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Gene Chips in Microbiology-its Various Diagnostic Applications

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ABSTRACT
The laboratory introduction of in vitro nucleic acid amplification techniques, led by real-time PCR, into the clinical microbiology laboratory has transformed the laboratory detection of viruses and select bacterial pathogens. However, the progression of the molecular diagnostic revolution currently relies on the ability to efficiently and accurately offer multiplex detection and characterization for a variety of infectious disease pathogens. Multiple microarray platforms exist, including printed double-stranded DNA and oligonucleotide arrays, in situ-synthesized arrays, high-density bead arrays, electronic microarrays, and suspension bead arrays. This review highlights uses of microarray technology that impact diagnostic microbiology, including the detection and identification of pathogens, determination of antimicrobial resistance, epidemiological strain typing, analysis of microbial infections using host genomic expression and polymorphism profiles and various other applications.

Other application of microarray include differentiations of mycobacterial species and detection of tuberculosis drug resistence, detection of virulence factors of microbial pathogens, detection of pathogens in food and water sample, typing of antimicrobial resistence genes ,and identification of fungal pathogens. This article has also reviewed the roll of microarray in the diagnosis of parasitic infection(identification of parasitic genes),industrial application of microarray(gene expression profiling to determine the effect of antibiotics,agrochemicals,pharmaceutical products on different organisms) and the roll of array in the detection of graft rejection.

Keywords: *Gene Chips,Oligonucleotide Microarray,Virochips.*

Micrarray in clinical microbiology is having various diagnostic and research applications. The table below shows the various microarray and its applications in the field of microbiology and molecular biology.

The photograph of microarray reader

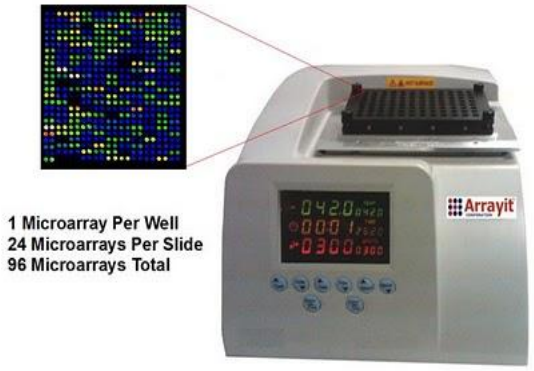

Table-1

MicroarrayApplication[1]	Synopsis
Gene expression profiling	Microarray-based gene expression profiling can be used to identify genes whose expression is changed in response to pathogens or other organisms by comparing gene expression in infected to that in uninfected cells or tissues.[8]
Comparative genomic hybridization	Assessing genome content in different cells or closely related organisms.[9][10]
Assessing genome content in different cells or closely related organisms.[9][10]	Small microarrays to check IDs of organisms in food and feed (like GMO[1]), mycoplasms in cell culture, or pathogens for disease detection, mostly combining PCR and microarray technology.
Chromatin immunoprecipitation on Chip	DNA sequences bound to a particular protein can be isolated by immunoprecipitating that protein (ChIP), these fragments can be then hybridized to a microarray (such as a tiling array) allowing the determination of protein binding site occupancy throughout the genome.
DamID	Analogously to ChIP, genomic regions bound by a protein of interest can be isolated and used to probe a microarray to determine binding site occupancy. Unlike ChIP, DamID does not require antibodies but makes use of adenine methylation near the protein's binding sites to selectively amplify those regions, introduced by expressing minute amounts of protein of interest fused to bacterial DNA adenine

	methyltransferase.
SNP detection	Identifying single nucleotide polymorphism among alleles within or between populations.[11] Several applications of microarrays make use of SNP detection, including Genotyping, forensic analysis, measuring predisposition to disease, identifying drug-candidates, evaluating germline mutations in individuals or somatic mutations in cancers, assessing loss of heterozygosity, or genetic linkage analysis.
Alternative splicing detection	An exon junction array design uses probes specific to the expected or potential splice sites of predicted exons for a gene. It is of intermediate density, or coverage, to a typical gene expression array (with 1-3 probes per gene) and a genomic tiling array (with hundreds or thousands of probes per gene). It is used to assay the expression of alternative splice forms of a gene. Exon arrays have a different design, employing probes designed to detect each individual exon for known or predicted genes, and can be used for detecting different splicing isoforms.
Fusion genes microarray	A Fusion gene microarray can detect fusion transcripts, e.g. from cancer specimens. The principle behind this is building on the alternative splicing microarrays. The oligo design strategy enables combined measurements of chimeric transcript junctions with exon-wise measurements of individual fusion partners.
Tiling microarray	Genome tiling arrays consist of overlapping probes designed to densely represent a genomic region of interest, sometimes as large as an entire human chromosome. The purpose is to empirically detect expression of transcripts or alternatively splice forms which may not have been previously known or predicted.

