently in clinical trials for selected cancers. Some cancers have proven resistant to Ad infection due to low expression of viral receptors. The authors sought to determine whether members of the Ewing sarcoma family of tumors (ESFTs) express Ad receptors and are sensitive to Ad-mediated oncolysis. METHODS: Using flow cytometry, the authors tested a panel of cell lines derived from ESFTs for expression of both the Ad receptor, coxsackie-adenovirus receptor (CAR), and the cellular mediator of Ad uptake, alpha(v)-integrins, as well as for Ad-mediated gene transduction. Cell survival assays were used to assess the sensitivity to Ad-mediated oncolysis. Immunohistochemistry was used to assess CAR expression in primary tumors. mRNA levels of CAR in cell lines and tumor samples were also queried from a cDNA expression database. RESULTS: The ESFT cell lines expressed CAR and alpha(v)-integrins, showed high levels of gene transduction, and were highly sensitive to viral oncolysis. Primary tumor samples were positive for CAR expression by immunohistochemistry. Microarray analysis confirmed CAR expression in ESFT cell lines and tumors. CONCLUSIONS: Ewing sarcoma cells express the Ad receptors and are sensitive to Ad oncolysis. Treatment of Ewing sarcoma using conditionally replicative adenoviruses should be explored.

Robertson, G. T., J. Zhao, et al. (2002). "Vancomycin tolerance induced by erythromycin but not by loss of vncRS, vex3, or pep27 function in Streptococcus pneumoniae." J Bacteriol **184**(24): 6987-7000.

Vancomycin-tolerant Streptococcus pneumoniae is a growing problem among drug-resistant human pathogens. Some vancomycin-tolerant pneumococci have been reported to carry mutations in loci encoding a two-component regulatory system designated VncRS or in a proximal ABC transporter, Vex. A model was advanced proposing that the tolerance phenotype resulted from the inability of a vncS mutant to respond to the Vex-transported Pep27 "death peptide" signal and dephosphorylate VncR, thereby preventing relief of repression of autolytic and other cell death functions in response to antibiotics. To explore this hypothesis, we constructed mutations in vncS, vncR, vex3, and pep27 in S. pneumoniae strain R6 and two additional genetic backgrounds. The lytic responses of the isogenic DeltavncS, Deltavex3, DeltavncR, and Deltapep27 mutants, but not a DeltalytA strain, to vancomycin were indistinguishable from that of the parent strain. DeltavncS strains also failed to exhibit tolerance to vancomycin at various doses in multiple media and showed wild-type sensitivity to other classes of autolysis-inducing antibiotics. In contrast, addition of subinhibitory levels of the antibiotic erythromycin led to tolerance to vancomycin during late, but not early, exponential-phase growth in a DeltavncS strain, in the parent strain R6, and in two other strains bearing erythromycin resistance markers, namely, a DeltavncR strain and an unrelated DeltacomD strain that is defective in competence-quorum sensing. Thus, this tolerance effect resulted from changes in cell growth or other erythromycin-dependent phenomena and not inactivation of vncS per se. Consistent with these results, and in contrast to a previous

report, we found that a synthetic form of Pep27 did not elicit lytic or nonlytic killing of pneumococci. Finally, microarray transcriptional analysis and beta-galactosidase reporter assays revealed VncS-dependent regulation of the vex123 gene cluster but did not support a role for VncRS in the regulation of autolytic or other putative cell death loci. Based on these findings, we propose that vancomycin tolerance in S. pneumoniae does not result from loss of vncS function alone.

Robinson, D. A. and M. C. Enright (2004). "Multilocus sequence typing and the evolution of methicillin-resistant Staphylococcus aureus." Clin Microbiol Infect **10**(2): 92-7.

The prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in many countries is increasing and, in hospitals in some areas, more than half of all S. aureus disease isolates are MRSA. MRSA strains are becoming increasingly multiresistant, and have recently developed resistance to vancomycin, used successfully to treat MRSA for more than 30 years. This review summarises recent studies that have elucidated the evolutionary history of MRSA. The first MRSA isolate evolved from a sensitive, epidemic strain prevalent in Europe, and its progeny-the first MRSA clone-quickly spread to other continents. Analyses of epidemic MRSA isolates from hospitals in different countries by molecular methods, including multilocus sequence typing (MLST) and DNA microarray analysis, reveal that MRSA strains have evolved separately within five distinct epidemic, sensitive lineages. However, resistance has been transferred to S. aureus on many more than five occasions, as some lineages have acquired different structural types of the element carrying the methicillin resistance gene. The emergence of MRSA as a community pathogen has been noted in several countries, and MLST and SCCmec typing have been used to demonstrate that community-acquired MRSA strains are typically related only distantly to hospital MRSA strains, and thus represent novel acquisitions of SCCmec.

Roth, S. B., J. Jalava, et al. (2004). "Use of an oligonucleotide array for laboratory diagnosis of bacteria responsible for acute upper respiratory infections." J Clin Microbiol **42**(9): 4268-74.

We developed a diagnostic array of oligonucleotide probes targeting species-specific variable regions of the genes encoding topoisomerases GyrB and ParE of respiratory bacterial pathogens. Suitable broad-range primer sequences were designed based on alignment of gyrB/parE sequences from nine different bacterial species. These species included Corynebacterium diphtheriae, Fusobacterium necrophorum, Haemophilus influenzae, Legionella pneumophila, Moraxella catarrhalis, Mycoplasma pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, and Streptococcus pyogenes. Specific probe sequences were selected by comparative analysis against the European Bioinformatics Database, as well as gyrB/parE sequences generated for this study. To verify specificity, at least six initial oligonucleotide probe sequences per bacterial species were tested by hybridization on a solid glass support using culture collection strains as templates. Finally, three oligonucleotide probes per bacterial species were utilized to examine 65 middle ear fluid and 29 throat swab samples. The sensitivities of the developed assay compared to classic culture from middle ear fluid samples for H. influenzae, M. catarrhalis, and S. pneumoniae were 96 (93 for culture), 73 (93 for culture), and 100% (78% for culture), respectively. No cross-reactivity with bacterial species belonging to the normal oral flora was observed when the 29 throat swab samples were studied. The sensitivity of the assay to detect S. pyogenes from these samples was 93% (80% for culture). These results provide a proof of concept for the diagnostic use of microarray technology based on broad-range topoisomerase gene amplification, followed by hybridization and specific detection of bacterial species.

Russo, G., P. P. Claudio, et al. (2003). "pRB2/p130 target genes in non-small lung cancer cells identified by microarray analysis." Oncogene **22**(44): 6959-69.

The retinoblastoma gene family consisting of RB/p105, p107, and RB2/p130 cooperate to regulate cell-cycle progression through the G1 phase of the cell cycle. Previous data demonstrated an independent role for the reduction or loss of pRb2/p130 expression in the formation and/or progression of lung carcinoma. Rb2/p130 is mutated in a human cell line of lung small cell carcinoma as well as in

primary lung tumors. To identify potential pRb2/p130 target genes in an unbiased manner, we have utilized an adenovirus-mediated expression system of pRb2/p130 in a non-small lung cancer cell line to identify specific genes that are regulated by pRb2/p130. Using oligonucleotide arrays, a number of Rb2/p130 downregulated genes were identified and their regulation was confirmed by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. As a result, 40 genes showed greater than 2.0-fold modification in their expression level after the RB2/p130 viral transduction. In conclusion, coupling adenoviral overexpression with microarray and semiquantitative RT-PCR analyses proved to be a versatile strategy for identifying pRb2/p130 target genes and for better understanding the expression profiles of these genes. Our results may also contribute to identifying novel therapeutic biomarkers in lung carcinoma.

Ruzsovics, A., B. Molnar, et al. (2004). "Review article: Deoxyribonucleic acid-based diagnostic techniques to detect Helicobacter pylori." Aliment Pharmacol Ther **19**(11): 1137-46.

Helicobacter pylori is an important cause of many gastrointestinal disorders, ranging from chronic gastritis to gastric lymphoma and adenocarcinoma. The deoxyribonucleic acid-based assays have the potential to be a powerful diagnostic tool given its ability to specifically identify H. pylori deoxyribonucleic acid. Markers used to include general H. pylori structures and pathogenetic factors like ureaseA, cagA, vacA, iceA. Deoxyribonucleic acid or bacterial ribonucleic acid for polymerase chain reaction assays can be collected from gastric biopsy, gastric juice, stool, buccal specimens. Polymerase chain reaction can yield quantitative and genotyping results with sensitivity and specificity that approaches 100%. A clear trend in the direction of the determination of quantitative H. pylori infection by real-time polymerase chain reaction can be observed. Fluorescent in situ hybridization is suggested for routine antibiotic resistance determination. To identify the organism, deoxyribonucleic acid structure and its virulence factors may be feasible by using oligonucleotide microarray specifically recognizing and discriminating bacterial deoxyribonucleic acid and various virulence factors. Deoxyribonucleic acid-based H. pylori diagnosis yields higher sensitivity, however, specificity requires sophisticated labour environment and associated with higher costs.

Samuel, C. E. (2001). "Antiviral actions of interferons." Clin Microbiol Rev **14**(4): 778-809, table of contents.

Tremendous progress has been made in understanding the molecular basis of the antiviral actions of interferons (IFNs), as well as strategies evolved by viruses to antagonize the actions of IFNs. Furthermore, advances made while elucidating the IFN system have contributed significantly to our understanding in multiple areas of virology and molecular cell biology, ranging from pathways of signal transduction to the biochemical mechanisms of transcriptional and translational control to the molecular basis of viral pathogenesis. IFNs are approved therapeutics and have moved from the basic research laboratory to the clinic. Among the IFN-induced proteins important in the antiviral actions of IFNs are the RNA-dependent protein kinase (PKR), the 2',5'-oligoadenylate synthetase (OAS) and RNase L, and the Mx protein GTPases. Double-stranded RNA plays a central role in modulating protein phosphorylation and RNA degradation catalyzed by the IFN-inducible PKR kinase and the 2'-5'-oligoadenylate-dependent RNase L, respectively, and also in RNA editing by the IFN-inducible RNA-specific adenosine deaminase (ADAR1). IFN also induces a form of inducible nitric oxide synthase (iNOS2) and the major histocompatibility complex class I and II proteins, all of which play important roles in immune response to infections. Several additional genes whose expression profiles are altered in response to IFN treatment and virus infection have been identified by microarray analyses. The availability of cDNA and genomic clones for many of the components of the IFN system, including IFN-alpha, IFN-beta, and IFN-gamma, their receptors, Jak and Stat and IRF signal transduction components, and proteins such as PKR, 2',5'-OAS, Mx, and ADAR, whose expression is regulated by IFNs, has permitted the generation of mutant proteins, cells that overexpress different forms of the proteins, and animals in which their expression has been disrupted by targeted gene disruption. The use of these IFN system reagents, both in cell culture and

in whole animals, continues to provide important contributions to our understanding of the virus-host interaction and cellular antiviral response.

Sato, T., H. Odagiri, et al. (2003). "Chemosensitivity of human pancreatic carcinoma cells is enhanced by IkappaBalpha super-repressor." Cancer Sci **94**(5): 467-72.

Pancreatic cancer has an unfavorable prognosis; surgery and chemotherapy at present have only limited value. To improve the prognosis of pancreatic cancer, effective non-surgical therapy is necessary. NF-kappaB is reported to be related to resistance to apoptosis, but its role in chemosensitivity remains controversial. We examined the effects on chemosensitivity of inhibition by induction of the super-repressor IkappaBalpha in pancreatic cancer cell lines, BxPC-3, Capan-1 and Panc-1. IkappaBalpha protein was transduced by infection of adenovirus vector AxCAhIkBDeltaN. Sensitivity to VP-16 and doxorubicin was increased significantly by IkappaBalpha induction in all three pancreatic cell lines. To investigate molecular events during IkappaBalpha induction, we examined the changes in expression of drug-resistance-related genes by real-time RT-PCR and those in apoptosis-related genes by cDNA microarray. There was no common change of gene expression before and after IkappaBalpha induction among the three pancreatic cancer cell lines, except for mdm2. Further examination of other genes is necessary for a better understanding of the molecular mechanisms of enhancement of chemosensitivity through IkappaBalpha induction. However, we have confirmed that IkappaBalpha induction leads to an increase of chemosensitivity of pancreatic cancer. Many problems remain before clinical application of this adenoviral system will be feasible, but our results may ultimately lead to an improved therapy of pancreatic cancer.

Seifarth, W., B. Spiess, et al. (2003). "Assessment of retroviral activity using a universal retrovirus chip." J Virol Methods **112**(1-2): 79-91.

A DNA chip-based assay is described for parallel detection and identification of a wide variety of human and mammalian exogenous and endogenous retroviruses. The assay combines multiplex polymerase chain reaction (PCR) using fluorochrome-modified primer mixtures and chip hybridization. The microarray is composed of retrovirus-specific synthetic oligonucleotides as capture probes deposited on glass slides. The retrovirus chip can be used to assess the occurrence of reverse transcriptase (RT)-related transcripts in biological samples of human and mammalian origin. For example, distinct expression profiles of human endogenous retroviruses (HERV) were established reproducibly in human white blood cells, mammary gland and other human tissues. In particles released by human cells, packaging of specific HERV transcripts could be observed. Monitoring of human exogenous retroviruses (HIV, HTLV) and detection of putative cross-species transmissions (MLV, PERV) in human samples was efficient and reliable. The DNA chip should be an excellent tool for the detection of most relevant retroviruses and offers insights into differential retroviral activities and replication strategies. Furthermore, it could improve significantly the safety of gene therapy, tissue engineering, xenotransplantation and production of therapeutic polypeptides in cell culture.

Semblat, J. P., O. Silvie, et al. (2002). "Laser capture microdissection of Plasmodium falciparum liver stages for mRNA analysis." Mol Biochem Parasitol **121**(2): 179-83.

Plasmodium falciparum liver-stages are important targets for vaccine-induced protective immune responses and prophylactic treatment against malaria. Little is known of the gene expression profile of malaria parasites during their development inside hepatocytes. The sequencing of the P. falciparum genome and the development of DNA microarray technology give new opportunities to identify genes expressed during the development of Plasmodium. However, transcriptome analysis cannot currently be applied to the hepatic stages, due to difficulties in obtaining sufficient amounts of parasite material that lie among the large excess of host cell RNA. Here, we describe the isolation of liver-stages by a modified laser capture microdissection approach applied to human hepatocyte cultures infected with P. falciparum. RT-PCR amplification of several P. falciparum transcripts demonstrated the high quality of the RNA

recovered after microdissection. This approach should enable analyses of P. falciparum transcriptome during its hepatic development and substantially assist the identification of new therapeutic and vaccine targets.

Sengupta, S., K. Onodera, et al. (2003). "Molecular detection and identification of influenza viruses by oligonucleotide microarray hybridization." J Clin Microbiol **41**(10): 4542-50.

Microarrays of virus-specific oligonucleotides may provide a method of screening samples for the presence or absence of a large variety of viruses simultaneously. Influenza viruses are ideal for evaluating such microarrays because of their genetic and host diversity, and the availability of an extensive sequence database. A collection of 476 influenza virus-specific oligonucleotides was spotted onto glass slides as probes. Viral RNAs were reverse transcribed and amplified by PCR, and the products were labeled with cyanine dyes. The presence of viruses and their identities were determined by hybridization. The fluorescence intensities of oligonucleotide spots were highly reproducible within each slide and satisfactorily proportional between experiments. However, the intensities of probe spots completely complementary to target sequences varied from background to saturation. The variations did not correlate with base composition, nucleotide sequence, or internal secondary structures. Therefore, thresholds for determining whether hybridization to a spot should be judged as positive were assigned individually. Considering only positive spots from probes predicted to be monospecific for influenza virus species, subtype, host source, or gene segment, this method made correct identifications at the species, hemagglutinin subtype, and gene segment levels. Monospecific neuraminidase (NA) subtype probes were insufficiently diverse to allow confident NA subtype assignment. Incorporating positive spots from polyspecific probes into the identification scheme gave similar results. Overall, the results demonstrate the potential of microarray-based oligonucleotide hybridization for multiple virus detection.

Sepulveda, A. R. and L. G. Coelho (2002). "Helicobacter pylori and gastric malignancies." Helicobacter **7 Suppl 1**: 37-42.

The epidemiological link of Helicobacter pylori and gastric carcinoma was confirmed and the reported rate of gastric carcinoma development in the Japanese population with H. pylori gastritis was 2.9% during a 7.8-year period. Studies showed that gastric atrophy and intestinal metaplasia may partially regress after H. pylori eradication, but whether this will result in a decrease in the development of malignant lesions remains to be confirmed in large studies. Little progress was made towards the identification of criteria applicable to individualized populations that would benefit from H. pylori screening and surveillance of gastric malignancy. Studies of low-grade MALT lymphoma reported clinical and molecular features that may be useful to identify cases that may be responsive to H. pylori eradication therapy, namely evaluation of lymphoma stage by EUS and t(11;18). The role of H. pylori eradication therapy in high-grade MALT lymphoma needs to be evaluated in larger prospective studies. The application of methods of global analysis of gene expression (microarray studies) resulted in the explosion of information on the molecular pathways activated by H. pylori in gastric epithelial cells. New pathways that may play an important role in H. pylori carcinogenesis have been discovered in several studies.

Sergeev, N., D. Volokhov, et al. (2004). "Simultaneous analysis of multiple staphylococcal enterotoxin genes by an oligonucleotide microarray assay." J Clin Microbiol **42**(5): 2134-43.

Staphylococcal enterotoxins (SEs) are a family of 17 major serological types of heat-stable enterotoxins that are one of the leading causes of gastroenteritis resulting from consumption of contaminated food. SEs are considered potential bioweapons. Many Staphylococcus aureus isolates contain multiple SEs. Because of the large number of SEs, serological typing and PCR typing are laborious and time-consuming. Furthermore, serological typing may not always be practical because of antigenic similarities among enterotoxins. We report on a microarray-based one-tube assay for the simultaneous detection and identification (genetic typing) of multiple enterotoxin (ent) genes. The

proposed typing method is based on PCR amplification of the target region of the ent genes with degenerate primers, followed by characterization of the PCR products by microchip hybridization with oligonucleotide probes specific for each ent gene. We verified the performance of this method by using several other techniques, including PCR amplification with gene-specific primers, followed by gel electrophoresis or microarray hybridization, and sequencing of the enterotoxin genes. The assay was evaluated by analysis of previously characterized staphylococcal isolates containing 16 ent genes. The microarray assay revealed that some of these isolates contained additional previously undetected ent genes. The use of degenerate primers allows the simultaneous amplification and identification of as many as nine different ent genes in one S. aureus strain. The results of this study demonstrate the usefulness of the oligonucleotide microarray assay for the analysis of multitoxigenic strains, which are common among S. aureus strains, and for the analysis of microbial pathogens in general.

Shibata, S. (2004). "Neural regulation of the hepatic Circadian rhythm." Anat Rec **280A**(1): 901.

A microarray analysis experiment has revealed that there are many genes, including so-called clock genes, expressing a circadian rhythm in the liver. The clock genes mentioned above are expressed not only in the suprachiasmatic nucleus (SCN) of the hypothalamus, where the master clock exists, but also in other brain regions and various peripheral tissues. In the liver, clock genes are abundantly expressed and show a clear circadian rhythm. Thus, clock genes seem to play a critical role in the molecular clockworks of both the SCN and the liver. Although oscillation of clock genes in the liver is controlled under the circadian clock mechanism in the SCN, we do not know the resetting signals on liver clock function. Over the past few years, use of the pseudorabies virus, a transsynaptic tract tracer, has allowed us to map neural connections between the SCN and peripheral tissues in several physiological systems. Communication between the SCN and peripheral tissues occurs through autonomic nervous systems involving the sympathetic and parasympathetic neurons. This review mainly describes both anatomical and physiological experiments to reveal the sympathetic control over liver clock function. Although further study is necessary to produce the precise mechanism underlying neural control of liver clock systems, evolution of this mechanism will help our understanding of liver clock functions such as drug metabolism and energy metabolism. Copyright 2004 Wiley-Liss, Inc.

Shieh, B. and C. Li (2004). "Multi-faceted, multi-versatile microarray: simultaneous detection of many viruses and their expression profiles." Retrovirology **1**(1): 11.

There are hundreds of viruses that infect different human organs and cause diseases. Some fatal emerging viral infections have become serious public health issues worldwide. Early diagnosis and subsequent treatment are therefore essential for fighting viral infections. Current diagnostic techniques frequently employ polymerase chain reaction (PCR)-based methods to quickly detect the pathogenic viruses and establish the etiology of the disease or illness. However, the fast PCR method suffers from many drawbacks such as a high false-positive rate and the ability to detect only one or a few gene targets at a time. Microarray technology solves the problems of the PCR limitations and can be effectively applied to all fields of molecular medicine. Recently, a report in Retrovirology described a multi-virus DNA array that contains more than 250 open reading frames from eight human viruses including human immunodeficiency virus type 1. This array can be used to detect multiple viral co-infections in cells and in vivo. Another benefit of this kind of multi-virus array is in studying promoter activity and viral gene expression and correlating such readouts with the progression of disease and reactivation of latent infections. Thus, the virus DNA-chip development reported in Retrovirology is an important advance in diagnostic application which could be a potent clinical tool for characterizing viral co-infections in AIDS as well as other patients.

Shih, S. R., V. Stollar, et al. (2004). "Identification of genes involved in the host response to enterovirus 71 infection." J Neurovirol **10**(5): 293-304.

Enterovirus 71 (EV71) infection may be asymptomatic or may cause diarrhea, rashes, and hand,

foot, and mouth disease (HFMD). However, EV71 also has the potential to cause severe neurological disease. To date, little is known about the molecular mechanisms of host response to EV71 infection. In this report, we utilized cDNA microarray to profile the kinetics and patterns of host gene expression in EV71-infected human neural SF268 cells. We have identified 157 genes with significant changes in mRNA expression and performed hierarchical clustering to classify these genes into five different groups based on their kinetics of expression. EV71 infection led to increases in the level of mRNAs encoding chemokines, proteins involved in protein degradation, complement proteins, and proapoptotis proteins. cDNA microarray expression comparisons of EV71- and mock-infected cells also revealed the down-regulation of several genes encoding proteins involved in host RNA synthesis. Expression of interferon-regulated proteins was increased early in the infection and then decreased. Expression of proteins involved in cellular development and differentiation, some oncogenes, and transcription and translation regulators were suppressed and then stimulated late in the infection. Our findings illustrate the overall host response to EV71 infection, and will aid in understanding the host response to this virus.

Shtrichman, R. and C. E. Samuel (2001). "The role of gamma interferon in antimicrobial immunity." Curr Opin Microbiol **4**(3): 251-9.

Gamma interferon (IFN-gamma) is an important cytokine in the host defense against infection by viral and microbial pathogens. IFN-gamma induces a variety of physiologically significant responses that contribute to immunity. Treatment of animal cells with IFN-gamma or infection with viral or microbial pathogens leads to changes in the level of expression of several target genes as revealed by DNA microarray analyses. The signaling pathways leading to the induction of IFN-gamma-regulated gene products and, in some cases, their biochemical functions have been defined in exquisite detail. Studies of transgenic mutant mice deficient in proteins of the IFN-gamma response pathway firmly establish the importance of IFN-gamma in immunity.

Sibley, L. D., D. G. Mordue, et al. (2002). "Genetic approaches to studying virulence and pathogenesis in Toxoplasma gondii." Philos Trans R Soc Lond B Biol Sci **357**(1417): 81-8.

Toxoplasma gondii is a common protozoan parasite that causes disease in immunocompromised humans. Equipped with a wide array of experimental tools, T. gondii has rapidly developed as a model parasite for genetic studies. The population structure of T. gondii is highly clonal, consisting of three distinct lineages that differ dramatically in virulence. Acute virulence is probably mediated by the genetic differences that distinguish strain types. We have utilized a combination of genetic approaches to investigate the acute virulence of toxoplasmosis using the mouse model. These studies reveal the surprising finding that pathogenicity is due to the over-stimulation of normally protective immune responses. Classical genetic linkage mapping studies indicate that genes that mediate acute virulence are linked to chromosome VII in the parasite. To increase the resolution of genetic mapping studies, single-nucleotide polymorphisms are being developed based on an extensive database of expressed sequence tags (ESTs) from T. gondii. Separately, DNA microarray studies are being used to examine the expression of parasite and host genes during infection. Collectively, these approaches should improve current understanding of virulence and pathogenicity in toxoplasmosis.

Signoretti, S., L. Di Marcotullio, et al. (2002). "Oncogenic role of the ubiquitin ligase subunit Skp2 in human breast cancer." J Clin Invest **110**(5): 633-41.

Estrogen receptor (ER) expression and Her-2 amplification define specific subsets of breast tumors for which specific therapies exist. The S-phase kinase-associated protein Skp2 is required for the ubiquitin-mediated degradation of the cdk-inhibitor p27 and is a bona fide proto-oncoprotein. Using microarray analysis and immunohistochemistry, we determined that higher levels of Skp2 are present more frequently in ER-negative tumors than in ER-positive cases. Interestingly, the subset of ER-negative breast carcinomas overexpressing Skp2 are also characterized by high tumor grade, negativity for Her-2, basal-like phenotype, high expression of certain cell cycle regulatory genes, and low levels of p27

protein. We also found that Skp2 expression is cell adhesion-dependent in normal human mammary epithelial cells but not in breast cancer cells and that an inhibition of Skp2 induces a decrease of adhesion-independent growth in both ER-positive and ER-negative cancer cells. Finally, forced expression of Skp2 abolished effects of antiestrogens, suggesting that deregulated Skp2 expression might play a role in the development of resistance to antiestrogens. We conclude that Skp2 has oncogenic potential in breast epithelial cells and is overexpressed in a subset of breast carcinomas (ER- and Her-2 negative) for which Skp2 inhibitors may represent a valid therapeutic option.