Other Application of micro array include the following.[2]

1. Evaluation of mental retardation and developmental delay
2. Cancer/tumor characterization
3. Cancer risk assessment
4. Diagnosis of aneuploidies
5. Prenatal diagnosis of congenital anomalies
6. Reproductive medicine
7. Identification of new syndromes
8. Genome-wide association studies

9. DNA expression Cancer/tumor characterization
10. Pharmacology and toxicology studies
11. Obesity research
12. DNA mi RNA Cancer research
13. Cell differentiation research
14. Protein Screening molecular interactions (protein protein, protein DNA/RNA, protein lipids, protein small molecule interactions)
15. Profiling and quantification of protein expression
16. Monitoring posttranslational modifications
17. antibody Profiling and quantification of protein expression
18. Evaluation of functional protein pathways
19. Enzymatic Evaluation of the activity of phosphatases, kinases,
    cysteine, proteases, and serine hydrolases
20. Evaluation of kinase inhibitors
21. Reverse protein Validation of biomarkers of survival prediction for pancreatic cancer patients receiving gemcitabine treatment
22. Drug development

Perhaps the most promising area of application of DNA microarray technology in clinical microbiology is the use of low- or middle-density microarrays for the simultaneous assessment of large numbers of microbial genetic targets [3,4]. Specific microbial gene amplification by either a broad-range or a multiplex PCR prior to microarray analysis enhances test sensitivity. The amplification of universal microorganism targets by broad-range PCR followed by sequencing analysis has been considered a standard procedure[5.] However, microarrays have emerged as potential tools for bacterial detection and identification given their high parallelism in screening for the presence of a wide diversity of genes. The most commonly used gene targets have been the 16S bacterial and 28S fungal and intergenic transcribed spacers (ITSs) in rRNA genes, and microarray technology has been incorporated to compensate for the time-consuming sequencing identification procedure [5]. An oligonucleotide microarray targeting the 16S rRNA gene was developed for the detection of a panel of 40 predominant human intestinal bacterial pathogens in human fecal samples[6] . Assays using broad-range PCR incorporated with microarrays have been shown to allow rapid bacterial detection and identification with positive blood cultures[7,8]. A similar procedure was developed and used for the rapid diagnosis of bloodstream infections caused by common bacterial pathogens in the paediatric and general populations[9,10] . PCR amplification, in combination with an oligonucleotide microarray, was used to identify Bacillus anthracis based on the rRNA ITS region[11].

Khodakov et al. described a novel microarray-based approach for the simultaneous identification and quantification of human immunodeficiency virus type 1 (HIV-1) and hepatitis B and C viruses in donor plasma specimens[12]. Bøving et al. reported the development of a novel multiplex PCR with product detection by the Luminex suspension array system covering a panel of

bacterial and viral pathogens causing meningitis. This system detected and identified nine microorganisms including Neisseria meningitidis, Streptococcus pneumoniae, E. coli, Staphylococcus aureus, L. monocytogenes, Streptococcus agalactiae, herpes simplex virus types 1 and 2, and varicella zoster virus directly from cerebrospinal fluid[13]. Respiratory infections caused by a panel of bacterial, viral, and fungal pathogens usually present with similar signs and symptoms that are nearly indistinguishable by clinical diagnosis. Simultaneous testing for all possible pathogens is an efficient means to obtain a conclusive result. In addition, assaying for all potential pathogens may yield information regarding possible coinfections or induced secondary infections. The first promising respiratory microarray system was described in 2002, which incorporated 1,600 unique 70-mer-long oligonucleotide probes covering approximately 140 viral genome sequences [14,15]. This Viro Chip system was used to identify the severe acute respiratory syndrome virus as a coronavirus16, for the discovery of a human parainfluenza virus type 4 infection associated with respiratory failure 17and human coronavirus and rhinovirus in nonasthmatic patients18 , and for the diagnosis of a human metapneumovirus causing critical respiratory illness19 .