Smith, M. W., Z. N. Yue, et al. (2003). "Identification of novel tumor markers in hepatitis C virus-associated hepatocellular carcinoma." Cancer Res **63**(4): 859-64.

Hepatocellular carcinoma (HCC) is a common primary cancer associated frequently with hepatitis C virus (HCV). To gain insight into the molecular mechanisms of hepatocarcinogenesis, and to identify potential HCC markers, we performed cDNA microarray analysis on surgical liver samples from 20 HCV-infected patients. RNA from individual tumors was compared with RNA isolated from adjacent nontumor tissue that was cirrhotic in all of the cases. Gene expression changes related to cirrhosis were filtered out using experiments in which pooled RNA from HCV-infected cirrhotic liver without tumors was compared with pooled RNA from normal liver. Expression of approximately 13,600 genes was analyzed using the advanced analysis tools of the Rosetta Resolver System. This analysis revealed a set of 50 potential HCC marker genes, which were up-regulated in the majority of the tumors analyzed, much more widely than common clinical markers such as cell proliferation-related genes. This HCC marker set contained several cancer-related genes, including serine/threonine kinase 15 (STK15), which has been implicated in chromosome segregation abnormalities but which has not been linked previously with liver cancer. In addition, a set of genes encoding secreted or plasma proteins was identified, including plasma glutamate carboxypeptidase (PGCP) and two secreted phospholipases A2 (PLA2G13 and PLA2G7). These genes may provide potential HCC serological markers because of their strong up-regulation in more than half of the tumors analyzed. Thus, high throughput methods coupled with high-order statistical analyses may result in the development of new diagnostic tools for liver malignancies.

Solnick, J. V., L. M. Hansen, et al. (2004). "Modification of Helicobacter pylori outer membrane protein expression during experimental infection of rhesus macaques." Proc Natl Acad Sci U S A **101**(7): 2106-11.

Clinical isolates of Helicobacter pylori show marked diversity, which may derive from genomic changes that occur during the often lifelong association of the bacterium with its human host. We used the rhesus macaque model, together with DNA microarrays, to examine genomic changes in H. pylori that occur early during experimental infection. Microarray analysis showed that H. pylori recovered from challenged macaques had deleted babA, a member of a large family of paralogous outer membrane proteins (OMPs) that mediates attachment of H. pylori to the Lewis B blood group antigen on gastric epithelium. In some cases the babA gene was replaced by babB, an uncharacterized OMP that is closely related to babA. In other cases the babA gene was present but was not expressed because of alteration in dinucleotide CT repeats in the 5' coding region. In either case, strains lacking babA did not adhere to Lewis B, which is expressed on macaque gastric epithelium. Absence of babA and duplication of babB was also seen in H. pylori isolates derived from human clinical samples, suggesting that this gene conversion event is not unique to experimentally infected rhesus monkeys. These results demonstrate in real time with a relevant animal model that H. pylori regulates OMP expression in vivo by using both antigenic variation and phase variation. We suggest that changes in babA and babB after experimental infection of macaques represent a dynamic response in the H. pylori outer membrane that facilitates adherence to the gastric epithelium and promotes chronic infection.

Song, J. W., J. S. Lin, et al. (2003). "[Clinical study of oligonucleotide microarray on monitoring the lamivudine-resistance mutations in hepatitis B virus]." Zhonghua Gan Zang Bing Za Zhi **11**(6): 361-3.

OBJECTIVE: To evaluate the mutations of lamivudine-resistance using oligonucleotide microarray in hepatitis B virus (HBV) infected patients. METHODS: A randomized clinical trial was conducted on 20 lamivudine-treated patients for 18 months and 10 patients as controls. The serum HBV DNA was amplified by PCR and the lamivudine-resistance mutations in YMDD region were assayed by 4 sites microarray developed before. RESULTS: This microarray could clearly differentiate the wide-type from mutated-type HBV with lamivudine-resistance mutations. The rate of mutations in YMDD region increased with the time of lamivudine treatment (chi2=6.69, P<0.01). The most common mutated type was M539V+L515M and next M539I. Continuous administration of lamivudine was no benefit for inhibiting the replication of HBV with YMDD mutation but helpful for wide-type HBV. CONCLUSION: The routine serum HBV DNA assay by PCR may introduce prejudice in monitoring HBV inhibitory effect by lamivudine, while the microarray technique can avoid this and is one of the best ways to monitor the lamivudine-resistance mutations in HBV. There is no effect of lamivudine on HBV with YMDD mutation in clinical practice.

Stephanopoulos, G., D. Hwang, et al. (2002). "Mapping physiological states from microarray expression measurements." Bioinformatics **18**(8): 1054-63.

MOTIVATION: The increasing use of DNA microarrays to probe cell physiology requires methods for visualizing different expression phenotypes and explicitly connecting individual genes to discriminating expression features. Such methods should be robust and maintain biological interpretability. RESULTS: We propose a method for the mapping of the physiological state of cells and tissues from multidimensional expression data such as those obtained with DNA microarrays. The method uses Fisher discriminant analysis to create a linear projection of gene expression measurements that maximizes the separation of different sample classes. Relative to other typical classification methods, this method provides insights into the discriminating characteristics of expression measurements in terms of the contribution of individual genes to the definition of distinct physiological states. This projection method also facilitates visualization of classification results in a reduced dimensional space. Examples from four different cases demonstrate the ability of the method to produce well-separated groups in the projection space and to identify important genes for defining physiological states. The method can be augmented to also include data from the proteomic and metabolic phenotypes and can be useful in disease diagnosis, drug screening and bioprocessing applications.

Stilwell, J. L., D. M. McCarty, et al. (2003). "Development and characterization of novel empty adenovirus capsids and their impact on cellular gene expression." J Virol **77**(23): 12881-5.

Adenovirus (Ad) has been extensively studied as a eukaryotic viral vector. As these vectors have evolved from first-generation vectors to vectors that contain either very few or no viral genes ("gutless" Ad), significant reductions in the host innate immune response upon infection have been observed. Regardless of these vector improvements an unknown amount of toxicity has been associated with the virion structural proteins. Here we demonstrate the ability to generate high particle numbers (10(11) to 10(12)) of Ad empty virions based on a modification of Cre/lox gutless Ad vectors. Using a battery of analyses (electron microscopy, atomic force microscopy, confocal images, and competition assays) we characterized this reagent and determined that it (i) makes intact virion particles, (ii) competes for receptor binding with wild-type Ad, and (iii) enters the cell proficiently, demonstrating an ability to carry out essential steps of viral entry. To further study the biological impact of these Ad empty virions on infected cells, we carried out DNA microarray analysis. Compared to that for recombinant Ad, the number of mRNAs modulated upon infection was significantly reduced but the expression signatures were similar. This reagent provides a valuable tool for studies of Ad in that researchers can examine the effect of infection in the presence of the virion capsid alone.

Stober, C. B. (2004). "From genomes to vaccines for leishmaniasis." Methods Mol Biol **270**: 423-38.

A total of 2183 clones derived from four life cycle stage-specific, spliced-leader cDNA libraries

of Leishmania major LV39 Neal strain were randomly picked and sequenced to generate expressed sequence tags (ESTs). Then 1094 unique genes were identified, with 18.2% having BLAST hits with known genes/proteins and 81.8% failing to match genes currently deposited in public databases. Approximately 250 unique genes were obtained from a lesion-derived amastigote complementary DNA (cDNA) library, the form of the parasite that is infective to the mammalian host. Polymerase chain reaction (PCR)-amplified ESTs were spotted onto glass slides, and DNA microarray used to identify a further approx 100 unique cDNAs highly expressed in amastigotes. One hundred unique, randomly selected amastigote-expressed genes were PCR amplified to exclude the 5'-spliced leader, and the full-length genes subcloned into the TOPO-TA cloning vector. The genes were sequence-verified, excised using restriction enzyme digestion, and cloned upstream of the eukaryotic cytomegalovirus promoter into the expression vector pcDNA3. Expression plasmids were sequence-verified and large-scale, endotoxin-free plasmids prepared. Then 100 microg of each expression plasmid (DNA vaccine) was delivered subcutaneously to the rump of susceptible BALB/c mice, the mice boosted 4 wk later and then challenged 2 wk post-boost with 2 x 10(6) L. major LV39 parasites to the hind footpad. Infection was monitored on a weekly basis by measuring footpad depth with digital calipers. Protection was scored by comparing the footpad depth of mice receiving empty vector DNA to those immunized with DNA containing L. major amastigote-expressed genes.

Straub, T. M. and D. P. Chandler (2003). "Towards a unified system for detecting waterborne pathogens." J Microbiol Methods **53**(2): 185-97.

Currently, there is no single method to collect, process, and analyze a water sample for all pathogenic microorganisms of interest. Some of the difficulties in developing a universal method include the physical differences between the major pathogen groups (viruses, bacteria, protozoa), efficiently concentrating large volume water samples to detect low target concentrations of certain pathogen groups, removing co-concentrated inhibitors from the sample, and standardizing a culture-independent endpoint detection method. Integrating the disparate technologies into a single, universal, simple method and detection system would represent a significant advance in public health and microbiological water quality analysis. Recent advances in sample collection, on-line sample processing and purification, and DNA microarray technologies may form the basis of a universal method to detect known and emerging waterborne pathogens. This review discusses some of the challenges in developing a universal pathogen detection method, current technology that may be employed to overcome these challenges, and the remaining needs for developing an integrated pathogen detection and monitoring system for source or finished water.

Striebel, H. M., E. Birch-Hirschfeld, et al. (2003). "Virus diagnostics on microarrays." Curr Pharm Biotechnol **4**(6): 401-15.

Whereas the majority of microarray applications still deal with expression analysis for gathering information about levels of gene products at certain cell states, other approaches simply ask the question whether particular genes, which are usually indicative for particular microorganisms and pathogens, are present in a sample or not. Investigations that are more detailed try to evaluate the presence of particular subtypes of a given pathogen. The combination of microarray technology and virus diagnostics promises to generate an ideal platform for fast, sensitive, specific, and parallelized virus diagnostics. Performing virus diagnostics on microarrays, however, requires other basic techniques to be optimized. This is necessary in order to obtain unambiguous and reproducible results, which are compatible with the needs for clinical routine. Parameters that have to be considered include supports, coupling chemistry, chemical oligonucleotide synthesis, signal enhancement strategies, and optimal coordination of PCR reactions, hybridizations, and signal detection, as well as interpretation strategies. Finally, considerations should be given to economic aspects, one chip-one patient strategies and low integrated arrays as a custom-tailored way to fast and accurate diagnostic tools.

Striebel, H. M., E. Birch-Hirschfeld, et al. (2004). "Enhancing sensitivity of human herpes virus diagnosis with DNA microarrays using dendrimers." Exp Mol Pathol **77**(2): 89-97.

DNA microarray technology has become a promising new tool for the detection and identification of viral pathogens in human plasma and cell cultures. For exploration of this technology, we have developed DNA microarrays that encode capture oligonucleotide probes for different human herpes viruses: herpes simplex virus (HSV) HSV-1, HSV-2, varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and HHV-6. The on-chip hybridization is accomplished with the PCR amplicons of the respective human herpes virus types. In this original article, we attached multiple Cy3-fluorophores to the branched 5' ends of the labeling oligonucleotide primers. For the first time, we experimentally demonstrated that the self-designed, knowledge-based, and focused microarrays specifically hybridized to fluorophore-labeled pathogenic DNAs using dendrimer technology. The fluorescence signal enhancement via the dendrimers was up to 30 times compared with the quenched single Cy3-fluorophore-labeled HSV-1 DNA. The on-chip signal-amplifying effect depended upon the number of branches and the concentration of fluorophore-labeled pathogenic DNAs. Treblers were superior to doublers, as trebler-labeled nucleic acids had fluorescence-signal-enhancing effects over a broad range of labeled DNA concentrations exemplified for the quenched single Cy3-fluorophore-labeled HSV-1 and non-quenched single Cy3-fluorophore-labeled CMV DNAs.

Sturmer, M., A. Berger, et al. (2004). "HIV-1 genotyping: comparison of two commercially available assays." Expert Rev Mol Diagn **4**(3): 281-91.

HIV-1 resistance testing has become an increasingly important feature in antiretroviral treatment and is commonly accomplished by genotyping. Currently, two different systems are being marketed and, despite being far from easy to use, have achieved a high degree of sophistication. Modifications of the standard-kit protocols may be advantageous in certain situations. Although resistance reports are issued by these systems through interpretation software based on decision rules, it nevertheless requires considerable knowledge and skills by the user to make useful clinical data out of detected resistance patterns. This review describes both systems in detail and discusses their advantages and disadvantages. A final decision on which system to use must be based on an individual's requirements. The future of this field may lie with the use of microarray systems.

Su, C., C. Hott, et al. (2004). "Typing single-nucleotide polymorphisms in Toxoplasma gondii by allele-specific primer extension and microarray detection." Methods Mol Biol **270**: 249-62.

Genotyping is an important tool for epidemiological and population genetic studies in protozoan parasites. The most commonly used method for genotyping is polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis of single nucleotide polymorphisms (SNPs). However, PCR-RFLP analysis is labor intensive, and only a proportion of the SNPs are recognized by currently available restriction enzymes. Here, we have developed a more efficient microarray-based method to genotype SNPs in the protozoan parasite Toxoplasma gondii. This method is sensitive, accurate, and capable of analyzing multiple SNPs simultaneously in a high-throughput format.

Su, Z. Z., Y. Chen, et al. (2003). "Customized rapid subtraction hybridization (RaSH) gene microarrays identify overlapping expression changes in human fetal astrocytes resulting from human immunodeficiency virus-1 infection or tumor necrosis factor-alpha treatment." Gene **306**: 67-78.

Genes displaying altered expression as a function of human immunodeficiency virus (HIV)-1 infection of cultured primary human fetal astrocytes (PHFA) were previously identified using a rapid subtraction hybridization (RaSH) method. This scheme identified both known and novel genes displaying elevated expression, astrocyte elevated genes (AEG), and decreased expression, astrocyte suppressed genes (ASG), in PHFA as a consequence of infection with HIV-1 or treatment with HIV-1 envelope glycoprotein (gp120). RaSH also identified both known and novel genes displaying enhanced (HR) or reduced (HS) expression in HIV-1 resistant versus HIV-1 susceptible human T-cell clones. In the present

study, a customized microarray approach employing these RaSH-derived genes was used to distinguish overlapping gene expression changes occurring in PHFA as a function of treatment with HIV-1 and the neurotoxic agent tumor necrosis factor (TNF)-alpha. RaSH cDNAs were spotted (microarrayed) on nylon membranes and probed with temporally isolated reverse transcribed cDNAs from HIV-1-infected and TNF-alpha-treated PHFA. This strategy identified genes displaying parallel changes after TNF-alpha treatment as observed following HIV-1 infection. Confirmation of genuine differential expression was achieved by Northern blotting. These studies document that TNF-alpha can induce a set of corresponding changes in specific AEGs and ASGs as does HIV-1 infection in PHFA. Furthermore, this customized microarray approach with RaSH-derived clones represents an efficient and sensitive methodology for elucidating molecular changes in PHFA occurring as a consequence of treatment with pharmacological agents affecting astrocyte physiology.

Sui, Y., R. Potula, et al. (2003). "Microarray analysis of cytokine and chemokine genes in the brains of macaques with SHIV-encephalitis." J Med Primatol **32**(4-5): 229-39.

Human immunodeficiency virus (HIV)-encephalitis results from a cascade of viral-host interactions that lead to cytokine and chemokine imbalance, which then leads to neuropathologic manifestations of the disease. These include macrophage/microglia activation, astrocytosis and neuronal dysfunction or death. As the molecular mechanisms of this process are poorly understood, we used Atlas human cytokine or cytokine receptor microarray analysis to highlight gene expression profiles that accompanied encephalitis in Simian human immunodeficiency virus (SHIV) 89.6P-infected macaques. Of the 277 genes screened, marked upregulation of monocyte chemoattractant protein-1, interferon-inducible peptide IP-10 and interleukin-4 were observed specifically in the encephalitic brains. These genes are collectively known to promote macrophage infiltration and activation and virus replication. In contrast, genes regulating neurotrophic functions, such as brain-derived neurotrophic factor were downregulated. We also found that some of the apoptosis genes were up- or down-regulated. These data provide a comprehensive spectrum of gene expression that underscores the two major clinical manifestations of this unique syndrome: enhanced virus replication in brain macrophages and dystrophic changes in neurons.

Sun, Y., P. L. Huang, et al. (2001). "Anti-HIV agent MAP30 modulates the expression profile of viral and cellular genes for proliferation and apoptosis in AIDS-related lymphoma cells infected with Kaposi's sarcoma-associated virus." Biochem Biophys Res Commun **287**(4): 983-94.

The anti-HIV agent MAP30 (Momordica anti-HIV protein, 30 kDa) inhibits the proliferation of BC-2, an AIDS-related primary effusion lymphoma (PEL) cell line derived from an AIDS patient. BC-2 cells are latently infected with Kaposi's sarcoma-associated herpes virus (KSHV), also known as human herpes virus 8 (HHV8). We examined the effect of MAP30 on the expression of viral and cellular genes in BC-2 during latent and lytic states of the viral life cycle. By Northern analysis and RT-PCR, we found that MAP30 downregulates the expression of viral cyclin D (vCD), viral interleukin-6 (vIL-6), and viral FLIP (vFLIP), genes involved in cell cycle regulation, viral pathogenesis, and apoptosis. By pathway-specific cDNA microarray analysis, we found that BC-2 cells express high levels of egr-1, ATF-2, hsp27, hsp90, IkappaB, mdm2, skp1, and IL-2, cellular genes involved in mitogenesis, tumorigenesis, and inhibition of apoptosis in NFkappaB and p53 signaling pathways. These results define for the first time the specific cellular pathways involved in AIDS-related tumorigenesis and suggest specific novel targets for the treatment. Furthermore, we found that MAP30 downregulates the expression of egr-1, ATF-2, hsp27, hsp90, IkappaB, mdm2, and Skp1, while it upregulates the pro-apoptotic-related genes Bax, CRADD, and caspase-3. Thus, MAP30 modulates the expression of both viral and cellular genes involved in KS pathogenesis. These results provide valuable insight into the molecular mechanisms of MAP30 anti-KS action and suggest its utility as a therapeutic agent against AIDS-related tumors.

Sun, Z. H., W. L. Zheng, et al. (2003). "Rapid preparation of DNA microarray using PCR for hepatitis B and D virus detection." Di Yi Jun Yi Da Xue Xue Bao **23**(7): 677-9.

OBJECTIVE: To prepare DNA microarray for detecting both hepatitis B and D virus as (HBV and HDV). METHODS: With the assistance of Oligo6.4 software, specific PCR primers targeting the conserved region of HBV and HDV were designed. The PCR products were purified and cloned into the pMD18-T vectors, followed by rapid identification. The recombinant plasmids were then extracted from positive clones and the target gene fragments underwent sequence analysis. RESULTS: The gene fragments of HBV and HDV were obtained by PCR, which were confirmed by sequence analysis to be specific gene fragments of HBV and HDV. CONCLUSION: Using PCR amplification products to prepare the DNA microarray is quick, simple and effective.

Sun, Z. H., W. L. Zheng, et al. (2004). "Detection of hepatitis D virus by cDNA microarray method." Hepatobiliary Pancreat Dis Int **3**(3): 423-7.

BACKGROUND: Viral hepatitis is considered a major public health problem in most areas of the world. In acute and chronic infections, hepatitis D virus (HDV) infection often leads to a more severe disease.This study was designed to prepare microarrays for HDV detection. METHODS: The specific primers of PCR were designed according to the conserved region of HDV. The cDNA microarrays were prepared by spotting PCR products onto the surface of glass slides by robotics. Restriction display PCR (RD-PCR) was used to label the samples. RESULTS: Sequences were aligned, and the results showed that the products of PCR amplification were the specific gene fragments of HDV. Hybridizing signals on gene chip showed the specificity and sensitivity in detecting HDV were satisfactory. CONCLUSION: Using PCR amplified products to construct gene chips for clinical diagnosis of HDV is a quick, simple and effective method.

Sun, Z. H., W. L. Zheng, et al. (2004). "[Preparation of the microarray for detecting hepatitis D virus]." Di Yi Jun Yi Da Xue Xue Bao **24**(1): 44-6, 49.

OBJECTIVE: To prepare the microarray for detection of hepatitis D virus (HDV). METHOD: Several pairs of specific PCR primers were designed according to the conserved region of HDV genome. The DNA microarray were prepared by blotting the PCR products onto the surface of glass slides with the use of robotics, and restriction display PCR (RD-PCR) was employed to label the samples. RESULT: Sequences analysis showed that the products of PCR amplification were the specific gene fragments of HDV. Hybridization signals on the gene chip demonstrated good specificity and sensitivity of the microarray for HDV detection. CONCLUSION: Microarray-based clinical HDV detection can be sensitive and effective.

Surendran, S., K. Michals-Matalon, et al. (2003). "Canavan disease: a monogenic trait with complex genomic interaction." Mol Genet Metab **80**(1-2): 74-80.

Canavan disease (CD) is an inherited leukodystrophy, caused by aspartoacylase (ASPA) deficiency, and accumulation of N-acetylaspartic acid (NAA) in the brain. The gene for ASPA has been cloned and more than 40 mutations have been described, with two founder mutations among Ashkenazi Jewish patients. Screening of Ashkenazi Jews for these two common mutations revealed a high carrier frequency, approximately 1/40, so that programs for carrier testing are currently in practice. The enzyme deficiency in CD interferes with the normal hydrolysis of NAA, which results in disruption of myelin and spongy degeneration of the white matter of the brain. The clinical features of the disease are macrocephaly, head lag, progressive severe mental retardation, and hypotonia in early life, which later changes to spasticity. A knockout mouse for CD has been generated, and used to study the pathophysiological basis for CD. Findings from the knockout mouse indicate that this monogenic trait leads to a series of genomic interaction in the brain. Changes include low levels of glutamate and GABA. Microarray expression analysis showed low level of expression of GABA-A receptor (GABRA6) and glutamate transporter (EAAT4). The gene Spi2, a gene involved in apoptosis and cell death, showed high level of expression. Such complexity of gene interaction results in the phenotype, the proteome, with spongy degeneration of the brain and neurological impairment of the mouse, similar to the human

counterpart. Aspartoacylase gene transfer trial in the mouse brain using adenoassociated virus (AAV) as a vector are encouraging showing improved myelination and decrease in spongy degeneration in the area of the injection and also beyond that site.

Suriawinata, A. and R. Xu (2004). "An update on the molecular genetics of hepatocellular carcinoma." Semin Liver Dis **24**(1): 77-88.

Hepatocellular carcinoma (HCC) is associated with multiple risk factors and is believed to arise from preneoplastic lesions, usually in the background of cirrhosis. Extensive studies on HCC and its precursors have demonstrated complex and heterogeneous genetic or chromosomal abnormalities along the way from preneoplastic lesions to HCCs. These genetic abnormalities include loss of heterozygosity, microsatellite instability, gene alterations, and aberrant global gene expression profiles. Although some genetic alterations involving the p53 family, Rb family, and Wnt pathways are particularly important in the development of HCCs, the molecular pathogenesis of HCC differs with etiology in some extent. Recent studies using DNA microarray technique have identified some unique gene expression profiles in hepatitis B virus (HBV)- and hepatitis C virus (HCV)-associated HCCs. Gene expression profiling also allows people to distinguish HCCs from normal tissue or preneoplastic lesions and to evaluate metastatic or recurrent potentials. These unique genes or gene products associated with malignant transformation and recurrent or metastatic potentials may serve as molecular markers for early diagnosis, prediction of prognosis, and responsiveness to therapy. To date, information that has accumulated for the past several decades is still incomplete, and we still are faced with a great challenge in deciphering the molecular mechanisms of HCCs.

Talaat, A. M., R. Lyons, et al. (2004). "The temporal expression profile of Mycobacterium tuberculosis infection in mice." Proc Natl Acad Sci U S A **101**(13): 4602-7.

Infection with Mycobacterium tuberculosis causes the illness tuberculosis with an annual mortality of approximately 2 million. Understanding the nature of the host-pathogen interactions at different stages of tuberculosis is central to new strategies for developing chemotherapies and vaccines. Toward this end, we adapted microarray technology to analyze the change in gene expression profiles of M. tuberculosis during infection in mice. This protocol provides the transcription profile of genes expressed during the course of early tuberculosis in immune-competent (BALB/c) and severe combined immune-deficient (SCID) hosts in comparison with growth in medium. The microarray analysis revealed clusters of genes that changed their transcription levels exclusively in the lungs of BALB/c, SCID mice, or medium over time. We identified a set of genes (n = 67) activated only in BALB/c and not in SCID mice at 21 days after infection, a key point in the progression of tuberculosis. A subset of the lung-activated genes was previously identified as induced during mycobacterial survival in a macrophage cell line. Another group of in vivo-expressed genes may also define a previously unreported genomic island. In addition, our analysis suggests the similarity between mycobacterial transcriptional machinery during growth in SCID and in broth, which questions the validity of using the SCID model for assessing mycobacterial virulence. The in vivo expression-profiling technology presented should be applicable to any microbial model of infection.

Tavares, C. A., A. P. Fernandes, et al. (2003). "Molecular diagnosis of leishmaniasis." Expert Rev Mol Diagn **3**(5): 657-67.

This review describes the worldwide situation of visceral and tegumentary leishmaniasis with an emphasis on diagnosis, including methods for the detection of antibodies, antigens, parasite DNA and of skin testing. The advantages and problems of each method are discussed and the need for a rapid, sensitive and low-cost diagnostic method for use in field conditions is highlighted. Recent advances in Leishmania genome sequencing, the use of DNA microarrays and protein microarray methodologies and their potential use for leishmaniasis diagnosis are presented.

Tzankov, A., A. Zimpfer, et al. (2003). "Expression of B-cell markers in classical hodgkin lymphoma: a tissue microarray analysis of 330 cases." Mod Pathol **16**(11): 1141-7.

Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma arise from B-lymphocytes. However, classical markers of the B-cell phenotype, such as CD20, are present only in about 25% of the cases. The aim of the present study was to assess expression of the B-cell-related antigens CD20, CD79a, and CD138 in classical Hodgkin lymphoma using a tissue microarray consisting of 330 classical Hodgkin lymphoma cases. Expression of CD15, CD20, CD30, CD79a, CD138, and latent membrane protein 1 of Epstein-Barr virus was assessed by immunohistochemistry, and the methodology was validated by direct comparison of CD20 expression on the tissue microarray cores with corresponding large sections. The influence of the number of arrayed sample cores on the obtained expression levels of CD20 was analyzed by comparing the results from single, duplicate, and triplicate cores. Two-hundred fifty-three (77%) of the 330 cases were morphologically representative. CD20 was expressed in 84 cases (33%), CD79a in 26 (10%), and CD138 in 2 (1%), respectively. CD20 and CD79a were co-expressed in 16 cases (P <.005), and expression of CD20 correlated inversely with CD15 (P <.01). Comparing the tissue microarray results with those from conventional sections for expression of CD20 yielded a concordance of 94% (63/67). Examining one, two, and three cores from individual cases revealed positivity for CD20 at 24% (61/253), 32% (82/253), and 33% (84/253), respectively. We conclude that B-cell markers are expressed in 38% of classical Hodgkin lymphoma in the following rank order: CD20>CD79a>>CD138. The use of two cores per tissue sample renders the tissue microarray technology effectively representative and thus very useful for high-throughput evaluation of heterogeneously expressed markers in classical Hodgkin lymphoma.

van Gijlswijk, R. P., E. G. Talman, et al. (2001). "Universal Linkage System: versatile nucleic acid labeling technique." Expert Rev Mol Diagn **1**(1): 81-91.

Over the last two decades nonradioactive nucleic acid labeling and detection systems have overcome the safety, disposal, stability and cost problems that are associated with radioactive techniques. Besides traditional, enzyme-mediated, nonradioactive labeling methods (e.g., random priming, nick translation or labeling by PCR), several chemical labeling systems have been developed (e.g., ULS, psoralen, alkylating agents). These methods provide fluorescent (or hapten) labeled probes for fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH) and microarray-based techniques. In this review the DNA-based molecular diagnostic applications and perspectives of the Universal Linkage System (ULS) technology will be described.

van Ijperen, C., P. Kuhnert, et al. (2002). "Virulence typing of Escherichia coli using microarrays." Mol Cell Probes **16**(5): 371-8.

We describe a microarray based broad-range screening technique for Escherichia coli virulence typing. Gene probes were amplified by PCR from a plasmid bank of characterised E. coli virulence genes and were spotted onto a glass slide to form an array of capture probes. Genomic DNA from E. coli strains which were to be tested for the presence of these virulence gene sequences was labelled with fluorescent cyanine dyes by random amplification and then hybridised against the array of probes. The hybridisation, washing and data analysis conditions were optimised for glass slides, and the applicability of the method for identifying the presence of the virulence genes was determined using reference strains and clinical isolates. It was found to be a sensitive screening method for detecting virulence genes, and a powerful tool for determining the pathotype of E. coli. It will be possible to expand and automate this microarray technique to make it suitable for rapid and reliable diagnostic screening of bacterial isolates.

van 't Wout, A. B., G. K. Lehrman, et al. (2003). "Cellular gene expression upon human immunodeficiency virus type 1 infection of CD4(+)-T-cell lines." J Virol **77**(2): 1392-402.

The expression levels of approximately 4,600 cellular RNA transcripts were assessed in CD4(+)-T-cell lines at different times after infection with human immunodeficiency virus type 1 strain BRU

(HIV-1(BRU)) using DNA microarrays. We found that several classes of genes were inhibited by HIV-1(BRU) infection, consistent with the G(2) arrest of HIV-1-infected cells induced by Vpr. These included genes involved in cell division and transcription, a family of DEAD-box proteins (RNA helicases), and all genes involved in translation and splicing. However, the overall level of cell activation and signaling was increased in infected cells, consistent with strong virus production. These included a subgroup of transcription factors, including EGR1 and JUN, suggesting they play a specific role in the HIV-1 life cycle. Some regulatory changes were cell line specific; however, the majority, including enzymes involved in cholesterol biosynthesis, of changes were regulated in most infected cell lines. Compendium analysis comparing gene expression profiles of our HIV-1 infection experiments to those of cells exposed to heat shock, interferon, or influenza A virus indicated that HIV-1 infection largely induced specific changes rather than simply activating stress response or cytokine response pathways. Thus, microarray analysis confirmed several known HIV-1 host cell interactions and permitted identification of specific cellular pathways not previously implicated in HIV-1 infection. Continuing analyses are expected to suggest strategies for impacting HIV-1 replication in vivo by targeting these pathways.

Volokhov, D., V. Chizhikov, et al. (2003). "Microarray analysis of erythromycin resistance determinants." J Appl Microbiol **95**(4): 787-98.

AIMS: To develop a DNA microarray for analysis of genes encoding resistance determinants to erythromycin and the related macrolide, lincosamide and streptogramin B (MLS) compounds. METHODS AND RESULTS: We developed an oligonucleotide microarray containing seven oligonucleotide probes (oligoprobes) for each of the six genes (ermA, ermB, ermC, ereA, ereB and msrA/B) that account for more than 98% of MLS resistance in Staphylococcus aureus clinical isolates. The microarray was used to test reference and clinical S. aureus and Streptococcus pyrogenes strains. Target genes from clinical strains were amplified and fluorescently labelled using multiplex PCR target amplification. The microarray assay correctly identified the MLS resistance genes in the reference strains and clinical isolates of S. aureus, and the results were confirmed by direct DNA sequence analysis. Of 18 S. aureus clinical strains tested, 11 isolates carry MLS determinants. One gene (ermC) was found in all 11 clinical isolates tested, and two others, ermA and msrA/B, were found in five or more isolates. Indeed, eight (72%) of 11 clinical isolate strains contained two or three MLS resistance genes, in one of the three combinations (ermA with ermC, ermC with msrA/B, ermA with ermC and msrA/B). CONCLUSIONS: Oligonucleotide microarray can detect and identify the six MLS resistance determinants analysed in this study. SIGNIFICANCE AND IMPACT OF THE STUDY: Our results suggest that microarray-based detection of microbial antibiotic resistance genes might be a useful tool for identifying antibiotic resistance determinants in a wide range of bacterial strains, given the high homology among microbial MLS resistance genes.

Volokhov, D., V. Chizhikov, et al. (2003). "Microarray-based identification of thermophilic Campylobacter jejuni, C. coli, C. lari, and C. upsaliensis." J Clin Microbiol **41**(9): 4071-80.

DNA microarrays are an excellent potential tool for clinical microbiology, since this technology allows relatively rapid identification and characterization of microbial and viral pathogens. In the present study, an oligonucleotide microarray was developed and used for the analysis of thermophilic Campylobacter spp., the primary food-borne pathogen in the United States. We analyzed four Campylobacter species: Campylobacter jejuni, C. coli, C. lari, and C. upsaliensis. Our assay relies on the PCR amplification of specific regions in five target genes (fur, glyA, cdtABC, ceuB-C, and fliY) as a first step, followed by microarray-based analysis of amplified DNAs. Alleles of two genes, fur and glyA, which are found in all tested thermophilic Campylobacter spp., were used for identification and discrimination among four bacterial species, the ceuB-C gene was used for discrimination between C. jejuni and C. coli, and the fliY and cdt genes were used as additional genetic markers specific either for C. upsaliensis and C. lari or for C. jejuni. The array was developed and validated by using 51 previously characterized Campylobacter isolates. All isolates were unambiguously identified on the basis of

hybridization patterns with 72 individual species-specific oligoprobes. Microarray identification of C. jejuni and C. coli was confirmed by PCR amplification of other genes used for identification (hipO and ask). Our results demonstrate that oligonucleotide microarrays are suitable for rapid and accurate simultaneous differentiation among C. jejuni, C. coli, C. lari, and C. upsaliensis.

Volokhov, D., A. Pomerantsev, et al. (2004). "Identification of Bacillus anthracis by multiprobe microarray hybridization." Diagn Microbiol Infect Dis **49**(3): 163-71.

We have developed a rapid assay based on microarray analysis of amplified genetic markers for reliable identification of Bacillus anthracis and its discrimination from other closely related bacterial species of the Bacillus cereus group. By combining polymerase chain reaction (PCR) amplification of six B. anthracis-specific genes (plasmid-associated genes encoding virulence factors (cyaA, pagA, lef, and capA, capB, capC) and one chromosomal marker BA-5449) with analysis of amplicons by microarray hybridization, we were able to unambiguously identify and discriminate B. anthracis among other closely related species. Bacillus identification relied on hybridization with multiple individual microarray oligonucleotide probes (oligoprobes) specific to each target B. anthracis gene. Evaluation of the assay was conducted using several B. anthracis strains (with or without pXO1 and pXO2 plasmids) as well as over 50 other species phylogenetically related to B. anthracis, including B. cereus, B. thuringiensis, B. mycoides, and B. subtilis. The developed microarray analysis of amplified genetic markers protocol provides an efficient method for (i) unambiguous identification and discrimination of B. anthracis from other Bacillus species and (ii) distinguishing between plasmid-containing and plasmid-free Bacillus anthracis strains.

Volokhov, D., A. Rasooly, et al. (2002). "Identification of Listeria species by microarray-based assay." J Clin Microbiol **40**(12): 4720-8.

We have developed a rapid microarray-based assay for the reliable detection and discrimination of six species of the Listeria genus: L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri, and L. grayi. The approach used in this study involves one-tube multiplex PCR amplification of six target bacterial virulence factor genes (iap, hly, inlB, plcA, plcB, and clpE), synthesis of fluorescently labeled single-stranded DNA, and hybridization to the multiple individual oligonucleotide probes specific for each Listeria species and immobilized on a glass surface. Results of the microarray analysis of 53 reference and clinical isolates of Listeria spp. demonstrated that this method allowed unambiguous identification of all six Listeria species based on sequence differences in the iap gene. Another virulence factor gene, hly, was used for detection and genotyping all L. monocytogenes, all L. ivanovii, and 8 of 11 L. seeligeri isolates. Other members of the genus Listeria and three L. seeligeri isolates did not contain the hly gene. There was complete agreement between the results of genotyping based on the hly and iap gene sequences. All L. monocytogenes isolates were found to be positive for the inlB, plcA, plcB, and clpE virulence genes specific only to this species. Our data on Listeria species analysis demonstrated that this microarray technique is a simple, rapid, and robust genotyping method that is also a potentially valuable tool for identification and characterization of bacterial pathogens in general.

Vondracek, M., D. A. Weaver, et al. (2002). "Transcript profiling of enzymes involved in detoxification of xenobiotics and reactive oxygen in human normal and simian virus 40 T antigen-immortalized oral keratinocytes." Int J Cancer **99**(6): 776-82.

The metabolic detoxification capacity may critically regulate the susceptibility of human tissues to cancer development. We used standardized and quantitative, reverse transcription-polymerase chain reaction (StaRT-PCR) and microarray chip techniques to analyze transcript levels of multiple detoxification enzymes in cultured normal human oral keratinocytes (NOK) and the Siman virus 40 T antigen-immortalized oral keratinocyte line SVpgC2a, viewing the latter as a model of a benign tumor state. With good agreement between the 2 methodologies, NOK and SVpgC2a were found to express transcripts for cytochrome P450 enzymes (CYPs), factors related to CYP induction and enzymes involved

in conjugation reactions or detoxification of reactive oxygen. The cell types expressed similar levels of CYP 2B6/7, CYP 2E1, P450 oxidoreductase, the aryl hydrocarbon receptor nuclear translocator, sulfotransferase 1A1, sulfotransferase 1A3, epoxide hydrolase, glutathione S-transferase M3, glutathione S-transferase pi and catalase, superoxide dismutase 1, glutathione peroxidase 1 and glutathione peroxidase 3. In contrast, SVpgC2a exhibited comparatively higher levels of CYP1A1, 1B1, aryl hydrocarbon receptor, glutathione S-transferase M1, 2, 4, 5, glutathione S-transferase theta 1 and superoxide dismutase 2 and comparatively lower levels of UDP glycosyltransferase 2 and microsomal glutathione S-transferase 1. Some transcripts, e.g., CYP 2A6/7, were not detected by either standard, non quantitative RT-PCR or the above methods, whereas others were barely quantifiable by StaRT-PCR, i.e., were present at 1-10 molecules/10(6) molecules of actin. Overall, the expression analysis demonstrated presence of mRNA for multiple enzymes involved in foreign compound metabolism and detoxification pathways, including several enzymes not previously reported for oral epithelium. Generally, the comparison of NOK from 2 individuals indicated relatively similar transcript levels of these enzymes. In contrast, differences between NOK and SVpgC2a, e.g., for CYP1B1, may reflect alteration caused by immortalization and aid identification of early stage tumor markers in oral epithelium.

Voyich, J. M., K. R. Braughton, et al. (2004). "Engagement of the pathogen survival response used by group A Streptococcus to avert destruction by innate host defense." J Immunol **173**(2): 1194-201.

Neutrophils are a critical component of human innate host defense and efficiently kill the vast majority of invading microorganisms. However, bacterial pathogens such as group A Streptococcus (GAS) successfully avert destruction by neutrophils to cause human infections. Relatively little is known about how pathogens detect components of the innate immune system to respond and survive within the host. In this study, we show that inactivation of a two-component gene regulatory system designated Ihk-Irr significantly attenuates streptococcal virulence in mouse models of soft tissue infection and bacteremia. Microarray analysis of wild-type and irr-negative mutant (irr mutant) GAS strains revealed that Ihk-Irr influenced expression of 20% of all transcripts in the pathogen genome. Notably, at least 11 genes involved in cell wall synthesis, turnover, and/or modification were down-regulated in the irr mutant strain. Compared with the wild-type strain, significantly more of the irr mutant strain was killed by human neutrophil components that destroy bacteria by targeting the cell envelope (cell wall and/or membrane). Unexpectedly, expression of ihk and irr was dramatically increased in the wild-type strain exposed to these same neutrophil products under conditions that favored cell envelope damage. We report a GAS mechanism for detection of innate host defense that initiates the pathogen survival response, in which cell wall synthesis is critical. Importantly, our studies identify specific genes in the pathogen survival response as potential targets to control human infections.

Wang, D., L. Coscoy, et al. (2002). "Microarray-based detection and genotyping of viral pathogens." Proc Natl Acad Sci U S A **99**(24): 15687-92.

The detection of viral pathogens is of critical importance in biology, medicine, and agriculture. Unfortunately, existing techniques to screen for a broad spectrum of viruses suffer from severe limitations. To facilitate the comprehensive and unbiased analysis of viral prevalence in a given biological setting, we have developed a genomic strategy for highly parallel viral screening. The cornerstone of this approach is a long oligonucleotide (70-mer) DNA microarray capable of simultaneously detecting hundreds of viruses. Using virally infected cell cultures, we were able to efficiently detect and identify many diverse viruses. Related viral serotypes could be distinguished by the unique pattern of hybridization generated by each virus. Furthermore, by selecting microarray elements derived from highly conserved regions within viral families, individual viruses that were not explicitly represented on the microarray were still detected, raising the possibility that this approach could be used for virus discovery. Finally, by using a random PCR amplification strategy in conjunction with the microarray, we were able to detect multiple viruses in human respiratory specimens without the use of sequence-specific or degenerate primers. This method is versatile and greatly expands the spectrum of

detectable viruses in a single assay while simultaneously providing the capability to discriminate among viral subtypes.

Wang, D., A. Urisman, et al. (2003). "Viral discovery and sequence recovery using DNA microarrays." PLoS Biol **1**(2): E2.

Because of the constant threat posed by emerging infectious diseases and the limitations of existing approaches used to identify new pathogens, there is a great demand for new technological methods for viral discovery. We describe herein a DNA microarray-based platform for novel virus identification and characterization. Central to this approach was a DNA microarray designed to detect a wide range of known viruses as well as novel members of existing viral families; this microarray contained the most highly conserved 70mer sequences from every fully sequenced reference viral genome in GenBank. During an outbreak of severe acute respiratory syndrome (SARS) in March 2003, hybridization to this microarray revealed the presence of a previously uncharacterized coronavirus in a viral isolate cultivated from a SARS patient. To further characterize this new virus, approximately 1 kb of the unknown virus genome was cloned by physically recovering viral sequences hybridized to individual array elements. Sequencing of these fragments confirmed that the virus was indeed a new member of the coronavirus family. This combination of array hybridization followed by direct viral sequence recovery should prove to be a general strategy for the rapid identification and characterization of novel viruses and emerging infectious disease.

Wang, J. and C. R. Taylor (2003). "Apoptosis and cell cycle-related genes and proteins in classical Hodgkin lymphoma: application of tissue microarray technique." Appl Immunohistochem Mol Morphol **11**(3): 206-13.

The etiology and pathogenesis of Hodgkin lymphoma (HL) are not yet known. There are implications of genes involved in programmed cell death (apoptosis), and there have been repeated suggestions of an association with Epstein-Barr virus (EBV). The aim of this study was to investigate the protein expression patterns of key cell cycle-related genes, together with evidence of apoptosis and EBV status, in relation to clinical stage in HLs. A double immunohistochemical and in situ hybridization technique was used to detect the expression of bcl-2, p53, retinoblastoma (Rb), p21, Ki67 (MIB 1), and topoisomerase IIalpha (TopoIIalpha), together with latent membrane protein-1 and EBER for EBV status and TdT-mediated dUTP-FITC nick end-labeling (TUNEL) as a measure of apoptosis, on tissue microarray sections of 62 cases of classic HL (35 NS, 17 MC, 8 LR, and 2 LD). A panel of phenotypic markers was used to facilitate recognition of Hodgkin and Reed-Sternberg (H-RS) cells: CD3, CD20, CD30, CD15, and EMA. The H-RS cells of 62 classic Hodgkin lymphomas were bcl-2-positive in 35 cases (56.45%), p53-positive in 14 (22.58%), and positive for both EBV latent membrane protein-1 and EBER in 37 (59.68%); there was complete concordance of results for EBV by both procedures. No correlation was found between expression of bcl-2, p53, or EBV markers in H-RS cells and clinical stage ($P > 0.05$). Expression of Rb, Ki67, p21, and TopoIIalpha did, however, show significant differences with clinical stage. Expression of Rb and p21 in CD30-positive H-RS cells decreased with more advanced stage ($P < 0.001$). In contrast, Ki67 and ToPoIIalpha expression increased with later stage ($P < 0.01$). No correlation was found between expression of any of these markers in H-RS cells and the subtypes of nodular sclerosis HL, mixed cellularity HL, and LRHL ($P > 0.05$). TUNEL was found in the nonneoplastic cellular background in all cases and in H-RS cells in only 10 of 62 cases (16.12%) (8 nodular sclerosis HL, 1 mixed cellularity HL, and 1 LRHL). There was a significant correlation between high expression of bcl-2 and a low score by TUNEL ($P < 0.05$). These data are consistent with the notion that overexpression of bcl-2 may be linked to blockage of apoptosis-mediated death of H-RS cells in classic HL. Abnormal expression of p53-related protein may not play a major role in HL, because it is present in H-RS cells in only a minority of cases. Increased expression of Ki67 and TopoIIalpha by H-RS cells is significantly associated with advanced stage and may indicate aggressive disease. Adverse clinical outcome in HL also is associated with loss of Rb and p21 protein expression, consistent with the possible

roles of Rb and p21 in inhibition of the growth of H-RS cells. Within the limitations of the methods used, almost two thirds of cases of HL provide evidence of an association with EBV. The tissue microarray technique is valuable not only for examination of large numbers of cases of a disease by a complex panel of markers but also potentially as a control for staining quality in immunohistochemistry and in situ hybridization.

Wang, R. F., M. L. Beggs, et al. (2004). "DNA microarray analysis of predominant human intestinal bacteria in fecal samples." Mol Cell Probes **18**(4): 223-34.

A microarray method was developed for the detection of 40 bacterial species reported in the literature to be predominant in the human gastrointestinal tract. The 40 species include seven species each of Bacteroides and Clostridium, six species of Ruminococcus, five species of Bifidobacterium, four species of Eubacterium, two species each of Fusobacterium, Lactobacillus and Enterococcus, and single species each of Collinsella, Eggerthella, Escherichia, Faecalibacterium and Finegoldia. Three 40-mer oligos specific for each bacterial species were designed based on comparison of the 16S rDNA sequences available in the GenBank database, and were used to make the DNA-array on epoxy slides. Using two universal primers, the 16S rRNA gene from bacteria present in fecal samples were amplified and labeled with Cyanine5-dCTP by PCR, and then hybridized to the DNA-array. After resolving some difficulties caused by sequence conflicts in GenBank and inaccurate reference strains, all 40 bacterial reference species gave positive results. The microarray method was used to screen fecal samples obtained from 11 healthy human volunteers for the presence of these intestinal bacteria. The results indicated that 25-37 of the 40 species could be detected in each fecal sample and that 33 of the species were found in a majority of the samples.

Wang, R. F., M. L. Beggs, et al. (2002). "Design and evaluation of oligonucleotide-microarray method for the detection of human intestinal bacteria in fecal samples." FEMS Microbiol Lett **213**(2): 175-82.

An oligonucleotide-microarray method was developed for the detection of intestinal bacteria in fecal samples collected from human subjects. The 16S rDNA sequences of 20 predominant human intestinal bacterial species were used to design oligonucleotide probes. Three 40-mer oligonucleotides specific for each bacterial species (total 60 probes) were synthesized and applied to glass slides. Cyanine5 (CY5)-labeled 16S rDNAs were amplified by polymerase chain reaction (PCR) from human fecal samples or bacterial DNA using two universal primers and were hybridized to the oligo-microarray. The 20 intestinal bacterial species tested were Bacteroides thetaiotaomicron, Bacteroides vulgatus, Bacteroides fragilis, Bacteroides distasonis, Clostridium clostridiiforme, Clostridium leptum, Fusobacterium prausnitzii, Peptostreptococcus productus, Ruminococcus obeum, Ruminococcus bromii, Ruminococcus callidus, Ruminococcus albus, Bifidobacterium longum, Bifidobacterium adolescentis, Bifidobacterium infantis, Eubacterium biforme, Eubacterium aerofaciens, Lactobacillus acidophilus, Escherichia coli, and Enterococcus faecium. The two universal primers were able to amplify full size 16S rDNA from all of the 20 bacterial species tested. The hybridization results indicated that the oligo-microarray method developed in this study is a reliable method for the detection of predominant human intestinal bacteria in the fecal samples.

Wang, R. F., S. J. Kim, et al. (2002). "Development of a membrane-array method for the detection of human intestinal bacteria in fecal samples." Mol Cell Probes **16**(5): 341-50.

A membrane-array method was developed for the detection of human intestinal bacteria in fecal samples without using the expensive microarray-arrayer and laser-scanner. The 16S rDNA sequences of 20 predominant human intestinal bacterial species were used to design oligonucleotide probes. Three 40-mer oligonucleotides specific for each bacterial species (total 60 probes) were synthesized and applied to nitrocellulose membranes. Digoxigenin (DIG)-labeled 16S rDNAs were amplified by polymerase chain reaction (PCR) from human fecal samples or pure cultured bacteria using two universal primers, and were hybridized to the membrane-array. Hybridization signals were read by NBT/BCIP color development.

The 20 intestinal bacterial species tested were Bacteroides thetaiotaomicron, B. vulgatus, B. fragilis, B. distasonis, Clostridium clostridiiforme, C. leptum, Fusobacterium prausnitzii, Peptostreptococcus productus, Ruminococcus obeum, R. bromii, R. callidus, R. albus, Bifidobacterium longum, B. adolescentis, B. infantis, Eubacterium biforme, E. aerofaciens, Lactobacillus acidophilus,Escherichia coli, and Enterococcus faecium. The two universal primers were able to amplify full size 16S rDNA from all of the 20 bacterial species tested. The hybridization results indicated that the membrane-array method is a reliable technique for the detection of predominant human intestinal bacteria in the fecal samples. The result was also confirmed by using specific PCR methods for these bacteria.

Wang, X., Z. H. Yuan, et al. (2004). "Gene expression profiles in an hepatitis B virus transfected hepatoblastoma cell line and differentially regulated gene expression by interferon-alpha." World J Gastroenterol **10**(12): 1740-5.

AIM: To study interactions between hepatitis B virus (HBV) and interferon-alpha in liver-derived cells. METHODS: mRNAs were separately isolated from an HBV-transfected cell line (HepG(2)2.2.15) and its parental cell line (HepG(2)) pre- and post-interferon-alpha (IFN-alpha) treatment at 6, 24 and 48 h, followed by hybridization with a cDNA microarray filter dotted with 14 000 human genes. After hybridization and scanning of the arrays, the data were analyzed using ArrayGauge software. The microarray data were further verified by Northern blot analysis. RESULTS: Compared to HepG(2) cells, 14 genes with known functions were down-regulated 3 to 12- magnitudes, while 7 genes were up-regulated 3-13 magnitudes in HepG(2)2.2.15 cells prior to IFN-alpha treatment. After interferon-alpha treatment, the expression of four genes (vascular endothelial growth factor, tyrosine phosphate 1E, serine protein with IGF-binding motif and one gene of clathrin light chain) in HepG(2)2.2.15 were up-regulated, while one gene encoding a GTP-binding protein, two genes of interferon-induced kinases and two proto-oncogenes were further down- regulated. Interestingly, under IFN-alpha treatment, a number of differentially regulated genes were new ESTs or genes with unknown functions. CONCLUSION: The up-regulated genes in HepG(2)2.2.15 cell line suggested that under IFN-alpha treatment, these repressed cellular genes in HBV infected hepatocytes could be partially restored, while the down- regulated genes were most likely the cellular genes which could not be restored under interferon treatment. These down-regulated genes identified by microarray analysis could serve as new targets for anti-HBV drug development or for novel therapies.

Wang, Y., W. L. Ma, et al. (2004). "Design and preparation of oligonucleotide microarray for vaccinia virus detection." Di Yi Jun Yi Da Xue Xue Bao **24**(2): 180-3.

OBJECTIVE: To study the preparation of oligonucleotide microarray for detecting vaccinia virus. METHODS: Oligonucleotide probes were designed and synthesized according to the specific genes of vaccinia virus. Sample DNA of the virus and the negative control sample were obtained and labeled by restriction display technique, followed by hybridization to the oligonucleotide microarray and scanned by Agilent scanner. RESULTS: Strong hybridization signals were detected from the viral DNA hybridized with the microarray, but were absent in the negative sample when positive probes were not used. CONCLUSION: Distinct differences in the hybridization signals between the virus sample and negative sample and between the samples obtained in different phase of infection demonstrate high specificity and sensitivity of the microarray for vaccinia virus detection.

Wang, Z., G. J. Vora, et al. (2004). "Detection and genotyping of Entamoeba histolytica, Entamoeba dispar, Giardia lamblia, and Cryptosporidium parvum by oligonucleotide microarray." J Clin Microbiol **42**(7): 3262-71.

Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum are the most frequently identified protozoan parasites causing waterborne disease outbreaks. The morbidity and mortality associated with these intestinal parasitic infections warrant the development of rapid and accurate detection and genotyping methods to aid public health efforts aimed at preventing and controlling

outbreaks. In this study, we describe the development of an oligonucleotide microarray capable of detecting and discriminating between E. histolytica, Entamoeba dispar, G. lamblia assemblages A and B, and C. parvum types 1 and 2 in a single assay. Unique hybridization patterns for each selected protozoan were generated by amplifying six to eight diagnostic sequences/organism by multiplex PCR; fluorescent labeling of the amplicons via primer extension; and subsequent hybridization to a set of genus-, species-, and subtype-specific covalently immobilized oligonucleotide probes. The profile-based specificity of this methodology not only permitted for the unequivocal identification of the six targeted species and subtypes, but also demonstrated its potential in identifying related species such as Cryptosporidium meleagridis and Cryptosporidium muris. In addition, sensitivity assays demonstrated lower detection limits of five trophozoites of G. lamblia. Taken together, the specificity and sensitivity of the microarray-based approach suggest that this methodology may provide a promising tool to detect and genotype protozoa from clinical and environmental samples.

Wells, S. I., B. J. Aronow, et al. (2003). "Transcriptome signature of irreversible senescence in human papillomavirus-positive cervical cancer cells." Proc Natl Acad Sci U S A **100**(12): 7093-8.

A frequent characteristic of human papillomavirus (HPV)-positive cervical cancers is the loss of viral E2 gene expression in HPV-infected cervical epithelial cells as a consequence of viral DNA integration into the cellular genome. The expression of E2 in HPV-positive cancer cells results in the repression of the viral E6/E7 oncogenes, activation of the p53 and pRB pathways, and a G1 cell cycle arrest, followed by induction of cellular senescence. The transcriptional consequences of E2-mediated cell cycle arrest that lead to senescence currently are unknown. Using conditional senescence induction in HeLa cells and microarray analysis, we describe here the expression profile of cells irreversibly committed to senescence. Our results provide insight into the molecular anatomy of senescence pathways and its regulation by HPV on-coproteins. These include the induction of the RAB vesicular transport machinery and a general down-regulation of chromatin regulatory molecules. The repression of tumor-specific G antigens during E2 senescence supports a reversal of the tumorigenic phenotype by E2 and the potential approach of tumor-specific G antigen-specific immunotherapy for cervical cancer.

Wenli, M., W. Yan, et al. (2004). "An oligonucleotide microarray for the detection of vaccinia virus." Br J Biomed Sci **61**(3): 142-5.

Vaccinia virus is a member of the orthopoxvirus group, to which also belongs variola virus, one of the most hazardous pathogens known to man. To establish a model system to detect orthopoxviruses, a vaccinia oligonucleotide microarray is designed, produced and tested. Vaccinia virus is used to test the prepared microarrays. The virus DNA samples in different propagation phases are extracted and hybridised with the oligonucleotide microarray. The results showed that the oligonucleotide microarray can detect vaccinia virus with high specificity and sensitivity.

West, M., C. Blanchette, et al. (2001). "Predicting the clinical status of human breast cancer by using gene expression profiles." Proc Natl Acad Sci U S A **98**(20): 11462-7.

Prognostic and predictive factors are indispensable tools in the treatment of patients with neoplastic disease. For the most part, such factors rely on a few specific cell surface, histological, or gross pathologic features. Gene expression assays have the potential to supplement what were previously a few distinct features with many thousands of features. We have developed Bayesian regression models that provide predictive capability based on gene expression data derived from DNA microarray analysis of a series of primary breast cancer samples. These patterns have the capacity to discriminate breast tumors on the basis of estrogen receptor status and also on the categorized lymph node status. Importantly, we assess the utility and validity of such models in predicting the status of tumors in crossvalidation determinations. The practical value of such approaches relies on the ability not only to assess relative probabilities of clinical outcomes for future samples but also to provide an honest assessment of the uncertainties associated with such predictive classifications on the basis of the selection of gene subsets for each

validation analysis. This latter point is of critical importance in the ability to apply these methodologies to clinical assessment of tumor phenotype.

Wilson, M., J. DeRisi, et al. (1999). "Exploring drug-induced alterations in gene expression in Mycobacterium tuberculosis by microarray hybridization." Proc Natl Acad Sci U S A **96**(22): 12833-8.

Tuberculosis is a chronic infectious disease that is transmitted by cough-propelled droplets that carry the etiologic bacterium, Mycobacterium tuberculosis. Although currently available drugs kill most isolates of M. tuberculosis, strains resistant to each of these have emerged, and multiply resistant strains are increasingly widespread. The growing problem of drug resistance combined with a global incidence of seven million new cases per year underscore the urgent need for new antituberculosis therapies. The recent publication of the complete sequence of the M. tuberculosis genome has made possible, for the first time, a comprehensive genomic approach to the biology of this organism and to the drug discovery process. We used a DNA microarray containing 97% of the ORFs predicted from this sequence to monitor changes in M. tuberculosis gene expression in response to the antituberculous drug isoniazid. Here we show that isoniazid induced several genes that encode proteins physiologically relevant to the drug's mode of action, including an operonic cluster of five genes encoding type II fatty acid synthase enzymes and fbpC, which encodes trehalose dimycolyl transferase. Other genes, not apparently within directly affected biosynthetic pathways, also were induced. These genes, efpA, fadE23, fadE24, and ahpC, likely mediate processes that are linked to the toxic consequences of the drug. Insights gained from this approach may define new drug targets and suggest new methods for identifying compounds that inhibit those targets.

Wilson, W. J., C. L. Strout, et al. (2002). "Sequence-specific identification of 18 pathogenic microorganisms using microarray technology." Mol Cell Probes **16**(2): 119-27.

We have developed a Multi-Pathogen Identification (MPID) microarray for high confidence identification of eighteen pathogenic prokaryotes, eukaryotes and viruses. Analysis of amplified products from pathogen genomic DNA using microarray hybridization allows for highly specific and sensitive detection, and allows the discrimination between true amplification products and false positive amplification products that might be derived from primers annealing to non-target sequences. Species-specific primer sets were used to amplify multiple diagnostic regions unique to each individual pathogen. Amplified products were washed over the surface of the microarray, and labelled with phycoerythrin-streptavidin for fluorescence detection. A series of overlapping 20-mer oligonucleotide probes hybridize to the entire diagnostic region, while parallel hybridizations on the same surface allow simultaneous screening for all organisms. Comparison to probes that differ by a single mismatch at the central position reduced the contribution of non-specific hybridization. Samples containing individual pathogens were analyzed in separate experiments and the corresponding species-specific diagnostic regions were identified by fluorescence among their highly redundant probe sets. On average, 91% of the 53 660 pathogen probes on the MPID microarray performed as predicted. The limit of detection was found to be as little as 10 fg of B. anthracis DNA in samples that were amplified with six diagnostic primer-pairs. In contrast, PCR products were not observed at this concentration when identical samples were prepared and visualized by agarose gel electrophoresis.

Wolfgang, M. C., J. Jyot, et al. (2004). "Pseudomonas aeruginosa regulates flagellin expression as part of a global response to airway fluid from cystic fibrosis patients." Proc Natl Acad Sci U S A **101**(17): 6664-8.

Cystic fibrosis (CF) patients are highly susceptible to chronic lung infections by the environmental bacterium Pseudomonas aeruginosa. The overproduction and accumulation of dehydrated viscous respiratory mucus and excessive inflammation represents a defining feature of CF and constitutes the major environment encountered by P. aeruginosa during chronic infections. We applied whole-genome microarray technology to investigate the ability of P. aeruginosa to respond to signals found in

muco-purulent airway liquids collected from chronically infected CF patients. Particularly notable was the activation of the Rhl-dependent quorum-sensing (QS) network and repression of fliC, which encodes flagellin. Activation of the Rhl branch of the QS network supports the observation that QS molecules are produced in the chronically infected CF lung. The shut-off of flagellin synthesis in response to CF airway liquids was rapid and independent of QS and the known regulatory networks controlling the hierarchical expression of flagellar genes. As flagellin is highly immunogenic and subject to detection by host pattern recognition receptors, its repression may represent an adaptive response that allows P. aeruginosa to avoid detection by host defense mechanisms and phagocytosis during the chronic phase of CF lung infections.

Wolfgang, M. C., B. R. Kulasekara, et al. (2003). "Conservation of genome content and virulence determinants among clinical and environmental isolates of Pseudomonas aeruginosa." Proc Natl Acad Sci U S A **100**(14): 8484-9.

Pseudomonas aeruginosa is a ubiquitous environmental bacterium capable of causing a variety of life-threatening human infections. The genetic basis for preferential infection of certain immunocompromised patients or individuals with cystic fibrosis by P. aeruginosa is not understood. To establish whether variation in the genomic repertoire of P. aeruginosa strains can be associated with a particular type of infection, we used a whole-genome DNA microarray to determine the genome content of 18 strains isolated from the most common human infections and environmental sources. A remarkable conservation of genes including those encoding nearly all known virulence factors was observed. Phylogenetic analysis of strain-specific genes revealed no correlation between genome content and infection type. Clusters of strain-specific genes in the P. aeruginosa genome, termed variable segments, appear to be preferential sites for the integration of novel genetic material. A specialized cloning vector was developed for capture and analysis of these genomic segments. With this capture system a site associated with the strain-specific ExoU cytotoxin-encoding gene was interrogated and an 80-kb genomic island carrying exoU was identified. These studies demonstrate that P. aeruginosa strains possess a highly conserved genome that encodes genes important for survival in numerous environments and allows it to cause a variety of human infections. The acquisition of novel genetic material, such as the exoU genomic island, through horizontal gene transfer may enhance colonization and survival in different host environments.

Wu, C. F., J. J. Valdes, et al. (2003). "DNA microarray for discrimination between pathogenic 0157:H7 EDL933 and non-pathogenic Escherichia coli strains." Biosens Bioelectron **19**(1): 1-8.

The primary technique currently used to detect biological agents is based on immunoassays. Although sensitive and specific, currently employed immunoassays generally rely on the detection of a single epitope, and therefore often cannot discriminate subtle strain-specific differences. Since DNA microarrays can hybridize hundreds to thousands of genomic targets simultaneously and do not rely on phenotypic expression of these genetic features for identification purposes, they have enormous potential to provide inexpensive, flexible and specific strain-specific detection and identification of pathogens. In this study, pathogenic Escherichia coli O157:H7-specific genes, non-pathogenic K12-specific genes, common E. coli genes, and negative control genes were polymerase chain reaction-amplified and spotted onto the surface of treated glass slides. After labeled bacterial cDNA samples were hybridized with probes on the microarray, specific fluorescence patterns were obtained, enabling identification of pathogenic E. coli O157:H7 and non-pathogenic E. coli K12. To test the utility of this microarray device to detect genetically engineered bacteria, E. coli BL21 (a B strain derivative with antibiotic resistance gene, ampR) and E. coli JM107 (a K12 strain derivative lacking the gene ompT) were also employed. The array successfully confirmed the strain genotypes and demonstrated that antibiotic resistance can also be detected. The ability to assess multiple data points makes this array method more efficient and accurate than a typical immunoassay, which detects a single protein product.

Wu, Y., X. Wang, et al. (2003). "Data-mining approaches reveal hidden families of proteases in the

genome of malaria parasite." Genome Res **13**(4): 601-16.

The search for novel antimalarial drug targets is urgent due to the growing resistance of Plasmodium falciparum parasites to available drugs. Proteases are attractive antimalarial targets because of their indispensable roles in parasite infection and development, especially in the processes of host erythrocyte rupture/invasion and hemoglobin degradation. However, to date, only a small number of proteases have been identified and characterized in Plasmodium species. Using an extensive sequence similarity search, we have identified 92 putative proteases in the P. falciparum genome. A set of putative proteases including calpain, metacaspase, and signal peptidase I have been implicated to be central mediators for essential parasitic activity and distantly related to the vertebrate host. Moreover, of the 92, at least 88 have been demonstrated to code for gene products at the transcriptional levels, based upon the microarray and RT-PCR results, and the publicly available microarray and proteomics data. The present study represents an initial effort to identify a set of expressed, active, and essential proteases as targets for inhibitor-based drug design.

Xiong, W., X. Wang, et al. (2003). "Analysis of gene expression in hepatitis B virus transfected cell line induced by interferon." Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) **35**(12): 1053-60.

Infection of hepatitis B virus (HBV) continues to be a significant health problem. alpha interferon (IFN-alpha) and gamma interferon (IFN-gamma) have been proven to be effective in inhibiting HBV replication. To study the global effect of HBV persistent existence on IFN induced cellular gene expression, cDNA microarrays dotted with 14 112 human genes were used to examine the transcriptional changes between an HBV DNA transfected cell line (HepG2.2.15) and its parental cell line (HepG2) after the treatment of IFN-alpha or IFN-gamma for 6 h. The results showed that many genes related to cell cycle, proliferation, apoptosis and new ESTs were regulated by IFN-alpha and/or IFN-gamma. Many genes involved in kinase and signal transduction, transcription regulation, antigen presentation and processing were differentially regulated between these two cell lines post IFN-alpha or IFN-gamma treatment. Interestingly, several IFN-differentially regulated genes, such as MyD88 and Diubiquitin, were found to inhibit HBV gene expression, and MyD88 was proved to inhibit HBV replication. Taken together, our results revealed the global effects of HBV persistent existence on IFN-induced cellular gene expression. The novel antiviral genes identified by microarray could be potentially developed as new anti-HBV drugs or for novel therapies.

Xu, D., D. Y. Tian, et al. (2004). "Effect of SEN virus coinfection on outcome of lamivudine therapy in patients with hepatitis B." World J Gastroenterol **10**(7): 968-71.

AIM: Interactions between hepatitis B virus (HBV) and other viral hepatitis infections are well known, whether the newly discovered SEN virus (SENV) has any effect on lamivudine antiHBV activity is unclear. Our aim was to clarify the effect on treatment outcome of coinfection with SEN virus in patients with hepatitis B during lamivudine therapy. METHODS: Nested polymerase chain reaction (PCR) amplification was used to detect SENV-D and SENV-H strains in serum from 45 patients with chronic hepatitis B treated with lamivudine 100 mg daily for 12 mo. HBV DNA load was detected with fluorescence quantitative PCR (FQ-PCR) and YMDD (tyrosine, methionine, aspartate, aspartate) motif mutation of HBV DNA was investigated with cDNA microarray. RESULTS: SENV DNA was detected in 5 of 45(11.1%) cases after 12 mo they received lamivudine treatment. SENV-D and SENV-H were 4.4% and 6.7% respectively. HBV DNA failed to respond to lamivudine therapy in 4 of 5 SENV coinfected patients while only 10 of 40 patients became SENV positive and the difference was statistically significant. Response of ALT and HBeAg to lamivudine had no significant difference between coinfection patients and single HBV infection ones. CONCLUSION: Coinfection with SEN virus in chronic hepatitis B patients may adversely affect the outcome of lamivudine treatment.

Yang, W. C., G. V. Devi-Rao, et al. (2002). "General and specific alterations in programming of global viral gene expression during infection by VP16 activation-deficient mutants of herpes simplex virus type

1." J Virol **76**(24): 12758-74.

During productive infection by herpes simplex virus 1 (HSV-1), viral gene expression occurs in a temporally regulated cascade in which transcription of the viral immediate-early (IE) genes is strongly stimulated by the virion protein VP16. We have employed an oligonucleotide microarray to examine the effect of VP16 mutations on the overall pattern of viral gene expression following infection of HeLa cells. This microarray detects essentially all HSV-1 transcripts with relative and absolute levels correlating well with known kinetics of expression. This analysis revealed that deletion of the VP16 activation domain sharply reduced overall viral gene expression; moreover, the pattern of this reduced expression varied greatly from the pattern of a wild-type (wt) infection. However, when this mutant virus was delivered at a high multiplicity of infection or in the presence of the cellular stress inducer hexamethylene bisacetamide, expression was largely restored to the wt levels and pattern. Infection with virions that deliver wt VP16 protein at the start of infection but synthesize only truncated VP16 resulted in a normal kinetic cascade. This suggests that newly synthesized VP16 does not play a significant role in the expression of later classes of transcripts. The VP16 activation domain comprises two subregions. Deletion of the C-terminal subregion resulted in minimal changes in the level and profile of gene expression compared to a normal (wt) cascade. In contrast, deletion of the N-terminal subregion reduced the overall expression levels and skewed the relative levels of IE transcripts but did not significantly alter the kinetic pattern of early and late transcript expression. We conclude that the general activation of IE gene transcription by VP16, but not the specific ratios of IE transcripts, is necessary for the subsequent ordered expression of viral genes. Moreover, this report establishes the feasibility of microarray analysis for globally assessing viral gene expression programs as a function of the conditions of infection.

Yin, D., B. Fox, et al. (2004). "Identification of antimicrobial targets using a comprehensive genomic approach." Pharmacogenomics **5**(1): 101-13.

Regulated antisense RNA enables the construction of a defined set of conditional growth-defective/lethal strains. In this study, we expanded the regulated antisense RNA interference technology and developed a high-throughput screening strategy to identify the potential drug targets of novel antimicrobials. To prove this concept, the specific antisense sublibrary of different essential open reading frames were pooled in the presence of an inducer, and treated with or without sublethal levels of mupirocin, triclosan, or gentamicin. Antisense RNA-expressing strains that were sensitized for increased susceptibility to the antibiotics were selectively detected via DNA subtractive hybridization, microarray, and whole-cell analyses. No strain was identified as supersensitive to gentamicin because there was no target-specific antisense strain in this sublibrary. In contrast, strains expressing antisense to isoleucine tRNA synthetase (ileS) and enoyl-[acyl-carrier-protein] reductase (fabI) were specifically identified as having increased susceptibility to mupirocin and triclosan, respectively. These results demonstrated that ileS and fabI antisense strains showed significant increases of susceptibility only to their specific inhibitors. This data demonstrates that a regulated antisense RNA expression library provides an effective tool to assist in the identification of potential targets for novel antibacterial agents.

Yoshikawa, Y., T. Ichihara, et al. (2003). "[Detection of drug-resistant Mycobacterium tuberculosis isolates using DNA microarray]." Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi **14**(1): 45-50.

Rapid identification of drug-resistant strains of Mycobacterium tuberculosis is an important problem to adequate patient treatment. However, current clinical assays for determining antibiotic susceptibility in M. tuberculosis require many weeks to complete due to the slow growth of the bacilli. We have developed simple and rapid drug susceptibility test using DNA microarray "Oligoarray TB," that allows the detection of rifampicin (RFP), isoniazide (INH), kanamycin (KM), streptomycin (SM), and ethambutol (EM)-resistant strains within 6 h with DNA extracted directly from sputum or cultured cells. The genes related to drug-resistance, results of detection using Oligoarray TB, comparative evaluation with media, and sequencing analysis of strains generating discrepant results in testing for INH-resistant will be discussed in this presentation.

Yu, S. L., H. W. Chen, et al. (2004). "Differential gene expression in gram-negative and gram-positive sepsis." Am J Respir Crit Care Med **169**(10): 1135-43.

Sepsis is the most common cause of death in patients in the intensive care unit. Genome-wide gene expression analysis can provide insights into the molecular alterations of sepsis. Total mRNA was extracted from the livers of 6 uninfected control mice and 60 septic mice after infusion of either live Escherichia coli or Staphylococcus aureus. Using a murine complementary DNA microarray system, changes in gene expression were monitored at six time points (uninfected, 2, 8, 24, 48, and 72 hours). Overall, 4.8% of 6,144 assessed genes were differentially regulated with a greater than twofold change across all time points. Most of the genes with altered expression were commonly present in gram-negative and gram-positive sepsis, but the expression levels of 17 genes were different between both types of sepsis at particular time points after infection. The microarray results support the hypothesis that both gram-positive and gram-negative sepsis share a final common pathway involved in the pathogenesis of sepsis, but certain genes are differentially expressed under distinct regulation. These results may provide insights into the pathogenesis of sepsis and may also help identify some altered genes that can serve as new targets for diagnostic tools and therapeutic strategies.

Yu, X., M. Susa, et al. (2004). "Development and validation of a diagnostic DNA microarray to detect quinolone-resistant Escherichia coli among clinical isolates." J Clin Microbiol **42**(9): 4083-91.

The incidence of resistance against fluoroquinolones among pathogenic bacteria has been increasing in accordance with the worldwide use of this drug. Escherichia coli is one of the most relevant species for quinolone resistance. In this study, a diagnostic microarray for single-base-mutation detection was developed, which can readily identify the most prevalent E. coli genotypes leading to quinolone resistance. Based on genomic sequence analysis using public databases and our own DNA sequencing results, two amino acid positions (83 and 87) on the A subunit of the DNA gyrase, encoded by the gyrA gene, have been identified as mutation hot spots and were selected for DNA microarray detection. Oligonucleotide probes directed against these two positions were designed so that they could cover the most important resistance-causing and silent mutations. The performance of the array was validated with 30 clinical isolates of E. coli from four different hospitals in Germany. The microarray results were confirmed by standard DNA sequencing and were in full agreement with phenotypic antimicrobial susceptibility testing.

Yue, J., W. Shi, et al. (2004). "Detection of rifampin-resistant Mycobacterium tuberculosis strains by using a specialized oligonucleotide microarray." Diagn Microbiol Infect Dis **48**(1): 47-54.

DNA microarray represents one of the major advances in diagnostic sequencing of polymerase chain reaction (PCR) products. Until now, arrays have been relatively expensive, complex to perform, and difficult to interpret, limiting their wide application in the clinical laboratory. A moderate-density oligonucleotide microarray that can rapidly identify Mycobacterium tuberculosis rifampin-resistant strains was developed. The method is based on the detection of point mutations and other rearrangements in the rpoB gene region determining rifampin resistance. Rifampin resistance was determined by hybridizing fluorescently labeled, amplified genetic material generated from bacterial colonies to the array. Fifty-three rifampin-resistant M. tuberculosis and 15 rifampin-susceptible M. tuberculosis were tested and results were concordant with those based on culture drug susceptibility testing and sequencing. Rifampin-resistant clinical isolates were detected in as little as 1.5 hours after PCR amplification with visual results. It is demonstrated that oligonucleotide microarray is an efficient, specialized technique to implement and can be used as a rapid method for detecting rifampin resistance to complement standard culture-based method.

Zhang, J., Y. Wang, et al. (2002). "[Detection of Mycoplasma hyorhinis in gastric cancer using bio-chip technology]." Zhonghua Yi Xue Za Zhi **82**(14): 961-5.

OBJECTIVE: To determine the prevalence of Mycoplasma hyorhinis in archived paraffin-

embedded gastric cancer tissue and to find whether Mycoplasma hyorhinis infection can influence gene expression level in gastric cancer cells. METHODS: A high-dense tissue microarray containing 105 gastric cancer samples, 101 benign margin samples and 62 non-cancerous gastric disease samples resected during operation was constructed. PD4, a specific anti-Mycoplasma-hyorhinis Mab, was used to detect the infection rate in all the samples in the tissue microarrays immunohistochemically. Then, cDNA microarray was used to pinpoint differentially the expressed genes between gastric cancer cell line MGC803 samples with and without Mycoplasma hyorhinis infection. RESULTS: The infection rate of M. hyorhinis was 54.1% 53/98 in gastric cancer samples, 51.7% 45/87 in benign margin samples, and 15.8 % 9/57 in non-cancerous disease samples respectively. The difference of infection rates between gastric cancer and non-cancerous gastric disease was statistically significant ($P = 0.001$). Highly differentiated adenocarcinomas had more opportunity (84.6%) to be infected with M. hyorhinis than poorly differentiated ones (45.9%)($P < 0.05$). Intestinal type of gastric cancers (according to Lauren's classification) got the infection more often than diffused type. About 409 gene expression alterations were detected in 48 000 sites from two gastric cancer cell lines and the expression levels of some genes correlating with cell apoptosis and cell adhesion were down regulated after Mycoplasma hyorhinis infection. CONCLUSION: The infection rate of M. hyorhinis is significantly higher in gastric cancer than in other gastric diseases, thus indicating the association between Mycoplasma infection and gastric cancer. Mycoplasma hyorhinis infection influences the gene expression level in gastric cancer cell line MGC803, which indicates that the infection could have something to do with the process of gastric cancer. The question whether M.hyorhinis has oncogenic potential remains to be elucidated.

Zhang, W., L. Bao, et al. (2002). "[Research on preparation of mycobacterium tuberculosis DNA microarray]." Hua Xi Yi Ke Da Xue Xue Bao **33**(2): 294-5, 308.