The capacity of microarrays to accommodate high probe element densities and the potential use of several multiplex pools of primer pairs permit the development of pan-pathogen-specific probe arrays that will encompass other, primarily bacterial, causes of CNS infection, in addition to viruses. Single-format pan-pathogen-specific microarrays will contribute significantly to the speed and accuracy of laboratory diagnosis. This will have a beneficial impact on clinical care and hospital economics. This study mentioned below has demonstrated the simplicity, accessibility, and applicability of such an approach in the context of CNS infections and is the first time that the diagnostic power of a microarray linked to multiplex PCR amplification of multiple pathogens has been demonstrated in a clinical laboratory.

Yury S. Boriskin[20] has constructed a low-density, high-resolution diagnostic DNA microarray comprising 38 gene targets for 13 viral causes of meningitis and encephalitis was constructed. The array has been used for the detection of multiplex PCR amplified viruses in cerebrospinal fluid (CSF) and non-CSF specimens. The clinical sensitivity, specificity, and negative and positive predictive values of the assay were 93, 100, 100, and83%, respectively, when the results were compared to those of the single-virus PCR, which was used as the “gold standard.” The microarray-based virus detection assay is qualitative and provides a single-format diagnostic tool for the detection of panviral CNS infections.)

Gene chip array for differentiation of mycobacterial species and detection of drug resistance[21]

SHI Xiao-chun, LIU Xiao-qing, XIE Xiu-li, etalselected 39 patients (54 clinical mycobacterium isolates), used gene chip array to identify the species ofthese isolates and detect drug resistance to isoniazid and rifampin in Mycobacterium tuberculosis isolates. Among these 39 patients whose mycobacterium culture were positive, 32 patients' isolates were identified as Mycobacterium tuberculosis, all of them were clinical infection. Seven patients' isolates were identified as non-tuberculosis mycobacterium. Isoniazid resistance was detected in two tuberculosis patients, while rifampin resistance was detected in one tuberculosis patient; there was another patient whose Mycobacterium tuberculosis isolate was resistant to both isoniazid and rifampin. So they concluded that the Gene chip array may be a simple, rapid, and reliable method for the identification of most mycobacterial species and detection of drug resistance in Mycobacterium tuberculosis and it may have a great potential for clinical application.

DNA microarray also provides information related to the presence of antibiotic markers and pathogenicity regions. The use of a DNA probe array for species identification and detection of rifampin resistance in M. tuberculosis has been described Troesch et al., [22]1999. Seventy mycobacterial isolates from 27 different species and 15 rifampin-resistant strains were tested. A total of 26 of the 27 species were correctly identified as well as all of the rifampin-resistant mutants.

Rick W. Ye[23], Tao Wang, Laura Bedzyk, had reviwed the topic on Applications of DNA microarrays in microbial systems where they have mentioned about the specific application

Specific applications includes

1. Determination of virulence factors of microbial pathogens

Many genes associated with virulence are regulatedby specific conditions. to determine the candidate virulence factors is to investigate the genome-wide gene expression profiles under relevant conditions. In a genome comparison study among H. pylori strains, a class of candidate virulence genes was identified by their coinheritance with a pathogenicity island by Salama et al24.,2000. The whole genome microarray of H. Pylori was also shown to be an effective method to identify differences in gene content between two H. Pylori strains that induce distinct pathological outcomes Israel et al[25]., 2001.. It is demonstrated that the ability of H. pylori to regulate epithelial cell responses related to inflammation depends on the presence of an intact cag pathogenicity island.

2. Host responses to pathogens or resident microflora

The host transcriptional profiles during the interaction of B. pertussis with a human bronchial epithelial cell line BEAS-2B were investigated using high-density DNA microarrays by Belcher et al., 2000.(26)The early transcriptional response to this pathogen is dominated by the altered expression of cytokines, DNA-binding proteins, and NFkB-regulated genes.It was found that B. pertussis induces mucin gene transcription by

BEAS-2B cells and then counters this defense by using mucin as a binding substrate. This result indicates the host defensive and pathogen counter-defensive strategies. A DNA microarray was also used to identify the host genes that were differentially expressed upon infection by P. aeruginosa to the A549 lung pneumocyte cell line as studied by Ichikawa etal., 2000.27. Differential expression of genes involved and in various cellular functions was found, and one of those genes encodes the transcription factor interferon regulatory factor

Both experiments with B.pertussis and P. aeruginosa demonstrate that host genomic transcriptional profiling, in combination with functional assays to evaluate subsequent biological events, provides insight into the complex interaction of host and human pathogens In addition to the studies of host response to pathogens, DNA microarrays were used to investigate the global intestinal transcriptional responses to the residential colonization of Bacteroidesthetaiotaomicron, a prominent component of the normal mouse and human intestinal microflora as studied by Hooper etal., 2001.[28].

3. Gene expression profiles of drugs, inhibitors and toxic compounds

Exploring the gene expression profiles with DNA microarrays may reveal information on the mode of action for drugs, inhibitors or toxic compounds. DNA microarray hybridization experiments have been conducted in M.tuberculosis to explore the changes in gene expression induced by the antituberculous drug isoniazid. Wilson et al.[29], 1999. INH selectively interrupts the synthesis of mycolic acids, which are branched b-hydroxy fatty acids. Microarray experiments showed that isoniazid induced several genes that encode proteins that are physiologically relevantto the drug's mode of action, including an operonic cluster of five genes encoding type II fatty acid synthase enzymes and fbp C, which encodes trehalosedimycolyl transferase. Insights gained from this approach may define new drug targets and suggest new methods for identifying compounds that inhibit those targets.

In addition to the alternation in gene expression patterns related to the drug's mode of action, drugs can induce changes in genes related to stress responses that are linked to the toxic consequences of the drug. The secondary effects of a drug may reveal information on the potential resistance mechanism, which may help design drugs that have less side effects but have high efficacy by reducing the bacterium's ability to neutralize the drug. Each type of compound often generates a signature pattern of gene expression. A database populated with these signature profiles can serve as a guide to elucidate the potential mode of action as well as side effects of uncharacterized compounds

4 Analysis of microbial evolution and epidemiology; DNA microarrays can be used to explore the variability in genetic content and in gene expression profiles within a natural population of the same orrelated species and between the ancestor and the descendents. As a

result, it provides very rich information on the molecular basis of microbial diversity, evolution and epidemiology. Genomes within the species of M. tuberculosis have been compared with a high density oligonucleotide microarray to detect small-scale genomic deletions among 19 clinically and epidemiologically well-characterized isolates Kato-Maeda30 et al., 2001. This study reveals that 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 and toxic compounds tends to diminish their pathogenicity.

5. Pathway engineering and process optimization

According to RW.etal31information obtained by the DNA microarrays can help pathway engineering and process optimization in several ways. i. Regulatory circuitry and coordination of gene expression among different pathways under different growth conditions can be measured by DNA microarray. ii. The physiological state of the cells during fermentation can be assessed by the genome-wide transcriptional patterns. iii. DNA microarray can help identify genes involved in a production process if they are coregulated. iv. The differences in genetic contents and expression profiles between wild-type and improved strains can be compared. v. The actual array data can be incorporated into the mathematical models to describe a cellular process. Finally, general applications of DNA microarray technology to understand microbial physiology will continue to generate very large amounts of information that will ultimately benefit the pathway engineering and fermentation optimization effort. Current research in using array information in pathway engineering and bio processing is at its early stage. Arrays containing genes involved central metabolism, key biosyntheses, some regulatory functions and stress response have been used to investigatethe metabolic responses to protein over production and metabolic fluxes in E. coli Oh and Liao,2000a,b;[32,33,] Gill et al., 2001.

Gene array analysis was also used as a tool to investigate the differences in the expression levels for 30 genes involved in xylose catabolism in the parent, strain B, and the engineered strain, KO11 Tao et al.[34], 2001.. Increased expression of genes involved in xylose catabolism is proposed as the basis for the increase in growth rate and glycolytic flux in ethanologenic KO11.