OBJECTIVE: To optimize and develop the technique for mycobacterium tuberculosis DNA microarray. METHODS: The process included preparation of DNA samples, spotting and past-spotting treatment of arrays. DNA microarrays were prepared by spotting fluorescence labeled PCR products of target genes onto specially treated glass slides with robotics. The fluorescent signals before and after treatment were scanned with a scanner, and the DNA attachment rate was calculated from the obtained data by software. RESULTS: A foundation for optimizing the conditions of Mycobacterium tuberculosis DNA microarrays has been laid. The support aldehyde-modified glass slide is useful for anchoring DNA at Some distance. DMSO as spotting solution is of benefit to preparation of Mycobacterium tuberculosis DNA microarray. Drying the chip at 37 degrees C temperature after spotting can enhance the DNA combination rate. CONCLUSION: Several key steps of this technique have been optimized. This study has provided a foundation for optimizing the DNA attachment conditions in creating mycobacterium tuberculosis DNA microarray.

Zhang, Y., M. Jamaluddin, et al. (2003). "Ribavirin treatment up-regulates antiviral gene expression via the interferon-stimulated response element in respiratory syncytial virus-infected epithelial cells." J Virol **77**(10): 5933-47.

Respiratory syncytial virus (RSV) is a mucosa-restricted virus that is a leading cause of epidemic respiratory tract infections in children. RSV replication is a potent activator of the epithelial-cell genomic response, influencing the expression of a spectrum of cellular pathways, including proinflammatory chemokines of the CC, CXC, and CX(3)C subclasses. Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a nontoxic antiviral agent currently licensed for the treatment of severe RSV lower respiratory tract infections. Because ribavirin treatment reduces the cytopathic effect in infected cells, we used high-density microarrays to investigate the hypothesis that ribavirin modifies the virus-induced epithelial genomic response to replicating virus. Ribavirin treatment administered in concentrations of 10 to 100 micro g/ml potently inhibited RSV transcription, thereby reducing the level of RSV N transcripts to approximately 13% of levels in nontreated cells. We observed that in both the absence and the presence of ribavirin, RSV infection induced global alterations in the host epithelial cell, affecting approximately

49% of the approximately 6,650 expressed genes detectable by the microarray. Ribavirin influences the expression of only 7.5% of the RSV-inducible genes (total number of genes, 272), suggesting that the epithelial-cell genetic program initiated by viral infection is independent of high-level RSV replication. Hierarchical clustering of the ribavirin-regulated genes identified four expression patterns. In one group, ribavirin inhibited the expression of the RSV-inducible CC chemokines MIP-1 alpha and -1 beta, which are important in RSV-induced pulmonary pathology, and interferon (IFN), a cytokine important in the mucosal immune response. In a second group, ribavirin further up-regulated a set of RSV- and IFN-stimulated response genes (ISGs) encoding antiviral proteins (MxA and p56), complement products, acute-phase response factors, and the STAT and IRF transcription factors. Because IFN-beta expression itself was reduced in the ribavirin-treated cells, we further investigated the mechanism for up-regulation of the IFN-signaling pathway. Enhanced expression of IFI 6-16, IFI 9-27, MxA/p78, STAT-1 alpha, STAT-1 beta, IRF-7B, and TAP-1-LMP2 transcripts were independently reproduced by Northern blot analysis. Ribavirin-enhanced TAP-1-LMP2 expression was a transcriptional event where site mutations of the IFN-stimulated response element (ISRE) blocked RSV and ribavirin-inducible promoter activity. Furthermore, ribavirin up-regulated the transcriptional activity of a reporter gene selectively driven by the ISRE. In specific DNA pull-down assays, we observed that ribavirin enhanced RSV-induced STAT-1 binding to the ISRE. We conclude that ribavirin potentiates virus-induced ISRE signaling to enhance the expression of antiviral ISGs, suggesting a mechanism for the efficacy of combined treatment with ribavirin and IFN in other chronic viral diseases.

Zhao, W., W. Liu, et al. (2002). "[Genotyping of hepatitis C virus by hepatitis gene diagnosis microarray]." Zhonghua Yi Xue Za Zhi **82**(18): 1249-53.

OBJECTIVE: To study the preparation of hepatitis C virus (HCV) diagnosis microarray and its accuracy in diagnosis of gene type of hepatitis C virus. METHODS: Probe and primer and primers were designed in 5'-untranslated region and C region of hepatitis C virus gene. The probes were synthesized by DNA synthesizer. Solutions of probe of the final concentration of 50 micromol/L were made by dissolving the probes into sodium carbonate buffer. Hepatitis C virus genotype array spotting was performed by pin-based spotting robot PixSys5500 with CMP3 pin. The gene chips were prepared by spotting the probes onto the specially treated glass sliders. Sixty HCV RNA positive serum samples were obtained from the in-patients of the Nanjing Second Hospital (experimental group), and 60 HCV RNA negative serum samples were obtained from the healthy people undergoing physical examination (control group). Quantitative examination of serum HCV RNA was made by fluorescent quantitation PCR. The HCV RNA in the serum specimens of the experimental group (with the HCV RNA concentration of more than 500 copies/ml) and of the control group (with the HCV RNA concentration of less than 500 copies/ml) was isolated and purified, underwent reversed transcription and nested PCR to be amplified, and then genotyped by gene microarray and HCV RNA sequencing. During the experiment, double blind method was used. RESULTS: Tested by the gene microarray, the serum specimens in the experimental group were all HCV RNA positive, out of which 46 cases were 1b type, 3 cases were 3a type, 3 cases were 3b type, 2 cases were 2a type, 2 cases were 2b type, 2 cases were 1b + 2a type, and 2 cases were 3a type. Tested by nucleotide sequencing assay, 50 cases were 1b type, 3 cases were 3a type, 3 cases were 3b type, 2 cases were 2a type, and 2 cases were 2b type. The double-blind test results showed a coincidence rate of 93.3% in genotyping HCV by these two methods. CONCLUSION: Hepatitis gene microarray can be used in detection of serum HCV RNA and in diagnostic genotyping with great accuracy.

Zhao, W., J. M. Wan, et al. (2003). "Hepatitis gene chip in detecting HBV DNA, HCV RNA in serum and liver tissue samples of hepatitis patients." Hepatobiliary Pancreat Dis Int **2**(2): 234-41.

OBJECTIVE: To study the preparation of diagnostic gene chip for detecting hepatitis B virus (HBV) and hepatitis C virus (HCV) and its accuracy in detecting HBV DNA and HCV RNA in serum and liver tissues. METHODS: The probes, which depend on the conservative gene fragment of hepatitis virus, was designed, synthesized and spotted on the modified glass. The probes and some other control probes

were assembled on the diagnostic microarray of hepatitis virus. The gene of hepatitis virus, purified from blood or tissue, was labeled with fluorescence and hybridized to the microarray. The hybridized microarry was scanned with microarray scanner and the diagnostic result was analyzed from the scanning data. Fourty patients with hepatitis B virus and 40 healthy people or 40 patients with hepatitis C virus were subjected to detection of HBV DNA and HCV RNA with the hepatitis virus gene chip by the double-blind method. Paraffin liver specimens obtained from 99 cases of posthepatitic cirrhosis were used to detect HBV DNA. The liver tissues and serum from 15 cases of chronic hepatitis B were used to detect HBV DNA. Simultaneously, HBsAg and HBcAg were detected in the serum by fluorescence microparticle quantitation, HBV DNA and HCV RNA in the serum by PCR, and HBcAg in liver tissues by immunocytochemistry or HBV DNA by in situ molecular hybridization. RESULTS: Chip detection of serum specimens showed that 30 patients were HBV DNA positive and 10 HBV DNA negative in the 40 patients with HBV positive, 25 patients were HCV RNA positive and 15 patients were HCV RNA negative in the 40 patients with HCV positive, and all were HBV and HCV negative in the 40 healthy people. In 15 patients with HBV marker positive who were subjected to liver biopsy, 15 patients were detected HBV DNA positive in serum by gene chip, 15 patients HBcAg positive in liver tissues by immunocytochemistry, 14 patients HBV DNA positive in liver tissues by in situ molecular hybridization, and 14 patients HBV DNA positive in liver tissues by gene chip. Paraffin liver tissues specimens from the 99 patients with posthepatitis B cirrhosis showed that 67 patients were detected HBcAg positive by immunocytochemistry, 53 patients HBV DNA positive by in situ molecular hybridization, and 46 patients HBV DNA positive by gene chip. In the 46 patients, 40 patients were detected HBV DNA and HBcAg positive by in situ molecular hybridization and immunocytochemistry, 6 patients only HBcAg positive, and 33 patients HBcAg negative. CONCLUSIONS: The designed diagnostic gene chip can be used to simultaneously detect serum HBV DNA and HCV RNA, but the positive rate of HCV RNA diagnosed by this chip is lower. The gene chip can detect HBV DNA in serum and in liver tissue.

Zhaohui, S., Z. Wenling, et al. (2004). "Microarrays for the detection of HBV and HDV." J Biochem Mol Biol **37**(5): 546-51.

The increasing pace of development in molecular biology during the last decade has had a direct effect on mass testing and diagnostic applications, including blood screening. We report the model Microarray that has been developed for Hepatitis B virus (HBV) and Hepatitis D virus (HDV) detection. The specific primer pairs of PCR were designed using the Primer Premier 5.00 program according to the conserved regions of HBV and HDV. PCR fragments were purified and cloned into pMD18-T vectors. The recombinant plasmids were extracted from positive clones and the target gene fragments were sequenced. The DNA microarray was prepared by robotically spotting PCR products onto the surface of glass slides. Sequences were aligned, and the results obtained showed that the products of PCR amplification were the required specific gene fragments of HBV, and HDV. Samples were labeled by Restriction Display PCR (RD-PCR). Gene chip hybridizing signals showed that the specificity and sensitivity required for HBV and HDV detection were satisfied. Using PCR amplified products to construct gene chips for the simultaneous clinical diagnosis of HBV and HDV resulted in a quick, simple, and effective method. We conclude that the DNA microarray assay system might be useful as a diagnostic technique in the clinical laboratory. Further applications of RD-PCR for the sample labeling could speed up microarray multi-virus detection.

Zhou, D., Y. Han, et al. (2004). "Identification of signature genes for rapid and specific characterization of Yersinia pestis." Microbiol Immunol **48**(4): 263-9.

Polymerase chain reaction (PCR) amplification of DNA-based unique markers, the signature sequences, is ideal for rapid detection and identification of pathogens. We described the discovery of twenty-eight signature genes of Yersinia pestis by DNA microarray-based comparative genome hybridization in conjunction with PCR validation. Three pairs of Y. pestis-specific primers designed from signature genes were demonstrated to have the expected specificity to this target bacterium, without

cross-reaction with the closely related Y. pseudotuberculosis or a large collection of genomic DNAs from other organisms.

Zhou, D., Y. Han, et al. (2004). "Defining the genome content of live plague vaccines by use of whole-genome DNA microarray." Vaccine **22**(25-26): 3367-74.

Yersinia pestis whole-genome DNA microarrays were developed to perform genomic comparison of a collection of live plague vaccines. By using the genomic DNA to probe the DNA microarrays, we detected dozens of deletions and amplifications of the genomic regions in the 19 vaccine strains analyzed. The revealed genomic differences within the vaccine strains of different origins provide us an unprecedented opportunity to understand the molecular background of the variability of the immunogenic and protective potency of plague live vaccine. The whole-genome DNA microarray also provides an ideal tool to perform the pre-evaluation of a vaccine strain for its high throughput to determine the genomic features essential or unallowable for the live vaccines.

Zhu, J. and J. J. Mekalanos (2003). "Quorum sensing-dependent biofilms enhance colonization in Vibrio cholerae." Dev Cell **5**(4): 647-56.

Vibrio cholerae is the causative agent of the diarrheal disease cholera. By an incompletely understood developmental process, V. cholerae forms complex surface-associated communities called biofilms. Here we show that quorum sensing-deficient mutants of V. cholerae produce thicker biofilms than those formed by wild-type bacteria. Microarray analysis of biofilm-associated bacteria shows that expression of the Vibrio polysaccharide synthesis (vps) operons is enhanced in hapR mutants. CqsA, one of two known autoinducer synthases in V. cholerae, acts through HapR to repress vps gene expression. Vibrio biofilms are more acid resistant than planktonic cells. However, quorum sensing-deficient biofilms have lower colonization capacities than those of wild-type biofilms, suggesting that quorum sensing may promote cellular exit from the biofilm once the organisms have traversed the gastric acid barrier of the stomach. These results shed light on the relationships among biofilm development, quorum sensing, infectivity, and pathogenesis in V. cholerae.

Environmental Applications

A-H

Andrews-Polymenis, H. L., W. Rabsch, et al. (2004). "Host restriction of Salmonella enterica serotype Typhimurium pigeon isolates does not correlate with loss of discrete genes." J Bacteriol **186**(9): 2619-28.

The definitive phage types (DT) 2 and 99 of Salmonella enterica serotype Typhimurium are epidemiologically correlated with a host range restricted to pigeons, in contrast to phage types with broader host ranges such as epidemic cattle isolates (DT104 and DT204). To determine whether phage types with broad host range possess genetic islands absent from host-restricted phage types, we compared the genomes of four pigeon isolates to serotype Typhimurium strain LT2 using a DNA microarray. Three of the four isolates tested caused fluid accumulation in bovine ligated ileal loops, but they had reduced colonization of liver and spleen in susceptible BALB/c mice and were defective for intestinal persistence in Salmonella-resistant CBA mice. The genomes of the DT99 and DT2 isolates were extremely similar to the LT2 genome, with few notable differences on the level of complete individual genes. Two large groups of genes representing the Fels-1 and Fels-2 prophages were missing from the DT2 and DT99 phage types we analyzed. One of the DT99 isolates examined was lacking a third cluster of five chromosomal genes (STM1555 to -1559). Results of the microarray analysis were extended using Southern analysis to a collection of 75 serotype Typhimurium clinical isolates of 24 different phage types. This analysis revealed no correlation between the presence of Fels-1, Fels-2, or STM1555 to -1559 and the association of phage types with different host reservoirs. We conclude that serotype Typhimurium phage types with broad host range do not possess genetic islands influencing host restriction, which are absent from the host-restricted pigeon isolates.

Bahl, A., B. Brunk, et al. (2002). "PlasmoDB: the Plasmodium genome resource. An integrated database providing tools for accessing, analyzing and mapping expression and sequence data (both finished and unfinished)." Nucleic Acids Res **30**(1): 87-90.

PlasmoDB (http://PlasmoDB.org) is the official database of the Plasmodium falciparum genome sequencing consortium. This resource incorporates finished and draft genome sequence data and annotation emerging from Plasmodium sequencing projects. PlasmoDB currently houses information from five parasite species and provides tools for cross-species comparisons. Sequence information is also integrated with other genomic-scale data emerging from the Plasmodium research community, including gene expression analysis from EST, SAGE and microarray projects. The relational schemas used to build PlasmoDB [Genomics Unified Schema (GUS) and RNA Abundance Database (RAD)] employ a highly structured format to accommodate the diverse data types generated by sequence and expression projects. A variety of tools allow researchers to formulate complex, biologically based queries of the database. A version of the database is also available on CD-ROM (Plasmodium GenePlot), facilitating access to the data in situations where Internet access is difficult (e.g. by malaria researchers working in the field). The goal of PlasmoDB is to enhance utilization of the vast quantities of data emerging from genome-scale projects by the global malaria research community.

Bahl, A., B. Brunk, et al. (2003). "PlasmoDB: the Plasmodium genome resource. A database integrating experimental and computational data." Nucleic Acids Res **31**(1): 212-5.

PlasmoDB (http://PlasmoDB.org) is the official database of the Plasmodium falciparum genome sequencing consortium. This resource incorporates the recently completed P. falciparum genome sequence and annotation, as well as draft sequence and annotation emerging from other Plasmodium sequencing projects. PlasmoDB currently houses information from five parasite species and provides tools for intra- and inter-species comparisons. Sequence information is integrated with other genomic-scale data emerging from the Plasmodium research community, including gene expression analysis from EST, SAGE and microarray projects and proteomics studies. The relational schema used to build

PlasmoDB, GUS (Genomics Unified Schema) employs a highly structured format to accommodate the diverse data types generated by sequence and expression projects. A variety of tools allow researchers to formulate complex, biologically-based, queries of the database. A stand-alone version of the database is also available on CD-ROM (P. falciparum GenePlot), facilitating access to the data in situations where internet access is difficult (e.g. by malaria researchers working in the field). The goal of PlasmoDB is to facilitate utilization of the vast quantities of genomic-scale data produced by the global malaria research community. The software used to develop PlasmoDB has been used to create a second Apicomplexan parasite genome database, ToxoDB (http://ToxoDB.org).

Berka, R. M., J. Hahn, et al. (2002). "Microarray analysis of the Bacillus subtilis K-state: genome-wide expression changes dependent on ComK." Mol Microbiol **43**(5): 1331-45.

In Bacillus subtilis, the competence transcription factor ComK activates its own transcription as well as the transcription of genes that encode DNA transport proteins. ComK is expressed in about 10% of the cells in a culture grown to competence. Using DNA microarrays representing approximately 95% of the protein-coding open reading frames in B. subtilis, we compared the expression profiles of wild-type and comK strains, as well as of a mecA mutant (which produces active ComK in all the cells of the population) and a comK mecA double mutant. In these comparisons, we identified at least 165 genes that are upregulated by ComK and relatively few that are downregulated. The use of reporter fusions has confirmed these results for several genes. Many of the ComK-regulated genes are organized in clusters or operons, and 23 of these clusters are preceded by apparent ComK-box promoter motifs. In addition to those required for DNA uptake, other genes that are upregulated in the presence of ComK are probably involved in DNA repair and in the uptake and utilization of nutritional sources. From this and previous work, we conclude that the ComK regulon defines a growth-arrested state, distinct from sporulation, of which competence for genetic transformation is but one notable feature. We suggest that this is a unique adaptation to stress and that it be termed the 'K-state'.

Bernstein, J. A., A. B. Khodursky, et al. (2002). "Global analysis of mRNA decay and abundance in Escherichia coli at single-gene resolution using two-color fluorescent DNA microarrays." Proc Natl Acad Sci U S A **99**(15): 9697-702.

Much of the information available about factors that affect mRNA decay in Escherichia coli, and by inference in other bacteria, has been gleaned from study of less than 25 of the approximately 4,300 predicted E. coli messages. To investigate these factors more broadly, we examined the half-lives and steady-state abundance of known and predicted E. coli mRNAs at single-gene resolution by using two-color fluorescent DNA microarrays. An rRNA-based strategy for normalization of microarray data was developed to permit quantitation of mRNA decay after transcriptional arrest by rifampicin. We found that globally, mRNA half-lives were similar in nutrient-rich media and defined media in which the generation time was approximately tripled. A wide range of stabilities was observed for individual mRNAs of E. coli, although approximately 80% of all mRNAs had half-lives between 3 and 8 min. Genes having biologically related metabolic functions were commonly observed to have similar stabilities. Whereas the half-lives of a limited number of mRNAs correlated positively with their abundance, we found that overall, increased mRNA stability is not predictive of increased abundance. Neither the density of putative sites of cleavage by RNase E, which is believed to initiate mRNA decay in E. coli, nor the free energy of folding of 5' or 3' untranslated region sequences was predictive of mRNA half-life. Our results identify previously unsuspected features of mRNA decay at a global level and also indicate that generalizations about decay derived from the study of individual gene transcripts may have limited applicability.

Berthier, D., R. Quere, et al. (2003). "Serial analysis of gene expression (SAGE) in bovine trypanotolerance: preliminary results." Genet Sel Evol **35 Suppl 1**: S35-47.

In Africa, trypanosomosis is a tsetse-transmitted disease which represents the most important

constraint to livestock production. Several indigenous West African taurine Bos taurus) breeds, such as the Longhorn (N'Dama) cattle are well known to control trypanosome infections. This genetic ability named "trypanotolerance" results from various biological mechanisms under multigenic control. The methodologies used so far have not succeeded in identifying the complete pool of genes involved in trypanotolerance. New post genomic biotechnologies such as transcriptome analyses are efficient in characterising the pool of genes involved in the expression of specific biological functions. We used the serial analysis of gene expression (SAGE) technique to construct, from Peripheral Blood Mononuclear Cells of an N'Dama cow, 2 total mRNA transcript libraries, at day 0 of a Trypanosoma congolense experimental infection and at day 10 post-infection, corresponding to the peak of parasitaemia. Bioinformatic comparisons in the bovine genomic databases allowed the identification of 187 up- and down- regulated genes, EST and unknown functional genes. Identification of the genes involved in trypanotolerance will allow to set up specific microarray sets for further metabolic and pharmacological studies and to design field marker-assisted selection by introgression programmes.

Bodrossy, L. and A. Sessitsch (2004). "Oligonucleotide microarrays in microbial diagnostics." Curr Opin Microbiol **7**(3): 245-54.

Oligonucleotide microarrays offer a fast, high-throughput alternative for the parallel detection of microbes from virtually any sample. The application potential spreads across most sectors of life sciences, including environmental microbiology and microbial ecology; human, veterinary, food and plant diagnostics; water quality control; industrial microbiology, and so on. The past two years have witnessed a rapid increase of research in this field. Many alternative techniques were developed and validated as seen in 'proof-of-concept' articles. Publications reporting on the application of oligonucleotide microarray technology for microbial diagnostics in microbiology driven projects have just started to appear. Current and future technical and bioinformatics developments will inevitably improve the potential of this technology further.

Bodrossy, L., N. Stralis-Pavese, et al. (2003). "Development and validation of a diagnostic microbial microarray for methanotrophs." Environ Microbiol **5**(7): 566-82.

The potential of DNA microarray technology in high-throughput detection of bacteria and quantitative assessment of their community structures is widely acknowledged but has not been fully realised yet. A generally applicable set of techniques, based on readily available technologies and materials, was developed for the design, production and application of diagnostic microbial microarrays. A microarray targeting the particulate methane monooxygenase (pmoA) gene was developed for the detection and quantification of methanotrophs and functionally related bacteria. A microarray consisting of a set of 59 probes that covers the whole known diversity of these bacteria was validated with a representative set of extant strains and environmental clones. The potential of the pmoA microarray was tested with environmental samples. The results were in good agreement with those of clone library sequence analyses. The approach can currently detect less dominant bacteria down to 5% of the total community targeted. Initial tests assessing the quantification potential of this system with artificial PCR mixtures showed very good correlation with the expected results with standard deviations in the range of 0.4-17.2%. Quantification of environmental samples with this method requires the design of a reference mixture consisting of very close relatives of the strains within the sample and is currently limited by biases inherent in environmental DNA extraction and universal PCR amplification.

Britton, R. A., P. Eichenberger, et al. (2002). "Genome-wide analysis of the stationary-phase sigma factor (sigma-H) regulon of Bacillus subtilis." J Bacteriol **184**(17): 4881-90.

Sigma-H is an alternative RNA polymerase sigma factor that directs the transcription of many genes that function at the transition from exponential growth to stationary phase in Bacillus subtilis. Twenty-three promoters, which drive transcription of 33 genes, are known to be recognized by sigma-H-containing RNA polymerase. To identify additional genes under the control of sigma-H on a genome-

wide basis, we carried out transcriptional profiling experiments using a DNA microarray containing >99% of the annotated B. subtilis open reading frames. In addition, we used a bioinformatics-based approach aimed at the identification of promoters recognized by RNA polymerase containing sigma-H. This combination of approaches was successful in confirming most of the previously described sigma-H-controlled genes. In addition, we identified 26 putative promoters that drive expression of 54 genes not previously known to be under the direct control of sigma-H. Based on the known or inferred function of most of these genes, we conclude that, in addition to its previously known roles in sporulation and competence, sigma-H controls genes involved in many physiological processes associated with the transition to stationary phase, including cytochrome biogenesis, generation of potential nutrient sources, transport, and cell wall metabolism.

Broekhuijsen, M., P. Larsson, et al. (2003). "Genome-wide DNA microarray analysis of Francisella tularensis strains demonstrates extensive genetic conservation within the species but identifies regions that are unique to the highly virulent F. tularensis subsp. tularensis." J Clin Microbiol **41**(7): 2924-31.

Francisella tularensis is a potent pathogen and a possible bioterrorism agent. Little is known, however, to explain the molecular basis for its virulence and the distinct differences in virulence found between the four recognized subspecies, F. tularensis subsp. tularensis, F. tularensis subsp. mediasiatica, F. tularensis subsp. holarctica, and F. tularensis subsp. novicida. We developed a DNA microarray based on 1,832 clones from a shotgun library used for sequencing of the highly virulent strain F. tularensis subsp. tularensis Schu S4. This allowed a genome-wide analysis of 27 strains representing all four subspecies. Overall, the microarray analysis confirmed a limited genetic variation within the species F. tularensis, and when the strains were compared, at most 3.7% of the probes showed differential hybridization. Cluster analysis of the hybridization data revealed that the causative agents of type A and type B tularemia, i.e., F. tularensis subsp. tularensis and F. tularensis subsp. holarctica, respectively, formed distinct clusters. Despite marked differences in their virulence and geographical origin, a high degree of genomic similarity between strains of F. tularensis subsp. tularensis and F. tularensis subsp. mediasiatica was apparent. Strains from Japan clustered separately, as did strains of F. tularensis subsp. novicida. Eight regions of difference (RD) 0.6 to 11.5 kb in size, altogether comprising 21 open reading frames, were identified that distinguished strains of the moderately virulent subspecies F. tularensis subsp. holarctica and the highly virulent subspecies F. tularensis subsp. tularensis. One of these regions, RD1, allowed for the first time the development of an F. tularensis-specific PCR assay that discriminates each of the four subspecies.

Brokx, S. J., M. Ellison, et al. (2004). "Genome-wide analysis of lipoprotein expression in Escherichia coli MG1655." J Bacteriol **186**(10): 3254-8.

To gain insight into the cell envelope of Escherichia coli grown under aerobic and anaerobic conditions, lipoproteins were examined by using functional genomics. The mRNA expression levels of each of these genes under three growth conditions--aerobic, anaerobic, and anaerobic with nitrate--were examined by using both Affymetrix GeneChip E. coli antisense genome arrays and real-time PCR (RT-PCR). Many genes showed significant changes in expression level. The RT-PCR results were in very good agreement with the microarray data. The results of this study represent the first insights into the possible roles of unknown lipoprotein genes and broaden our understanding of the composition of the cell envelope under different environmental conditions. Additionally, these data serve as a test set for the refinement of high-throughput bioinformatic and global gene expression methods.

Brooks, C. S., P. S. Hefty, et al. (2003). "Global analysis of Borrelia burgdorferi genes regulated by mammalian host-specific signals." Infect Immun **71**(6): 3371-83.

Lyme disease is a tick-borne infection that can lead to chronic, debilitating problems if not recognized or treated appropriately. Borrelia burgdorferi, the causative agent of Lyme disease, is maintained in nature by a complex enzootic cycle involving Ixodes ticks and mammalian hosts. Many

previous studies support the notion that B. burgdorferi differentially expresses numerous genes and proteins to help it adapt to growth in the mammalian host. In this regard, several studies have utilized a dialysis membrane chamber (DMC) cultivation system to generate "mammalian host-adapted" spirochetes for the identification of genes selectively expressed during mammalian infection. Here, we have exploited the DMC cultivation system in conjunction with microarray technology to examine the global changes in gene expression that occur in the mammalian host. To identify genes regulated by only mammal-specific signals and not by temperature, borrelial microarrays were hybridized with cDNA generated either from organisms temperature shifted in vitro from 23 degrees C to 37 degrees C or from organisms cultivated by using the DMC model system. Statistical analyses of the combined data sets revealed that 125 genes were expressed at significantly different levels in the mammalian host, with almost equivalent numbers of genes being up- or down-regulated by B. burgdorferi within DMCs compared to those undergoing temperature shift. Interestingly, during DMC cultivation, the vast majority of genes identified on the plasmids were down-regulated (79%), while the differentially expressed chromosomal genes were almost entirely up-regulated (93%). Global analysis of the upstream promoter regions of differentially expressed genes revealed that several share a common motif that may be important in transcriptional regulation during mammalian infection. Among genes with known or putative functions, the cell envelope category, which includes outer membrane proteins, was found to contain the most differentially expressed genes. The combined findings have generated a subset of genes that can now be further characterized to help define their role or roles with regard to B. burgdorferi virulence and Lyme disease pathogenesis.

Dennis, P., E. A. Edwards, et al. (2003). "Monitoring gene expression in mixed microbial communities by using DNA microarrays." Appl Environ Microbiol **69**(2): 769-78.

A DNA microarray to monitor the expression of bacterial metabolic genes within mixed microbial communities was designed and tested. Total RNA was extracted from pure and mixed cultures containing the 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacterium Ralstonia eutropha JMP134, and the inducing agent 2,4-D. Induction of the 2,4-D catabolic genes present in this organism was readily detected 4, 7, and 24 h after the addition of 2,4-D. This strain was diluted into a constructed mixed microbial community derived from a laboratory scale sequencing batch reactor. Induction of two of five 2,4-D catabolic genes (tfdA and tfdC) from populations of JMP134 as low as 10(5) cells/ml was clearly detected against a background of 10(8) cells/ml. Induction of two others (tfdB and tfdE) was detected from populations of 10(6) cells/ml in the same background; however, the last gene, tfdF, showed no significant induction due to high variability. In another experiment, the induction of resin acid degradative genes was statistically detectable in sludge-fed pulp mill effluent exposed to dehydroabietic acid in batch experiments. We conclude that microarrays will be useful tools for the detection of bacterial gene expression in wastewaters and other complex systems.

Dorrell, N., J. A. Mangan, et al. (2001). "Whole genome comparison of Campylobacter jejuni human isolates using a low-cost microarray reveals extensive genetic diversity." Genome Res **11**(10): 1706-15.

Campylobacter jejuni is the leading cause of bacterial food-borne diarrhoeal disease throughout the world, and yet is still a poorly understood pathogen. Whole genome microarray comparisons of 11 C. jejuni strains of diverse origin identified genes in up to 30 NCTC 11168 loci ranging from 0.7 to 18.7 kb that are either absent or highly divergent in these isolates. Many of these regions are associated with the biosynthesis of surface structures including flagella, lipo-oligosaccharide, and the newly identified capsule. Other strain-variable genes of known function include those responsible for iron acquisition, DNA restriction/modification, and sialylation. In fact, at least 21% of genes in the sequenced strain appear dispensable as they are absent or highly divergent in one or more of the isolates tested, thus defining 1300 C. jejuni core genes. Such core genes contribute mainly to metabolic, biosynthetic, cellular, and regulatory processes, but many virulence determinants are also conserved. Comparison of the capsule biosynthesis locus revealed conservation of all the genes in this region in strains with the same Penner serotype as strain NCTC 11168. By contrast, between 5 and 17 NCTC 11168 genes in this region are

either absent or highly divergent in strains of a different serotype from the sequenced strain, providing further evidence that the capsule accounts for Penner serotype specificity. These studies reveal extensive genetic diversity among C. jejuni strains and pave the way toward identifying correlates of pathogenicity and developing improved epidemiological tools for this problematic pathogen.

Goodman, A. L. and S. Lory (2004). "Analysis of regulatory networks in Pseudomonas aeruginosa by genomewide transcriptional profiling." Curr Opin Microbiol **7**(1): 39-44.

Transcriptional profiling using DNA microarrays has proved to be a valuable tool for dissecting bacterial adaptation to various environments, including human hosts. Analysis of genomes and transcriptomes of Pseudomonas aeruginosa shows that this bacterium possesses and expresses a core set of genes, including virulence factors, which allow it to thrive in a range of environments. Transcriptional regulators previously thought to control single virulence traits are now shown to regulate complex global signaling networks. Microarray-based research has led to the discovery of upstream regulators and downstream components of these pathways, as well as probed the response to antibiotics, environmental stresses and other bacteria. Independent studies have highlighted the role of media composition, the makeup of the physical environment and experimental methods in the outcome of microarray analyses. A compilation of all the published data clearly shows transcriptional regulation of genes in all functional classes. Under conditions examined to date, slightly more than a quarter of the genome is regulated, suggesting that P. aeruginosa may use much of its genome for conditions unexplored in the laboratory.

Hihara, Y., A. Kamei, et al. (2001). "DNA microarray analysis of cyanobacterial gene expression during acclimation to high light." Plant Cell **13**(4): 793-806.

DNA microarrays bearing nearly all of the genes of the unicellular cyanobacterium Synechocystis sp PCC 6803 were used to examine the temporal program of gene expression during acclimation from low to high light intensity. A complete pattern is provided of gene expression during acclimation of a photosynthetic organism to changing light intensity. More than 160 responsive genes were identified and classified into distinct sets. Genes involved in light absorption and photochemical reactions were downregulated within 15 min of exposure to high light intensity, whereas those associated with CO(2) fixation and protection from photoinhibition were upregulated. Changes in the expression of genes involved in replication, transcription, and translation, which were induced to support cellular proliferation, occurred later. Several unidentified open reading frames were induced or repressed. The possible involvement of these genes in the acclimation to high light conditions is discussed.

Hihara, Y., M. Muramatsu, et al. (2004). "A cyanobacterial gene encoding an ortholog of Pirin is induced under stress conditions." FEBS Lett **574**(1-3): 101-5.

Pirin is a recently identified protein in eukaryotes as a transcription cofactor or as an apoptosis-related protein. Although Pirin is highly conserved from bacteria to human, there have been no reports on prokaryotic Pirin orthologs. We show here that pirA (sll1773) encoding an ortholog of Pirin together with an adjacent gene, pirB (ssl3389), was upregulated under high salinity and some other stress conditions in a cyanobacterium Synechocystis sp. PCC 6803. Induction of the pirAB genes was not related to cell death and disruption of pirA did not affect the gene expression profile. Expression of the pirAB genes was negatively regulated by a LysR family transcriptional regulator encoded by pirR (slr1871) located immediately upstream of pirAB in the divergent direction. DNA microarray analysis indicated that PirR repressed expression of closely located ORFs, slr1870 and mutS (sll1772), in addition to pirAB and pirR itself.

Hihara, Y., K. Sonoike, et al. (2003). "DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium Synechocystis sp. strain PCC 6803." J Bacteriol **185**(5): 1719-25.

Whole-genome DNA microarrays were used to evaluate the effect of the redox state of the photosynthetic electron transport chain on gene expression in Synechocystis sp. strain PCC 6803. Two

specific inhibitors of electron transport, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), were added to the cultures, and changes in accumulation of transcripts were examined. About 140 genes were highlighted as reproducibly affected by the change in the redox state of the photosynthetic electron transport chain. It was shown that some stress-responsive genes but not photosynthetic genes were under the control of the redox state of the plastoquinone pool in Synechocystis sp. strain PCC 6803.

Hirsh, A. E., A. G. Tsolaki, et al. (2004). "Stable association between strains of Mycobacterium tuberculosis and their human host populations." Proc Natl Acad Sci U S A **101**(14): 4871-6.

Mycobacterium tuberculosis is an important human pathogen in virtually every part of the world. Here we investigate whether distinct strains of M. tuberculosis infect different human populations and whether associations between host and pathogen populations are stable despite global traffic and the convergence of diverse strains of the pathogen in cosmopolitan urban centers. The recent global movement and transmission history of 100 M. tuberculosis isolates was inferred from a molecular epidemiologic study of tuberculosis that spans 12 years. Genetic relationships among these isolates were deduced from the distribution of large genomic deletions, which were identified by DNA microarray and confirmed by PCR and sequence analysis. Phylogenetic analysis of these deletions indicates that they are unique event polymorphisms and that horizontal gene transfer is extremely rare in M. tuberculosis. In conjunction with the epidemiological data, phylogenies reveal three large phylogeographic regions. A host's region of origin is predictive of the strain of tuberculosis he or she carries, and this association remains strong even when transmission takes place in a cosmopolitan urban center outside of the region of origin. Approximate dating of the time since divergence of East Asian and Philippine clades of M. tuberculosis suggests that these lineages diverged centuries ago. Thus, associations between host and pathogen populations appear to be highly stable.

Hurt, R. A., X. Qiu, et al. (2001). "Simultaneous recovery of RNA and DNA from soils and sediments." Appl Environ Microbiol **67**(10): 4495-503.

Recovery of mRNA from environmental samples for measurement of in situ metabolic activities is a significant challenge. A robust, simple, rapid, and effective method was developed for simultaneous recovery of both RNA and DNA from soils of diverse composition by adapting our previous grinding-based cell lysis method (Zhou et al., Appl. Environ. Microbiol. 62:316-322, 1996) for DNA extraction. One of the key differences is that the samples are ground in a denaturing solution at a temperature below 0 degrees C to inactivate nuclease activity. Two different methods were evaluated for separating RNA from DNA. Among the methods examined for RNA purification, anion exchange resin gave the best results in terms of RNA integrity, yield, and purity. With the optimized protocol, intact RNA and high-molecular-weight DNA were simultaneously recovered from 19 soil and stream sediment samples of diverse composition. The RNA yield from these samples ranged from 1.4 to 56 microg g of soil(-1) dry weight), whereas the DNA yield ranged from 23 to 435 microg g(-1). In addition, studies with the same soil sample showed that the DNA yield was, on average, 40% higher than that in our previous procedure and 68% higher than that in a commercial bead milling method. For the majority of the samples, the DNA and RNA recovered were of sufficient purity for nuclease digestion, microarray hybridization, and PCR or reverse transcription-PCR amplification.

Environmental Applications

I-P

Kakinuma, K., M. Fukushima, et al. (2003). "Detection and identification of Escherichia coli, Shigella, and Salmonella by microarrays using the gyrB gene." Biotechnol Bioeng **83**(6): 721-8.

Commonly, 16S ribosome RNA (16S rRNA) sequence analysis has been used for identifying

enteric bacteria. However, it may not always be applicable for distinguishing closely related bacteria. Therefore, we selected gyrB genes that encode the subunit B protein of DNA gyrase (a topoisomerase type II protein) as target genes. The molecular evolution rate of gyrB genes is higher than that of 16S rRNA, and gyrB genes are distributed universally among bacterial species. Microarray technology includes the methods of arraying cDNA or oligonucleotides on substrates such as glass slides while acquiring a lot of information simultaneously. Thus, it is possible to identify the enteric bacteria easily using microarray technology. We devised a simple method of rapidly identifying bacterial species through the combined use of gyrB genes and microarrays. Closely related bacteria were not identified at the species level using 16S rRNA sequence analysis, whereas they were identified at the species level based on the reaction patterns of oligonucleotides on our microarrays using gyrB genes.

Leach, J. E., M. Ryba-White, et al. (2001). "Plants, plant pathogens, and microgravity--a deadly trio." Gravit Space Biol Bull **14**(2): 15-23.

Plants grown in spaceflight conditions are more susceptible to colonization by plant pathogens. The underlying causes for this enhanced susceptibility are not known. Possibly the formation of structural barriers and the activation of plant defense response components are impaired in spaceflight conditions. Either condition would result from altered gene expression of the plant. Because of the tools available, past studies focused on a few physiological responses or biochemical pathways. With recent advances in genomics research, new tools, including microarray technologies, are available to examine the global impact of growth in the spacecraft on the plant's gene expression profile. In ground-based studies, we have developed cDNA subtraction libraries of rice that are enriched for genes induced during pathogen infection and the defense response. Arrays of these genes are being used to dissect plant defense response pathways in a model system involving wild-type rice plants and lesion mimic mutants. The lesion mimic mutants are ideal experimental tools because they erratically develop defense response-like lesions in the absence of pathogens. The gene expression profiles from these ground-based studies will provide the molecular basis for understanding the biochemical and physiological impacts of spaceflight on plant growth, development and disease defense responses. This, in turn, will allow the development of strategies to manage plant disease for life in the space environment.

Liu, R. H., J. Yang, et al. (2004). "Self-contained, fully integrated biochip for sample preparation, polymerase chain reaction amplification, and DNA microarray detection." Anal Chem **76**(7): 1824-31.

A fully integrated biochip device that consists of microfluidic mixers, valves, pumps, channels, chambers, heaters, and DNA microarray sensors was developed to perform DNA analysis of complex biological sample solutions. Sample preparation (including magnetic bead-based cell capture, cell preconcentration and purification, and cell lysis), polymerase chain reaction, DNA hybridization, and electrochemical detection were performed in this fully automated and miniature device. Cavitation microstreaming was implemented to enhance target cell capture from whole blood samples using immunomagnetic beads and accelerate DNA hybridization reaction. Thermally actuated paraffin-based microvalves were developed to regulate flows. Electrochemical pumps and thermopneumatic pumps were integrated on the chip to provide pumping of liquid solutions. The device is completely self-contained: no external pressure sources, fluid storage, mechanical pumps, or valves are necessary for fluid manipulation, thus eliminating possible sample contamination and simplifying device operation. Pathogenic bacteria detection from approximately milliliters of whole blood samples and single-nucleotide polymorphism analysis directly from diluted blood were demonstrated. The device provides a cost-effective solution to direct sample-to-answer genetic analysis and thus has a potential impact in the fields of point-of-care genetic analysis, environmental testing, and biological warfare agent detection.

Loy, A., A. Lehner, et al. (2002). "Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment." Appl Environ Microbiol **68**(10): 5064-81.

For cultivation-independent detection of sulfate-reducing prokaryotes (SRPs) an oligonucleotide microarray consisting of 132 16S rRNA gene-targeted oligonucleotide probes (18-mers) having hierarchical and parallel (identical) specificity for the detection of all known lineages of sulfate-reducing prokaryotes (SRP-PhyloChip) was designed and subsequently evaluated with 41 suitable pure cultures of SRPs. The applicability of SRP-PhyloChip for diversity screening of SRPs in environmental and clinical samples was tested by using samples from periodontal tooth pockets and from the chemocline of a hypersaline cyanobacterial mat from Solar Lake (Sinai, Egypt). Consistent with previous studies, SRP-PhyloChip indicated the occurrence of Desulfomicrobium spp. in the tooth pockets and the presence of Desulfonema- and Desulfomonile-like SRPs (together with other SRPs) in the chemocline of the mat. The SRP-PhyloChip results were confirmed by several DNA microarray-independent techniques, including specific PCR amplification, cloning, and sequencing of SRP 16S rRNA genes and the genes encoding the dissimilatory (bi)sulfite reductase (dsrAB).

Matsunaga, T., H. Nakayama, et al. (2001). "Fluorescent detection of cyanobacterial DNA using bacterial magnetic particles on a MAG-microarray." Biotechnol Bioeng **73**(5): 400-5.

Bacterial magnetic particles (BMPs) were used for the identification of cyanobacterial DNA. Genus-specific oligonucleotide probes for the detection of Anabaena spp., Microcystis spp., Nostoc spp., Oscillatoria spp., and Synechococcus spp. were designed from the variable region of the cyanobacterial 16S rDNA of 148 strains. These oligonucleotide probes were immobilized on BMPs via streptavidin-biotin conjugation and employed for magnetic-capture hybridization against digoxigenin-labeled cyanobacterial 16S rDNA. Bacterial magnetic particles were magnetically concentrated, spotted in 100-microm-size microwell on MAG-microarray, and the fluorescent detection was performed. This work details the development of an automated technique for the magnetic isolation, the concentration of hybridized DNA, and the detection of specific target DNA on MAG-microarray. The entire process of hybridization and detection was automatically performed using a magnetic-separation robot and all five cyanobacterial genera were successfully discriminated.

Meibom, K. L., X. B. Li, et al. (2004). "The Vibrio cholerae chitin utilization program." Proc Natl Acad Sci U S A **101**(8): 2524-9.

Chitin, an insoluble polymer of GlcNAc, is an abundant source of carbon, nitrogen, and energy for marine microorganisms. Microarray expression profiling and mutational studies of Vibrio cholerae growing on a natural chitin surface, or with the soluble chitin oligosaccharides (GlcNAc)(2-6), GlcNAc, or the glucosamine dimer (GlcN)2 identified three sets of differentially regulated genes. We show that (i) ChiS, a sensor histidine kinase, regulates expression of the (GlcNAc)(2-6) gene set, including a (GlcNAc)2 catabolic operon, two extracellular chitinases, a chitoporin, and a PilA-containing type IV pilus, designated ChiRP (chitin-regulated pilus) that confers a significant growth advantage to V. cholerae on a chitin surface; (ii) GlcNAc causes the coordinate expression of genes involved with chitin chemotaxis and adherence and with the transport and assimilation of GlcNAc; (iii) (GlcN)2 induces genes required for the transport and catabolism of nonacetylated chitin residues; and (iv) the constitutively expressed MSHA pilus facilitates adhesion to the chitin surface independent of surface chemistry. Collectively, these results provide a global portrait of a complex, multistage V. cholerae program for the efficient utilization of chitin.

Musarrat, J. and S. A. Hashsham (2003). "Customized cDNA microarray for expression profiling of environmentally important genes of Pseudomonas stutzeri strain KC." Teratog Carcinog Mutagen **Suppl 1**: 283-94.

DNA microarray is a powerful tool for parallel detection of multiple target genes in biological systems. In this study, a low-density DNA microarray has been custom designed by using Pseudomonas stutzeri strain KC ORFs that are implicated in carbon tetrachloride degradation. PCR amplified strain KC probes of varying lengths were obtained using ORF-specific primers. Purified short probes (80-120 bp)

and full-length amplicons were directly immobilized on gamma-aminosilane coated and superaldehyde trade mark glass substrates without any chemical modification. The full-length amplicons exhibited a much higher signal compared to the shorter probes upon hybridization with the Cy5/Cy3-labeled unfragmented cDNA targets. The meager signal with the shorter probes limits the advantage of using the multiple probes of the same genes for enhancing the specificity of hybridization with environmental samples. Nevertheless, expression analysis of strain KC genome, under controlled laboratory conditions, revealed the constitutive expression of at least 11 putative ORFs of the pdt operon. Comparatively weaker hybridization signals with the cDNA from mutant cells suggested a low abundance of mRNA transcripts in the KC 1896 mutant. Similar expression levels of the pdt ORFs I, J, K, M, N, O, P, and fur gene both under iron-limiting conditions and in presence of iron (20 micro M Fe(3+)) suggested metal ion-independent regulation of the pdt operon. The tailor-made array with strain KC gene-specific probes served as a model for demonstrating the utility of cDNA microarray technology in monitoring the expression of environmentally important genes in bacteria.

Parro, V. and M. Moreno-Paz (2003). "Gene function analysis in environmental isolates: the nif regulon of the strict iron oxidizing bacterium Leptospirillum ferrooxidans." Proc Natl Acad Sci U S A **100**(13): 7883-8.

A random genomic library from an environmental isolate of the Gram-negative bacterium Leptospirillum ferrooxidans has been printed on a microarray. Gene expression analysis was carried out with total RNA extracted from L. ferrooxidans cultures in the presence or absence of ammonium as nitrogen source under aerobic conditions. Although practically nothing is known about the genome sequence of this bacterium, this approach allowed us the selection and sequencing of only those clones bearing genes that showed an altered expression pattern. By sequence comparison, we have identified most of the genes of nitrogen fixation regulon in L. ferrooxidans, like the nifHDKENX operon, encoding the structural components of Mo-Fe nitrogenase; nifSU-hesB-hscBA-fdx operon, for Fe-S cluster assembly; the amtB gene (ammonium transporter); modA (molybdenum ABC type transporter); some regulatory genes like ntrC, nifA (the specific activator of nif genes); or two glnB-like genes (encoding the PII regulatory protein). Our results show that shotgun DNA microarrays are very powerful tools to accomplish gene expression studies with environmental bacteria whose genome sequence is still unknown, avoiding the time and effort necessary for whole genome sequencing projects.

Peplies, J., S. C. Lau, et al. (2004). "Application and validation of DNA microarrays for the 16S rRNA-based analysis of marine bacterioplankton." Environ Microbiol **6**(6): 638-45.

An oligonucleotide probe-based DNA microarray was evaluated for its ability to detect 16S rRNA targets in marine bacterioplankton samples without prior amplification by polymerase chain reaction (PCR). The results obtained were compared with those of quantitative fluorescence in situ hybridization (FISH). For extraction and direct labelling of total RNA, a fast and efficient protocol based on commercially available kits was established. A set of redundant and hierarchically structured probes was applied, and specificity of hybridization was assessed by additional control oligonucleotides comprising single central mismatches. The protocol was initially tested by microarray analysis of bacterial pure cultures. Complete discrimination of all control oligonucleotides was achieved, indicating a high degree of hybridization specificity. In a co-culture, abundant members were detected by microarray analysis, but signal ratios of positive probes did not correlate well with quantitative data from FISH experiments. A marine picoplankton sample from the German Bight was analysed. Bacterial populations with relative abundances of at least 5% were detected by hybridizing 0.1 microg of total RNA extracted from a sample of 375 ml equivalent to 4.1 x 10(8) cells. Our results demonstrate that major populations of marine bacterioplankton can be identified by microarray analysis in a fast and reliable way, even in relatively low volumes of sea water.

Pysz, M. A., S. B. Conners, et al. (2004). "Transcriptional Analysis of Biofilm Formation Processes in the

Anaerobic, Hyperthermophilic Bacterium Thermotoga maritima." Appl Environ Microbiol **70**(10): 6098-112.

Thermotoga maritima, a fermentative, anaerobic, hyperthermophilic bacterium, was found to attach to bioreactor glass walls, nylon mesh, and polycarbonate filters during chemostat cultivation on maltose-based media at 80 degrees C. A whole-genome cDNA microarray was used to examine differential expression patterns between biofilm and planktonic populations. Mixed-model statistical analysis revealed differential expression (twofold or more) of 114 open reading frames in sessile cells (6% of the genome), over a third of which were initially annotated as hypothetical proteins in the T. maritima genome. Among the previously annotated genes in the T. maritima genome, which showed expression changes during biofilm growth, were several that corresponded to biofilm formation genes identified in mesophilic bacteria (i.e., Pseudomonas species, Escherichia coli, and Staphylococcus epidermidis). Most notably, T. maritima biofilm-bound cells exhibited increased transcription of genes involved in iron and sulfur transport, as well as in biosynthesis of cysteine, thiamine, NAD, and isoprenoid side chains of quinones. These findings were all consistent with the up-regulation of iron-sulfur cluster assembly and repair functions in biofilm cells. Significant up-regulation of several beta-specific glycosidases was also noted in biofilm cells, despite the fact that maltose was the primary carbon source fed to the chemostat. The reasons for increased beta-glycosidase levels are unclear but are likely related to the processing of biofilm-based polysaccharides. In addition to revealing insights into the phenotype of sessile T. maritima communities, the methodology developed here can be extended to study other anaerobic biofilm formation processes as well as to examine aspects of microbial ecology in hydrothermal environments.

Environmental Applications

Q-Z

Ren, D., L. A. Bedzyk, et al. (2004). "Gene expression in Escherichia coli biofilms." Appl Microbiol Biotechnol **64**(4): 515-24.

DNA microarrays were used to study the gene expression profile of Escherichia coli JM109 and K12 biofilms. Both glass wool in shake flasks and mild steel 1010 plates in continuous reactors were used to create the biofilms. For the biofilms grown on glass wool, 22 genes were induced significantly (p< or =0.05) compared to suspension cells, including several genes for the stress response (hslS, hslT, hha, and soxS), type I fimbriae (fimG), metabolism (metK), and 11 genes of unknown function (ybaJ, ychM, yefM, ygfA, b1060, b1112, b2377, b3022, b1373, b1601, and b0836). The DNA microarray results were corroborated with RNA dot blotting. For the biofilm grown on mild steel plates, the DNA microarray data showed that, at a specific growth rate of 0.05/h, the mature biofilm after 5 days in the continuous reactors did not exhibit differential gene expression compared to suspension cells although genes were induced at 0.03/h. The present study suggests that biofilm gene expression is strongly associated with environmental conditions and that stress genes are involved in E. coli JM109 biofilm formation.