Application of Microbial Diagnostic Microarrays for Pathogen Detection in Food, Environmental and Water Sample

Wang and co-workers [35] reported on the development and application of a 16S rRNA gene-based microarray for the detection of food-borne pathogens. Twenty-eight short oligonucleotide probes were designed (including positive and negative control probes) based on 128 bacterial 16S rDNA sequences (ranging from 1 to >10 sequences per targeted species). The specificity of the microarray was validated using more than 200 strains of target organisms. Since microarray could not differentiate between strains belonging

to Shigella spp./E. coli and Salmonella spp. a second microarray, based on species specific virA and inv A genes, was developed. microarray results agreed with those obtained by employing conventional methods. The absolute limit of detection was determined with spiked food samples and was reported to be at the level of 101 to 103 cfu/g food. A 16S rRNA gene-based MDM for the detection of wastewater bacterial pathogens was developed by Lee and co-workers [36]. To demonstrate the applicability of the proposed detection system the authors used wastewater samples collected from two municipal waste water treatment plants (both prior and after the disinfection step). Microarray results were compared to those obtained by species-specific TaqManq PCR and in general good agreement between both methods was observed. A major drawback of 16S rRNA gene-based arrays is the limited differentiation potential of the 16S rRNA marker gene.

Cremonesi and co-workers [37] developed a 16S rRNA gene-based microarray employing a ligation detection reaction (LDR). This approach utilizes the discriminatory power of ligase that joins ends of two discriminatory probes, thus allowing single nucleotide differentiation. Validation results confirmed the high differentiation potential of the LDR approach. Results obtained with the microarray correlated well with conventional, microbiological analysis, although the array detected contamination with multiple pathogens, which was not the case with standard methods. However, one disadvantage of the LDR system is the high cost of the probes. Microarray sensitivity was shown to be target-specific and ranged between 0.001 and 0.1 nggDNA and 104 cfu/mL in a pure culture. Utilization of more specific alternative marker genes (e.g., virulence or toxin genes) allows for additional typing of the detected microorganisms. For example, Call and co-workers [39] reported on the development of a microarray for the detection and typing of E. coli O157:H7

microarray which was established on the ArrayTube platform and included 39 virulence, 7 bacteriocin and 15 control short oligonucleotide probes. Subsequently, the microarray was used to type human and animal clinical E. coli isolates and the results mostly matched clinical diagnosis. Several isolates displayed a novel combination of genes. A more comprehensive system, based on long oligonucleotide probes, was described by Bruant and co-workers[40]. This microarray enabled the detection of E. coli virulence genes and antimicrobial resistance genes. After detailed validation with a set of reference strains the microarray was used for a screening of E. coli in river waters[41].Different Salmonella spp. serotyping microarrays were developed. The Salmonella PremiTest employs a ligation detection reaction and targets different, undisclosed genomic loci. Wattiau and co-workers reported on two performance studies [42,43]. In the first one 754 Salmonella strains belonging to 58 different serovars were analyzed. By classical serotyping 685 tested isolates (90.8%) could be identified. Performance of the Premi Test was influenced by the quality of DNA extraction (crude extracts vs. purified gDNA) and better

results (714 typeable isolates, 94.7%) were obtained with purified gDNA. Remaining isolates yielded either non-interpretable (19, 2.5%), dual (16, 2.1%) or wrong (5, 0.6%) results. The second study included 443 Salmonella strains. Eighty-four unique profiles were identified with the Premi Test, whereas classical serotyping identified 62 serovars. The sensitivity issue of long oligonucleotide microarrays was also addressed by Sou and co-workers [44]. The developed microarray was limited to only four highly relevant food-borne pathogens, E. coli O157:H7, Salmonella enterica, Listeria monocyto genes and Campylobacter jejuni and encompassed 14 virulence genes (on average 2 per pathogen). This allowed for the implementation of a multiplex PCR amplification in the analytical chain and resulted in the absolute sensitivity of 0.1 pgg DNA, corresponding to approximately 20 genome equivalents. An additional advantage of PCR-based amplification is enhanced specificity

MDMs(microbial diagnostic microarray) for Typing of Microorganism Anjum and co-workers [45] developed an E. coli pathotyping microarray.

More comprehensive antimicrobial resistance MDMs were later described for gram-positive [46] and gram-negative [47] bacteria. Both systems were developed on the Array Tube platform and DNA amplification/labelling protocols were selected to enable a high degree of multiplexing (randomly primed polymerization and linear amplification). The MDM developed by Perreten and co-workers [46] targets 90 antimicrobial resistance genes (coverage: 1 to 2 probes per gene). The validation with 36 strains carrying specific antimicrobial resistances allowed testing ofthe sensitivity and specificity of 125 probes (out of 137) and the microarray results corresponded to the phenotype.

Forty-seven antimicrobial resistance genes were targeted with the microarray developed by Batchelor and co-workers [47] and the system was validated with a set of selected reference strains. PCR was used as a reference method and correlation between two methods was 98.8%.