Rhee, S. K., X. Liu, et al. (2004). "Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays." Appl Environ Microbiol **70**(7): 4303-17.

To effectively monitor biodegrading populations, a comprehensive 50-mer-based oligonucleotide microarray was developed based on most of the 2,402 known genes and pathways involved in biodegradation and metal resistance. This array contained 1,662 unique and group-specific probes with <85% similarity to their nontarget sequences. Based on artificial probes, our results showed that under hybridization conditions of 50 degrees C and 50% formamide, the 50-mer microarray hybridization can differentiate sequences having <88% similarity. Specificity tests with representative pure cultures indicated that the designed probes on the arrays appeared to be specific to their corresponding target

genes. The detection limit was approximately 5 to 10 ng of genomic DNA in the absence of background DNA and 50 to 100 ng of pure-culture genomic DNA in the presence of background DNA or 1.3 x 10(7) cells in the presence of background RNA. Strong linear relationships between the signal intensity and the target DNA and RNA were observed (r(2) = 0.95 to 0.99). Application of this type of microarray to analyze naphthalene-amended enrichment and soil microcosms demonstrated that microflora changed differently depending on the incubation conditions. While the naphthalene-degrading genes from Rhodococcus-type microorganisms were dominant in naphthalene-degrading enrichments, the genes involved in naphthalene (and polyaromatic hydrocarbon and nitrotoluene) degradation from gram-negative microorganisms, such as Ralstonia, Comamonas, and Burkholderia, were most abundant in the soil microcosms. In contrast to general conceptions, naphthalene-degrading genes from Pseudomonas were not detected, although Pseudomonas is widely known as a model microorganism for studying naphthalene degradation. The real-time PCR analysis with four representative genes showed that the microarray-based quantification was very consistent with real-time PCR (r(2) = 0.74). In addition, application of the arrays to both polyaromatic-hydrocarbon- and benzene-toluene-ethylbenzene-xylene-contaminated and uncontaminated soils indicated that the developed microarrays appeared to be useful for profiling differences in microbial community structures. Our results indicate that this technology has potential as a specific, sensitive, and quantitative tool in revealing a comprehensive picture of the compositions of biodegradation genes and the microbial community in contaminated environments, although more work is needed to improve detection sensitivity.

Sato, N., M. Ohmori, et al. (2004). "Use of segment-based microarray in the analysis of global gene expression in response to various environmental stresses in the cyanobacterium Anabaena sp. PCC 7120." J Gen Appl Microbiol **50**(1): 1-8.

We prepared microarrays that contain genomic sequences of a heterocyst-forming filamentous cyanobacterium Anabaena sp. PCC 7120. The complete genome of this cyanobacterium codes for about 5,368 protein-coding genes in the main chromosome of 6.4 Mbp. In total, 2,407 DNA segments were selected from the sequencing clones, and amplified by PCR, then spotted on glass slides in duplicate. These microarrays differ from the widely used commercial or custom-made ones for other microorganisms in that each DNA segment was 3-4 kbp long, and contained about 3-4 predicted genes on average. This feature, however, did not decrease the usefulness of the microarrays, since we were able to detect a number of potentially novel genes that are induced in response to nitrogen deprivation, low temperature and drought. In addition, we found some genomic regions in which dozens of contiguous genes are simultaneously regulated. These results suggest that these segment-based microarrays are useful especially for such large genomes as Anabaena, for which the number of genes exceeds either technical or practical limitations.

Schembri, M. A., K. Kjaergaard, et al. (2003). "Global gene expression in Escherichia coli biofilms." Mol Microbiol **48**(1): 253-67.

It is now apparent that microorganisms undergo significant changes during the transition from planktonic to biofilm growth. These changes result in phenotypic adaptations that allow the formation of highly organized and structured sessile communities, which possess enhanced resistance to antimicrobial treatments and host immune defence responses. Escherichia coli has been used as a model organism to study the mechanisms of growth within adhered communities. In this study, we use DNA microarray technology to examine the global gene expression profile of E. coli during sessile growth compared with planktonic growth. Genes encoding proteins involved in adhesion (type 1 fimbriae) and, in particular, autoaggregation (Antigen 43) were highly expressed in the adhered population in a manner that is consistent with current models of sessile community development. Several novel gene clusters were induced upon the transition to biofilm growth, and these included genes expressed under oxygen-limiting conditions, genes encoding (putative) transport proteins, putative oxidoreductases and genes associated with enhanced heavy metal resistance. Of particular interest was the observation that many of the genes

altered in expression have no current defined function. These genes, as well as those induced by stresses relevant to biofilm growth such as oxygen and nutrient limitation, may be important factors that trigger enhanced resistance mechanisms of sessile communities to antibiotics and hydrodynamic shear forces.

Small, J., D. R. Call, et al. (2001). "Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays." Appl Environ Microbiol **67**(10): 4708-16.

We report on the development and validation of a simple microarray method for the direct detection of intact 16S rRNA from unpurified soil extracts. Total RNAs from Geobacter chapellei and Desulfovibrio desulfuricans were hybridized to an oligonucleotide array consisting of universal and species-specific 16S rRNA probes. PCR-amplified products from Geobacter and Desulfovibrio were easily and specifically detected under a range of hybridization times, temperatures, and buffers. However, reproducible, specific hybridization and detection of intact rRNA could be accomplished only by using a chaperone-detector probe strategy. With this knowledge, assay conditions were developed for rRNA detection using a 2-h hybridization time at room temperature. Hybridization specificity and signal intensity were enhanced using fragmented RNA. Formamide was required in the hybridization buffer in order to achieve species-specific detection of intact rRNA. With the chaperone detection strategy, we were able to specifically hybridize and detect G. chapellei 16S rRNA directly from a total-RNA soil extract, without further purification or removal of soluble soil constituents. The detection sensitivity for G. chapellei 16S rRNA in soil extracts was at least 0.5 microg of total RNA, representing approximately 7.5 x 10(6) Geobacter cell equivalents of RNA. These results suggest that it is now possible to apply microarray technology to the direct detection of microorganisms in environmental samples, without using PCR.

Stahl, D. A. (2004). "High-throughput techniques for analyzing complex bacterial communities." Adv Exp Med Biol **547**: 5-17.

A more complete understanding of microbial diversity and the environmental processes they control will require much more than a biotic inventory. It will require a deeper understanding of the basic features of systems organization and inter-population interactions. Communities, not total biomass, control net process rates driving the biogeochemical cycles sustaining the biosphere. Although the general patterns of macroorganismal diversity are relatively well known, spatial and temporal patterns of microorganismal diversity are essentially unknown. Having tools capable of resolving these patterns is a prerequisite to developing an understanding of the relationship between community structure and function. This talk discusses conceptual and technical developments that now provide the framework for systematically resolving temporal and spatial patterns of microorganisms and relating those patterns to processes at local and system levels. Of particular emphasis will be ongoing studies using highly parallel analyses with DNA microarrays for intensive monitoring of microbial populations in environmental systems. Although microarray technology is reasonably well established for studies of model organisms in well-defined laboratory settings, the application of this technology to environmental systems of uncharacterized diversity imposes additional demands on implementation; in particular, the requirement for optimized discrimination between target and non-target nucleic acids in complex, and undefined, mixtures. To increase the resolving power (information content) of our DNA microarray format, we are investigating the use of thermal dissociation of hybrids immobilized on individual array elements to resolve target and non-target sequences that differ by a single nucleotide. These studies, combined with specialized algorithms for optimizing the readout of the microarray should serve for informed environmental application. Initial studies have validated the general approach for analyses of sediment systems.

Stin, O. C., A. Carnahan, et al. (2003). "Characterization of microbial communities from coastal waters using microarrays." Environ Monit Assess **81**(1-3): 327-36.

Molecular methods, including DNA probes, were used to identify and enumerate pathogenic

Vibrio species in the Chesapeake Bay; our data indicated that Vibrio vulnificus exhibits seasonal fluctuations in number. Our work included a characterization of total microbial communities from the Bay; development of microarrays that identify and quantify the diversity of those communities; and observation of temporal changes in those communities. To identify members of the microbial community, we amplified the 16S rDNA gene from community DNA isolated from a biofilm sample collected from the Chesapeake Bay in February, 2000. The resultant 75 sequences were 95% or more similar to 7 species including two recently described Shewanella species, baltica and frigidimarina, that have not been previously isolated from the Chesapeake. When the genera of bacteria from biofilm after culturing are compared to those detected by subcloning amplified 16S fragments from community DNA, the cultured sample exhibited a strong bias. In oysters collected in February, the most common bacteria were previously unknown. Based on our 16S findings, we are developing microarrays to detect these and other microbial species in these estuarine communities. The microarrays will detect each species using four distinct loci, with the multiple loci serving as an internal control. The accuracy of the microarray will be measured using sentinel species such as Aeromonas species, Escherichia coli, and Vibrio vulnificus. Using microarrays, it should be possible to determine the annual fluctuations of bacterial species (culturable and non-culturable, pathogenic and non-pathogenic). The data may be applied to understanding patterns of environmental change; assessing the "health" of the Bay; and evaluating the risk of human illness associated with exposure to and ingestion of water and shellfish.

Stralis-Pavese, N., A. Sessitsch, et al. (2004). "Optimization of diagnostic microarray for application in analysing landfill methanotroph communities under different plant covers." Environ Microbiol **6**(4): 347-63.

Landfill sites are responsible for 6-12% of global methane emission. Methanotrophs play a very important role in decreasing landfill site methane emissions. We investigated the methane oxidation capacity and methanotroph diversity in lysimeters simulating landfill sites with different plant vegetations. Methane oxidation rates were 35 g methane m-2 day-1 or higher for planted lysimeters and 18 g methane m-2 day-1 or less for bare soil controls. Best methane oxidation, as displayed by gas depth profiles, was found under a vegetation of grass and alfalfa. Methanotroph communities were analysed at high throughput and resolution using a microbial diagnostic microarray targeting the particulate methane monooxygenase (pmoA) gene of methanotrophs and functionally related bacteria. Members of the genera Methylocystis and Methylocaldum were found to be the dominant members in landfill site simulating lysimeters. Soil bacterial communities in biogas free control lysimeters, which were less abundant in methanotrophs, were dominated by Methylocaldum. Type Ia methanotrophs were found only in the top layers of bare soil lysimeters with relatively high oxygen and low methane concentrations. A competetive advantage of type II methanotrophs over type Ia methanotrophs was indicated under all plant covers investigated. Analysis of average and individual results from parallel samples was used to identify general trends and variations in methanotroph community structures in relation to depth, methane supply and plant cover. The applicability of the technology for the detection of environmental perturbations was proven by an erroneous result, where an unexpected community composition detected with the microarray indicated a potential gas leakage in the lysimeter being investigated.

Straub, T. M. and D. P. Chandler (2003). "Towards a unified system for detecting waterborne pathogens." J Microbiol Methods **53**(2): 185-97.

Currently, there is no single method to collect, process, and analyze a water sample for all pathogenic microorganisms of interest. Some of the difficulties in developing a universal method include the physical differences between the major pathogen groups (viruses, bacteria, protozoa), efficiently concentrating large volume water samples to detect low target concentrations of certain pathogen groups, removing co-concentrated inhibitors from the sample, and standardizing a culture-independent endpoint detection method. Integrating the disparate technologies into a single, universal, simple method and detection system would represent a significant advance in public health and microbiological water quality

analysis. Recent advances in sample collection, on-line sample processing and purification, and DNA microarray technologies may form the basis of a universal method to detect known and emerging waterborne pathogens. This review discusses some of the challenges in developing a universal pathogen detection method, current technology that may be employed to overcome these challenges, and the remaining needs for developing an integrated pathogen detection and monitoring system for source or finished water.

Straub, T. M., D. S. Daly, et al. (2002). "Genotyping Cryptosporidium parvum with an hsp70 single-nucleotide polymorphism microarray." Appl Environ Microbiol **68**(4): 1817-26.

We investigated the application of an oligonucleotide microarray to (i) specifically detect Cryptosporidium spp., (ii) differentiate between closely related C. parvum isolates and Cryptosporidium species, and (iii) differentiate between principle genotypes known to infect humans. A microarray of 68 capture probes targeting seven single-nucleotide polymorphisms (SNPs) within a 190-bp region of the hsp70 gene of Cryptosporidium parvum was constructed. Labeled hsp70 targets were generated by PCR with biotin- or Cy3-labeled primers. Hybridization conditions were optimized for hybridization time, temperature, and salt concentration. Two genotype I C. parvum isolates (TU502 and UG502), two C. parvum genotype II isolates (Iowa and GCH1), and DNAs from 22 non-Cryptosporidium sp. organisms were used to test method specificity. Only DNAs from C. parvum isolates produced labeled amplicons that could be hybridized to and detected on the array. Hybridization patterns between genotypes were visually distinct, but identification of SNPs required statistical analysis of the signal intensity data. The results indicated that correct mismatch discrimination could be achieved for all seven SNPs for the UG502 isolate, five of seven SNPs for the TU502 isolate, and six of seven SNPs for both the Iowa and GCH1 isolates. Even without perfect mismatch discrimination, the microarray method unambiguously distinguished between genotype I and genotype II isolates and demonstrated the potential to differentiate between other isolates and species on a single microarray. This method may provide a powerful new tool for water utilities and public health officials for assessing point and nonpoint source contamination of water supplies.

Taroncher-Oldenburg, G., E. M. Griner, et al. (2003). "Oligonucleotide microarray for the study of functional gene diversity in the nitrogen cycle in the environment." Appl Environ Microbiol **69**(2): 1159-71.

The analysis of functional diversity and its dynamics in the environment is essential for understanding the microbial ecology and biogeochemistry of aquatic systems. Here we describe the development and optimization of a DNA microarray method for the detection and quantification of functional genes in the environment and report on their preliminary application to the study of the denitrification gene nirS in the Choptank River-Chesapeake Bay system. Intergenic and intragenic resolution constraints were determined by an oligonucleotide (70-mer) microarray approach. Complete signal separation was achieved when comparing unrelated genes within the nitrogen cycle (amoA, nifH, nirK, and nirS) and detecting different variants of the same gene, nirK, corresponding to organisms with two different physiological modes, ammonia oxidizers and denitrifying halobenzoate degraders. The limits of intragenic resolution were investigated with a microarray containing 64 nirS sequences comprising 14 cultured organisms and 50 clones obtained from the Choptank River in Maryland. The nirS oligonucleotides covered a range of sequence identities from approximately 40 to 100%. The threshold values for specificity were determined to be 87% sequence identity and a target-to-probe perfect match-to-mismatch binding free-energy ratio of 0.56. The lower detection limit was 10 pg of DNA (equivalent to approximately 10(7) copies) per target per microarray. Hybridization patterns on the microarray differed between sediment samples from two stations in the Choptank River, implying important differences in the composition of the denitirifer community along an environmental gradient of salinity, inorganic nitrogen, and dissolved organic carbon. This work establishes a useful set of design constraints (independent of the target gene) for the implementation of functional gene microarrays for environmental

applications.

Tiquia, S. M., L. Wu, et al. (2004). "Evaluation of 50-mer oligonucleotide arrays for detecting microbial populations in environmental samples." Biotechniques **36**(4): 664-70, 672, 674-5.

Microarrays fabricated with oligonucleotides longer than 40 bp have been introduced for monitoring whole genome expression but have not been evaluated with environmental samples. To determine the potential of this type of microarray for environmental studies, a 50-mer oligonucleotide microarray was constructed using 763 genes involved in nitrogen cycling: nitrite reductase (nirS and nirK), ammonia monooxygenase (amoA), nitrogenase (nifH), methane monooxygenase (pmoA), and sulfite reductase (dsrAB) from public databases and our own sequence collections. The comparison of the sequences from pure cultures indicated that the developed microarrays could provide species-level resolution for analyzing microorganisms involved in nitrification, denitrification, nitrogen fixation, methane oxidation, and sulfite reduction. Sensitivity tests suggested that the 50-mer oligonucleotide arrays could detect dominant populations in the environments, although sensitivity still needs to be improved. A significant quantitative relationship was also obtained with a mixture of DNAs from eight different bacteria. These results suggest that the 50-mer oligonucleotide array can be used as a specific and quantitative parallel tool for the detection of microbial populations in environmental samples.

Voelckel, C. and I. T. Baldwin (2004). "Herbivore-induced plant vaccination. Part II. Array-studies reveal the transience of herbivore-specific transcriptional imprints and a distinct imprint from stress combinations." Plant J **38**(4): 650-63.

Summary Microarray technology has given plant biologists the ability to simultaneously monitor changes in the expression of hundreds of genes, and yet, to date, this technology has not been applied to ecological phenomena. In native tobacco (Nicotiana attenuata), prior attack of sap-feeding mirids (Tupiocoris notatus) results in vaccination of the plant against subsequent attacks by chewing hornworms (Manduca sexta). This vaccination is mediated by a combination of direct and indirect defenses and tolerance responses, which act in concert with the attack preferences of a generalist predator. Here, we use microarrays enriched in herbivore-elicited genes with a principal components analysis (PCA) to characterize transcriptional 'imprints' of single, sequential, or simultaneous attacks by these two main herbivores of N. attenuata. The PCA identified distinctly different imprints left by individual attack from the two species after 24 h, but not after 5 days. Moreover, imprints of sequential or simultaneous attacks differed significantly from those of single attack, suggesting the existence of a distinct gene expression program responsive to the combination of biological stressors. A dissection of the transcriptional imprints revealed responses in direct and indirect defense genes that were well correlated with observed increases in defense metabolites. Attack from both herbivores elicits a switch from growth- to defense-related transcriptional processes, and herbivore-specific changes occur largely in primary metabolism and signaling cascades. PCA of these polygenic transcriptional imprints characterizes the ephemeral changes in the transcriptome that occur during the maturation of ecologically relevant phenotypic responses.

Vora, G. J., C. E. Meador, et al. (2004). "Nucleic acid amplification strategies for DNA microarray-based pathogen detection." Appl Environ Microbiol **70**(5): 3047-54.

DNA microarray-based screening and diagnostic technologies have long promised comprehensive testing capabilities. However, the potential of these powerful tools has been limited by front-end target-specific nucleic acid amplification. Despite the sensitivity and specificity associated with PCR amplification, the inherent bias and limited throughput of this approach constrain the principal benefits of downstream microarray-based applications, especially for pathogen detection. To begin addressing alternative approaches, we investigated four front-end amplification strategies: random primed, isothermal Klenow fragment-based, phi29 DNA polymerase-based, and multiplex PCR. The utility of each amplification strategy was assessed by hybridizing amplicons to microarrays consisting of 70-mer oligonucleotide probes specific for enterohemorrhagic Escherichia coli O157:H7 and by

quantitating their sensitivities for the detection of O157:H7 in laboratory and environmental samples. Although nearly identical levels of hybridization specificity were achieved for each method, multiplex PCR was at least 3 orders of magnitude more sensitive than any individual random amplification approach. However, the use of Klenow-plus-Klenow and phi29 polymerase-plus-Klenow tandem random amplification strategies provided better sensitivities than multiplex PCR. In addition, amplification biases among the five genetic loci tested were 2- to 20-fold for the random approaches, in contrast to >4 orders of magnitude for multiplex PCR. The same random amplification strategies were also able to detect all five diagnostic targets in a spiked environmental water sample that contained a 63-fold excess of contaminating DNA. The results presented here underscore the feasibility of using random amplification approaches and begin to systematically address the versatility of these approaches for unbiased pathogen detection from environmental sources.

Wang, Z., G. J. Vora, et al. (2004). "Detection and genotyping of Entamoeba histolytica, Entamoeba dispar, Giardia lamblia, and Cryptosporidium parvum by oligonucleotide microarray." J Clin Microbiol **42**(7): 3262-71.

Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum are the most frequently identified protozoan parasites causing waterborne disease outbreaks. The morbidity and mortality associated with these intestinal parasitic infections warrant the development of rapid and accurate detection and genotyping methods to aid public health efforts aimed at preventing and controlling outbreaks. In this study, we describe the development of an oligonucleotide microarray capable of detecting and discriminating between E. histolytica, Entamoeba dispar, G. lamblia assemblages A and B, and C. parvum types 1 and 2 in a single assay. Unique hybridization patterns for each selected protozoan were generated by amplifying six to eight diagnostic sequences/organism by multiplex PCR; fluorescent labeling of the amplicons via primer extension; and subsequent hybridization to a set of genus-, species-, and subtype-specific covalently immobilized oligonucleotide probes. The profile-based specificity of this methodology not only permitted for the unequivocal identification of the six targeted species and subtypes, but also demonstrated its potential in identifying related species such as Cryptosporidium meleagridis and Cryptosporidium muris. In addition, sensitivity assays demonstrated lower detection limits of five trophozoites of G. lamblia. Taken together, the specificity and sensitivity of the microarray-based approach suggest that this methodology may provide a promising tool to detect and genotype protozoa from clinical and environmental samples.

Warsen, A. E., M. J. Krug, et al. (2004). "Simultaneous discrimination between 15 fish pathogens by using 16S ribosomal DNA PCR and DNA microarrays." Appl Environ Microbiol **70**(7): 4216-21.

We developed a DNA microarray suitable for simultaneous detection and discrimination between multiple bacterial species based on 16S ribosomal DNA (rDNA) polymorphisms using glass slides. Microarray probes (22- to 31-mer oligonucleotides) were spotted onto Teflon-masked, epoxy-silane-derivatized glass slides using a robotic arrayer. PCR products (ca. 199 bp) were generated using biotinylated, universal primer sequences, and these products were hybridized overnight (55 degrees C) to the microarray. Targets that annealed to microarray probes were detected using a combination of Tyramide Signal Amplification and Alexa Fluor 546. This methodology permitted 100% specificity for detection of 18 microbes, 15 of which were fish pathogens. With universal 16S rDNA PCR (limited to 28 cycles), detection sensitivity for purified control DNA was equivalent to <150 genomes (675 fg), and this sensitivity was not adversely impacted either by the presence of competing bacterial DNA (1.1 x 10(6) genomes; 5 ng) or by the addition of up to 500 ng of fish DNA. Consequently, coupling 16S rDNA PCR with a microarray detector appears suitable for diagnostic detection and surveillance for commercially important fish pathogens.

Wolfgang, M. C., B. R. Kulasekara, et al. (2003). "Conservation of genome content and virulence determinants among clinical and environmental isolates of Pseudomonas aeruginosa." Proc Natl Acad Sci

U S A **100**(14): 8484-9.

Pseudomonas aeruginosa is a ubiquitous environmental bacterium capable of causing a variety of life-threatening human infections. The genetic basis for preferential infection of certain immunocompromised patients or individuals with cystic fibrosis by P. aeruginosa is not understood. To establish whether variation in the genomic repertoire of P. aeruginosa strains can be associated with a particular type of infection, we used a whole-genome DNA microarray to determine the genome content of 18 strains isolated from the most common human infections and environmental sources. A remarkable conservation of genes including those encoding nearly all known virulence factors was observed. Phylogenetic analysis of strain-specific genes revealed no correlation between genome content and infection type. Clusters of strain-specific genes in the P. aeruginosa genome, termed variable segments, appear to be preferential sites for the integration of novel genetic material. A specialized cloning vector was developed for capture and analysis of these genomic segments. With this capture system a site associated with the strain-specific ExoU cytotoxin-encoding gene was interrogated and an 80-kb genomic island carrying exoU was identified. These studies demonstrate that P. aeruginosa strains possess a highly conserved genome that encodes genes important for survival in numerous environments and allows it to cause a variety of human infections. The acquisition of novel genetic material, such as the exoU genomic island, through horizontal gene transfer may enhance colonization and survival in different host environments.

Wu, L., D. K. Thompson, et al. (2001). "Development and evaluation of functional gene arrays for detection of selected genes in the environment." Appl Environ Microbiol **67**(12): 5780-90.

To determine the potential of DNA array technology for assessing functional gene diversity and distribution, a prototype microarray was constructed with genes involved in nitrogen cycling: nitrite reductase (nirS and nirK) genes, ammonia mono-oxygenase (amoA) genes, and methane mono-oxygenase (pmoA) genes from pure cultures and those cloned from marine sediments. In experiments using glass slide microarrays, genes possessing less than 80 to 85% sequence identity were differentiated under hybridization conditions of high stringency (65 degrees C). The detection limit for nirS genes was approximately 1 ng of pure genomic DNA and 25 ng of soil community DNA using our optimized protocol. A linear quantitative relationship (r(2) = 0.89 to 0.94) was observed between signal intensity and target DNA concentration over a range of 1 to 100 ng for genomic DNA (or genomic DNA equivalent) from both pure cultures and mixed communities. However, the quantitative capacity of microarrays for measuring the relative abundance of targeted genes in complex environmental samples is less clear due to divergent target sequences. Sequence divergence and probe length affected hybridization signal intensity within a certain range of sequence identity and size, respectively. This prototype functional gene array did reveal differences in the apparent distribution of nir and amoA and pmoA gene families in sediment and soil samples. Our results indicate that glass-based microarray hybridization has potential as a tool for revealing functional gene composition in natural microbial communities; however, more work is needed to improve sensitivity and quantitation and to understand the associated issue of specificity.

Food Applications

A-H

Al-Khaldi, S. F., S. A. Martin, et al. (2002). "DNA microarray technology used for studying foodborne pathogens and microbial habitats: minireview." J AOAC Int **85**(4): 906-10.

Microarray analysis is an emerging technology that has the potential to become a leading trend in bacterial identification in food and feed improvement. The technology uses fluorescent-labeled probes amplified from bacterial samples that are then hybridized to thousands of DNA sequences immobilized on chemically modified glass slides. The whole gene or open reading frame(s) is represented by a polymerase chain reaction fragment of double-strand DNA, approximately 1000 base pair (bp) or 20-70 bp single-strand oligonucleotides. The technology can be used to identity bacteria and to study gene expression in complex microbial populations, such as those found in food and gastrointestinal tracts. Data generated by microarray analysis can be potentially used to improve the safety of our food supply as well as ensure the efficiency of animal feed conversion to human food, e.g., in meat and milk production by ruminants. This minireview addresses the use of microarray technology in bacterial identification and gene expression in different microbial systems and in habitats containing mixed populations of bacteria.

Alvarez, J., S. Porwollik, et al. (2003). "Detection of a Salmonella enterica serovar California strain spreading in spanish feed mills and genetic characterization with DNA microarrays." Appl Environ Microbiol **69**(12): 7531-4.

We performed an epidemiological study on Salmonella isolated from raw plant-based feed in Spanish mills. Overall, 32 different Salmonella serovars were detected. Despite its rare occurrence in humans and animals, Salmonella enterica serovar California was found to be the predominant serovar in Spanish feed mills. Different typing techniques showed that isolates of this serovar were genetically closely related, and comparative genomic hybridization using microarray technology revealed 23 S. enterica serovar Typhimurium LT2 gene clusters that are absent from serovar California.

Backhus, L. E., J. DeRisi, et al. (2001). "Functional genomic analysis of a commercial wine strain of Saccharomyces cerevisiae under differing nitrogen conditions." FEMS Yeast Res **1**(2): 111-25.

DNA microarray analysis was used to profile gene expression in a commercial isolate of Saccharomyces cerevisiae grown in a synthetic grape juice medium under conditions mimicking a natural environment for yeast: High-sugar and variable nitrogen conditions. The high nitrogen condition displayed elevated levels of expression of genes involved in biosynthesis of macromolecular precursors across the time course as compared to low-nitrogen. In contrast, expression of genes involved in translation and oxidative carbon metabolism were increased in the low-nitrogen condition, suggesting that respiration is more nitrogen-conserving than fermentation. Several genes under glucose repression control were induced in low-nitrogen in spite of very high (17%) external glucose concentrations, but there was no general relief of glucose repression. Expression of many stress response genes was elevated in stationary phase. Some of these genes were expressed regardless of the nitrogen concentration while others were found at higher levels only under high nitrogen conditions. A few genes, FSP2, RGS2, AQY1, YFL030W, were expressed more strongly with nitrogen limitation as compared to other conditions.

Bodrossy, L. and A. Sessitsch (2004). "Oligonucleotide microarrays in microbial diagnostics." Curr Opin Microbiol **7**(3): 245-54.

Oligonucleotide microarrays offer a fast, high-throughput alternative for the parallel detection of microbes from virtually any sample. The application potential spreads across most sectors of life sciences, including environmental microbiology and microbial ecology; human, veterinary, food and plant diagnostics; water quality control; industrial microbiology, and so on. The past two years have witnessed

a rapid increase of research in this field. Many alternative techniques were developed and validated as seen in 'proof-of-concept' articles. Publications reporting on the application of oligonucleotide microarray technology for microbial diagnostics in microbiology driven projects have just started to appear. Current and future technical and bioinformatics developments will inevitably improve the potential of this technology further.

Boonham, N., K. Walsh, et al. (2003). "Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis." J Virol Methods **108**(2): 181-7.

Currently, most diagnostic methodology is geared towards detection of a very specific target species and often a number of assays need to be run in parallel to reach a result. The generic methods that are available for virus testing tends to give identification to the genus level only. The method described in this paper addresses this problem by exploiting a technology that has potential to test for a large number of targets in a single assay. Using the array constructed, the method was able to detect several common potato viruses (PVY, PVX, PVA, PVS) in single and mixed infections. The method was shown to be able to discriminate sequences with less than 80% sequence identity but was able to detect sequence variants with greater than 90% sequence identity. Thus the method should be useful for discriminating at the species level, but able to cope well with the intrinsic variability found within the genomes of RNA viruses. The sensitivity of the assay was found to be comparable with ELISA. The paper illustrates a significant step forward in the development of diagnostic methodologies by presenting for the first time a method that could theoretically be used not just for viruses, but for all the plant pathogens and pests that a modern diagnostic laboratory would want to test for, in a single completely generic and highly parallel format.

Call, D. R., F. J. Brockman, et al. (2001). "Detecting and genotyping Escherichia coli O157:H7 using multiplexed PCR and nucleic acid microarrays." Int J Food Microbiol **67**(1-2): 71-80.

Rapid detection and characterization of food borne pathogens such as Escherichia coli O157:H7 is crucial for epidemiological investigations and food safety surveillance. As an alternative to conventional technologies, we examined the sensitivity and specificity of nucleic acid microarrays for detecting and genotyping E. coli O157:H7. The array was composed of oligonucleotide probes (25-30 mer) complementary to four virulence loci (intimin, Shiga-like toxins I and II, and hemolysin A). Target DNA was amplified from whole cells or from purified DNA via single or multiplexed polymerase chain reaction (PCR), and PCR products were hybridized to the array without further modification or purification. The array was 32-fold more sensitive than gel electrophoresis and capable of detecting amplification products from < 1 cell equivalent of genomic DNA (1 fg). Immunomagnetic capture, PCR and a microarray were subsequently used to detect 55 CFU ml(-1) (E. coli O157:H7) from chicken rinsate without the aid of pre-enrichment. Four isolates of E. coli O157:H7 and one isolate of O91:H2, for which genotypic data were available, were unambiguously genotyped with this array. Glass-based microarrays are relatively simple to construct and provide a rapid and sensitive means to detect multiplexed PCR products; the system is amenable to automation

Chandler, D. P., J. Brown, et al. (2001). "Automated immunomagnetic separation and microarray detection of E. coli O157:H7 from poultry carcass rinse." Int J Food Microbiol **70**(1-2): 143-54.

We describe the development and application of an electromagnetic flow cell and fluidics system for automated immunomagnetic separation (IMS) of Escherichia coli O157:H7 directly from poultry carcass rinse. We further describe the biochemical coupling of automated sample preparation with nucleic acid microarrays. Both the cell concentration system and microarray detection method did not require cell growth or enrichment from the poultry carcass rinse prior to IMS. Highly porous Ni foam was used to enhance the magnetic field gradient within the flow path, providing a mechanism for immobilizing immunomagnetic particles throughout the fluid rather than the tubing wall. A maximum of 32% recovery efficiency of non-pathogenic E. coli was achieved within the automated system with 6 s cell contact times

using commercially available antibodies targeted against the O and K antigens. A 15-min protocol (from sample injection though elution) provided a cell recovery efficiency that was statistically similar to > I h batch captures. O157:H7 cells were reproducibly isolated directly from poultry carcass rinse with 39% recovery efficiency at 10(3) CFU ml(-1) inoculum. Direct plating of washed beads showed positive recovery of O157:H7 directly from poultry carcass rinse at an inoculum of 10 CFU ml(-1). Recovered beads were used for direct polymerase chain reaction (PCR) amplification and microarray detection, with a process-level detection limit (automated cell concentration though microarray detection) of < 10(3)CFU ml(-1) in poultry carcass rinse.

Food Applications

I-P

Keramas, G., D. D. Bang, et al. (2003). "Development of a sensitive DNA microarray suitable for rapid detection of Campylobacter spp." Mol Cell Probes **17**(4): 187-96.

Campylobacter is the most common cause of human acute bacterial gastroenteritis worldwide, widely distributed and isolated from human clinical samples as well as from many other different sources. To comply with the demands of consumers for food safety, there is a need for development of a rapid, sensitive and specific detection method for Campylobacter. In this study, we present the development of a novel sensitive DNA-microarray based detection method, evaluated on Campylobacter and non-Campylobacter reference strains, to detect Campylobacter directly from the faecal cloacal swabs. The DNA-microarray method consists of two steps: first, both universal bacterial sequences and specific Campylobacter sequences (size range: 149-307 bp) are amplified and fluorescently labeled using multiplex-PCR, targeting the 16S rRNA, the 16S-23S rRNA intergenic region and specific Campylobacter genes. Secondly, the Cy5 labeled PCR-amplicons are hybridised to immobilised capture probes on the microarray. The method allows detection of three to thirty genome equivalents (6-60 fg DNA) of Campylobacter within 3 h, with a hands on time of only 15 min. Using the DNA-microarrays, two closely related Campylobacter species, Campylobacter jejuni and Campylobacter coli could be detected and differentiated directly from chicken faeces. The DNA-microarray method has a high potential for automation and incorporation into a dedicated mass screening microsystem.

Food Applications

Q-Z

Stin, O. C., A. Carnahan, et al. (2003). "Characterization of microbial communities from coastal waters using microarrays." Environ Monit Assess **81**(1-3): 327-36.

Molecular methods, including DNA probes, were used to identify and enumerate pathogenic Vibrio species in the Chesapeake Bay; our data indicated that Vibrio vulnificus exhibits seasonal fluctuations in number. Our work included a characterization of total microbial communities from the Bay; development of microarrays that identify and quantify the diversity of those communities; and observation of temporal changes in those communities. To identify members of the microbial community, we amplified the 16S rDNA gene from community DNA isolated from a biofilm sample collected from the Chesapeake Bay in February, 2000. The resultant 75 sequences were 95% or more similar to 7 species including two recently described Shewanella species, baltica and frigidimarina, that have not been previously isolated from the Chesapeake. When the genera of bacteria from biofilm after culturing are compared to those detected by subcloning amplified 16S fragments from community DNA, the cultured sample exhibited a strong bias. In oysters collected in February, the most common bacteria were previously unknown. Based on our 16S findings, we are developing microarrays to detect these and other microbial species in these estuarine communities. The microarrays will detect each species using four

distinct loci, with the multiple loci serving as an internal control. The accuracy of the microarray will be measured using sentinel species such as Aeromonas species, Escherichia coli, and Vibrio vulnificus. Using microarrays, it should be possible to determine the annual fluctuations of bacterial species (culturable and non-culturable, pathogenic and non-pathogenic). The data may be applied to understanding patterns of environmental change; assessing the "health" of the Bay; and evaluating the risk of human illness associated with exposure to and ingestion of water and shellfish.

Warsen, A. E., M. J. Krug, et al. (2004). "Simultaneous discrimination between 15 fish pathogens by using 16S ribosomal DNA PCR and DNA microarrays." Appl Environ Microbiol **70**(7): 4216-21.

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Review Articles

A-H

Amick, J. D. and Y. V. Brun (2001). "Anatomy of a bacterial cell cycle." Genome Biol **2**(7): REVIEWS1020.

Two recent reports describe mRNA and protein expression patterns in the bacterium Caulobacter crescentus. The combined use of DNA microarray and proteomic analyses provides a powerful new perspective for unraveling the global regulatory networks of this complex bacterium.

Arendt, C. W. and D. R. Littman (2001). "HIV: master of the host cell." Genome Biol **2**(11): REVIEWS1030.

The human immunodeficiency virus has evolved various mechanisms to exploit its host cells, including the interruption and augmentation of signal transduction pathways. Recently, two DNA microarray studies have illustrated a remarkably broad-based perturbation in host transcriptional responses, which is in part mediated by the HIV-encoded Nef protein. HIV therefore seems to function as a 'master regulator' of cellular gene expression.

Speaker Suggested Articles

A-H

Baeumner, A. J., Humiston, M.C., Montagna, R.A., and Durst, R.A. "Detection of Viable Oocysts of Cryptosporidium parvum Following Nucleic Acid Sequence-Based Amplification," Anal. Chem. 73, no. 6: 1176-1180 (2001).

A reliable method using nucleic acid sequence based amplification (NASBA) with subsequent electrochemiluminescent detection for the specific and sensitive detection of viable oocysts of Cryptosporidium parvum in environmental samples was developed. The target molecule was a 121-nt sequence from the C. parvum heat shock protein hsp70 mRNA. Oocysts of C. parvum were isolated from environmental water via vortex flow filtration and immunomagnetic separation. A brief heat shock was applied to the oocysts and the nucleic acid purified using an optimized very simple but efficient nucleic acid extraction method. The nucleic acid was amplified in a water bath for 60-90 min with NASBA, an isothermal technique that specifically amplifies RNA molecules. Amplified RNA was hybridized with specific DNA probes and quantified with an electrochemiluminescence (ECL) detection system. We optimized the nucleic acid extraction and purification, the NASBA reaction, amplification, and detection probes. We were able to amplify and detect as few as 10 mRNA molecules. The NASBA primers as well as the ECL probes were highly specific for C. parvum in buffer and in environmental samples. Our detection limit was 5 viable oocysts/sample for the assay procedure, including nucleic acid extraction, NASBA, and ECL detection. Nonviable oocysts were not detected.

Bekal-Si Ali, S., R. Brousseau, L. Masson, G. Prefontaine, J. Fairbrother and J Harel. (2003). Rapid identification of E. coli pathotypes through virulence gene detection by DNA microarrays J. Clin. Microbiol. 41: 2113-2125.

One approach to the accurate determination of the pathogenic potential (pathotype) of isolated Escherichia coli strains would be through a complete assessment of each strain for the presence of all known E. coli virulence factors. To accomplish this, an E. coli virulence factor DNA microarray composed of 105 DNA PCR amplicons printed on glass slides and arranged in eight subarrays corresponding to different E. coli pathotypes was developed. Fluorescently labeled genomic DNAs from E. coli strains representing known pathotypes were initially hybridized to the virulence gene microarrays for both chip optimization and validation. Hybridization pattern analysis with clinical isolates permitted a rapid assessment of their virulence attributes and determination of the pathogenic group to which they belonged. Virulence factors belonging to two different pathotypes were detected in one human E. coli isolate (strain H87-5406). The microarray was also tested for its ability to distinguish among phylogenetic groups of genes by using gene probes derived from the attaching-and-effacing locus (espA, espB, tir). After hybridization with these probes, we were able to distinguish E. coli strains harboring espA, espB, and tir sequences closely related to the gene sequences of an enterohemorrhagic strain (EDL933), a human enteropathogenic strain (E2348/69), or an animal enteropathogenic strain (RDEC-1). Our results show that the virulence factor microarray is a powerful tool for diagnosis-based studies and that the concept is useful for both gene quantitation and subtyping. Additionally, the multitude of virulence genes present on the microarray should greatly facilitate the detection of virulence genes acquired by horizontal transfer and the identification of emerging pathotypes.

Call, DR, MK Borucki and FJ Loge. 2003. Detection of bacterial pathogens in environmental samples using DNA microarrays. Journal of Microbiological Methods 53:235-243.

Polymerase chain reaction (PCR) is an important tool for pathogen detection, but historically, it has not been possible to accurately identify PCR products without sequencing, Southern blots, or dot-blots. Microarrays can be coupled with PCR where they serve as a set of parallel dot-blots to enhance product detection and identification. Microarrays are composed of many discretely located probes on a

solid substrate such as glass. Each probe is composed of a sequence that is complimentary to a pathogen-specific gene sequence. PCR is used to amplify one or more genes and the products are then hybridized to the array to identify species-specific polymorphism within one or more genes. We illustrate this type of array using 16S rDNA probes suitable for distinguishing between several salmonid pathogens. We also describe the use of microarrays for direct detection of either RNA or DNA without the aid of PCR, although the sensitivity of these systems currently limits their application for pathogen detection. Finally, microarrays can also be used to ''fingerprint'' bacterial isolates and they can be used to identify diagnostic markers suitable for developing new PCR-based detection assays. We illustrate this type of array for subtyping an important food-borne pathogen, Listeria monocytogenes.

Speaker Suggested Articles

I-P

Lemarchand, K.,Masson, L. and Brousseau, R. 2004. Molecular biology and DNA microarray technology for microbial quality monitoring of water. Crit. Rev. Microbiol. 30:145-172.

Public concern over polluted water is a major environmental issue worldwide. Microbial contamination of water arguably represents the most significant risk to human health on a global scale. An important challenge in modern water microbial quality monitoring is the rapid, specific, and sensitive detection of microbial indicators and waterborne pathogens. Presently, microbial tests are based essentially on time-consuming culture methods. Rapid microbiological analyses and detection of rare events in water systems are important challenges in water safety assessment since culture methods present serious limitations from both quantitative and qualitative points of view. To circumvent lengthy culture methods, newer enzymatic, immunological, and genetic methods are being developed as an alternative. DNA microarray technology is a new and promising tool that allows the detection of several hundred or even thousands DNA sequences simultaneously. Recent advances in sample processing and DNA microarray technologies provide new perspectives to assess microbial water quality. The aims of this review are to (1) summarize what is currently known about microbial indicators, (2) describe the most important waterborne pathogens, (3) present molecular methods used to monitor the presence of pathogens in water, and (4) show the potential of DNA microarrays in water quality monitoring.

Letowski, J., Brousseau, R. and Masson, L. (2003) DNA microarray applications in environmental microbiology. Analytical Letters, 36, 3147-3166.

Although the majority of microarray reports are concerned with gene expression profiling in health-related studies, the use of DNA microarray technology is expanding into new fields and new applications. In environmental microbiology, developments are also focusing on the detection of specific sequences in complex environmental samples and on genomic comparisons. Despite the fact that some limitations still exist, microarrays offer several advantages over the more traditional approaches. In this review, we will present examples of specific applications of this exciting new technology relating to the better understanding of the microbial world, with particular emphasis on strain detection, as well as the assessment of microbial diversity, adaptation, and evolution.

Letowski, J., Brousseau, R., and Masson, L. 2004 Designing better probes: effect of probe size, mismatch position and number on hybridization in DNA oligonucleotide microarrays. J. Microbiol. Methods 57: 269-278

DNA microarrays represent a powerful technology whose use has been hampered by the uncertainty of whether the same principles, established on a scale typical for membrane hybridizations, apply when using the smaller, rigid support of microarrays. Our goal was to understand how the number and position of base pair mismatches, probe length and their G+ C content affect the intensity and specificity of the hybridization signal. One set of oligonucleotides (50-mers) based on three regions of the

Bacillus thuringiensis cry1Aa1 gene possessing 30%, 42%, and 56% G+ C content, a second set with similar G+C content (37% to 40%) but different lengths (30 to 100 bases), and finally amplicon probes (101 to 3000 base pairs) with G+C contents of 37% to 39%, were used. Probes with mismatches distributed over their entire length were the most specific, while those with mismatches grouped at either the 3V or 5V-end were the least specific. Hybridizations done at 8 to 13 jC below the calculated Tm of perfectly matched probes, as compared to the widely used lower temperatures of 20 to 25 jC, enhanced probe discrimination. Longer probes produced higher fluorescent hybridization signals than shorter ones. These results should help to optimize the design of oligonucleotide-based DNA microarrays.

Li J, Chen S, Evans DH. Typing and subtyping influenza virus using DNA microarrays and multiplex reverse transcriptase PCR. J Clin Microbiol. 2001 Feb;39(2):696-704.

A model DNA microarray has been prepared and shown to facilitate typing and subtyping of human influenza A and B viruses. Reverse transcriptase PCR was used to prepare cDNAs encoding ;500-bp influenza virus gene fragments, which were then cloned, sequenced, reamplified, and spotted to form a glass-bound microarray. These target DNAs included multiple fragments of the hemagglutinin, neuraminidase, and matrix protein genes. Cy3- or Cy5-labeled fluorescent probes were then hybridized to these target DNAs, and the arrays were scanned to determine the probe binding site(s). The hybridization pattern agreed perfectly with the known grid location of each target, and the signal-to-background ratio varied from 5 to 30. No crosshybridization could be detected beyond that expected from the limited degree of sequence overlap between different probes and targets. At least 100 to 150 bp of homology was required for hybridization under the conditions used in this study. Combinations of Cy3- and Cy5-labeled DNAs can also be hybridized to the same chip, permitting further differentiation of amplified molecules in complex mixtures. In a more realistic test of the technology, several sets of multiplex PCR primers that collectively target influenza A and B virus strains were identified and were used to type and subtype several previously unsequenced influenza virus isolates. The results show that DNA microarray technology provides a useful supplement to PCR-based diagnostic methods.

Pemov, A., Modi, H., Chandler, D., and Bavykin, S.. 2005. DNA analysis with multiplex microarray-enhanced PCR. Nucl. Acids Res. 33(2): e11.

We have developed a highly sensitive method forDNA analysis on 3D gel element microarrays, a technique we call multiplex microarray-enhanced PCR (MMEPCR). Two amplification strategies are carried out simultaneously in the reaction chamber: on or within gel elements, and in bulk solution over the gel element array. MME-PCR is initiated by multiple complex primers containing gene-specific, forward and reverse, sequences appended to the 30 end of a universal amplification primer. The complex primer pair is covalently tethered through its 50 end to the polyacrylamide backbone. In the bulk solution above the gel element array, a single pair of unattached universal primers simultaneously directs pseudo-monoplex PCR of all targets according to normal solution phase PCR. The presence of a single universal PCR primer pair in solution accelerates amplification within gel elements and eliminates the problem of primer interference that is common to conventional multiplex PCR. We show 106-fold amplification of targeted DNA after 50 cycles with average amplification efficiency 1.34 per cycle, and demonstrate specific on-chip amplification of six genes in Bacillus subtilis. All six genes were detected at 4.5 pg of bacterial genomic DNA (equivalent to 103 genomes) in 60 independent amplification reactions performed simultaneously in single reaction chamber.

Speaker Suggested Articles

Q-Z

Stine OC, Carnahan A, Singh R, Powell J, Furuno JP, Dorsey A, Silbergeld E, Williams HN, Morris JG. Characterization of microbial communities from coastal waters using microarrays. Environmental

Monitoring and Assessment 81:331-340, 2003

Molecular methods, including DNA probes, were used to identify and enumerate pathogenic Vibrio species in the Chesapeake Bay; our data indicated that V. vulnificus exhibits seasonal fluctuations in number. Our work included a characterization of total microbial communities from the Bay; development of microarrays that identify and quantify the diversity of those communities; and observation of temporal changes in those communities. To identify members of the microbial community, we amplified the 16S rDNA gene from community DNA isolated from a biofilm sample collected from the Chesapeake Bay in February. The resultant 75 sequences were 95% or more similar to 7 species including two recently described Shewanella species, baltica and frigidimarina, that have not been previously isolated from the Chesapeake. When the genera of bacteria from biofilm after culturing are compared to those detected by subcloning amplified 16S fragments from community DNA, the cultured sample exhibited a strong bias. In oysters collected in February, the most common bacteria were previously unknown. Based on our 16S findings, we are developing microarrays to detect these and other microbial species in these estuarine communities. The microarrays will detect each species using four distinct loci, with the multiple loci serving as an internal control. The accuracy of the microarray will be measured using sentinel species such as Aeromonas species, E. coli, and Vibrio vulnificus. Using microarrays, it should be possible to determine the annual fluctuations of bacterial species (culturable and non-culturable, pathogenic and non-pathogenic). The data may be applied to understanding patterns of environmental change; assessing the "health" of the Bay; and evaluating the risk of human illness associated with exposure to and ingestion of water and shellfish.

Vora, G., Meador, C., Stenger, D. and Andreadis, J. Nucleic Acid Amplification Strategies for DNA Microarray-Based Pathogen Detection APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2004, p. 3047—3054.

DNA microarray-based screening and diagnostic technologies have long promised comprehensive testing capabilities. However, the potential of these powerful tools has been limited by front-end target-specific nucleic acid amplification. Despite the sensitivity and specificity associated with PCR amplification, the inherent bias and limited throughput of this approach constrain the principal benefits of downstream microarray-based applications, especially for pathogen detection. To begin addressing alternative approaches, we investigated four front-end amplification strategies: random primed, isothermal Klenow fragment-based, 29 DNA polymerase- based, and multiplex PCR. The utility of each amplification strategy was assessed by hybridizing amplicons to microarrays consisting of 70-mer oligonucleotide probes specific for enterohemorrhagic Escherichia coli O157:H7 and by quantitating their sensitivities for the detection of O157:H7 in laboratory and environmental samples. Although nearly identical levels of hybridization specificity were achieved for each method, multiplex PCR was at least 3 orders of magnitude more sensitive than any individual random amplification approach. However, the use of Klenow-plus-Klenow and 29 polymerase-plus-Klenow tandem random amplification strategies provided better sensitivities than multiplex PCR. In addition, amplification biases among the five genetic loci tested were 2- to 20-fold for the random approaches, in contrast to >4 orders of magnitude for multiplex PCR. The same random amplification strategies were also able to detect all five diagnostic targets in a spiked environmental water sample that contained a 63-fold excess of contaminating DNA. The results presented here underscore the feasibility of using random amplification approaches and begin to systematically address the versatility of these approaches for unbiased pathogen detection from environmental sources.

Wang D, Coscoy L, Zylberberg M, Avila PC, Boushey HA, Ganem D, DeRisi JL. Microarray-based detection and genotyping of viral pathogens. (2002) Proc Natl Acad Sci U S A 99(24), 15687-92

Wang, D, Urisman, A, Liu, YT, Springer, M, Ksiazek, TG, Erdman, DD, Mardis, ER, Hickenbotham, M, Magrini, V, Eldred, J, Latreille, JP, Wilson, RK, Ganem, D and DeRisi, JL. Viral Discovery and

Sequence Recovery Using DNA Microarrays. PLoS Biol 1:2, E2 (2003).

Warsen, A, MJ Krug, S LaFrentz, DR Stanek, FJ Loge, and DR Call. 2004. Simultaneous discrimination between 15 fish pathogens using 16S rDNA PCR and DNA microarrays. Applied and Environmental Microbiology 70:4216-4221.

Waterborne outbreak of gastroenetritis associated with a norovirus. Parshionikar SU, Willian-True S, Fout GS, Robbins DE, Seys SA, Cassady JD, Harris R.Appl Environ Microbiol. 2003 Sep;69(9):5263-8.