DNA microarray was used to detect and identify DNA from 14 fungal pathogens (Aspergillusfumigatus, Aspergillusflavus, Aspergillusterreus, Candida albicans, Candida dubliniensis, Candida glabrata, Candida lusitaniae, Candida tropicalis, Fusariumoxysporum, Fusariumsolani, Mucorracemosus, Rhizopusmicrosporus, Scedosporiumprolificans, and Trichosporonasahii) in blood, bronchoalveolar lavage, and tissue samples from high-risk patients.Birgit Spiess,et al 48. The assay combines multiplex PCR and consecutive DNA microarray hybridization. PCR primers and capture probes were derived from unique sequences of the 18S, 5.8S, and internal transcribed spacer 1 regions of the fungal r RNA genes. Hybridization with genomic DNA of fungal species resulted in species-specific hybridization patterns. By testing clinical samples from 46 neutropenic patients with proven, probable, or possible IFI or without IFI, we detected A. flavus, A. fumigatus, C. albicans, C. dubliniensis, C. glabrata, F. oxysporum, F. solani, R. microsporus, S. prolificans, and T. asahii.

The assay detected genomic DNA from 14 fungal pathogens from the clinical samples, pointing to a high significance for improving the diagnosis of IFI.

The established multifungal DNA microarray detects clinically relevant fungal pathogens specifically and at low detection thresholds from noncultured clinical samples, detecting different fungal pathogens with one test. In view of the changing spectrum of clinically relevant and emerging fungal pathogens causing IFI, this new diagnostic approach meets urgent clinical needs, at least concerning the high-risk group of patients with hematologic malignancies.

Application of Microarrays in the detection of Parasitic Diseases

Due to the increasing reliance upon genetic tests for identification and differentiation, the low concentration or number of organisms required in causing the disease, and the often-found presence of multiple parasites in a single environmental or clinical sample, such methods would be ideally suited for the detection of parasites Traditional molecular methods work on a 'one gene - one experiment' basis and because of the limited throughput of such methods, the whole picture of gene function is difficult to obtain. Scientists have long studied gene expression as a hallmark of gene activities reflecting current cell conditions and response to host immune defense systems. These studies are cumbersome, technically demanding and time-consuming. Application of microarrays is new for parasites. Most of these applications are done to monitor the parasite gene expression, predict the functions of uncharacterized genes, probe the physiologic adaptations made under various environmental conditions, identify virulence-associated genes and to test the effects of drug targets. Similarly, by using host-specific gene microarrays, one can explore host response at the level of gene expression and provide a molecular description of the events that follow the parasitic infection. Host-specific gene profiling might also identify gene expression signatures unique for each pathogen or for specific life stage of the parasite. The best examples are vector-borne parasites like Plasmodium, Trypanosoma and Leishmania, in which genes expressed, during mammalian and insect host stages, have been elucidated.[49,50,51] Microarrays have also been successfully applied to understand the factors responsible to induce transformation from tachyzoite-to-bradyzoite and vice versa in Toxoplasma gondii .[52] In malaria and Trypanosoma cruzi , parasitic genes have been identified, which are expressed during insect phase and mammalian host stages.[53]

Yeast has been the micro-organism of choice for many research groups to analyse cell-cycle-associated gene expression and the effects of various environmental changes, such as osmotic shock, temperature shock, presence of DNA-damaging agents and growth in minimal or rich media (Table 1⇑). The new level of analysis provided by whole-genome expression profiling has revealed the complexity of the cellular

response to major changes in metabolism, exemplified by work on yeast diauxic shift De Risi et al., .[55] The expression levels of 1840 genes (30% of a total of 6116 genes tested) were found to be affected by the transition from anaerobic to aerobic growth. Similar complexity of gene expression in the transcriptional program was reported for yeast going through the mitotic cell cycle Cho et al., [56] or sporulation Chu et al., .[57] Many of the responsive genes that were identified had previously been designated as FUN. To understand the large amount of data created by microarrays, Mike Eisen developed a computer program to cluster genes according to their expression profiles Eisenet al.,.[58] Based on the important observation that functionally related genes show similar patterns of expression, identification of well-characterized genes that are co-expressed with FUN genes can give important clues towards function. Using this tool, Chu et al. [57] defined seven sequential temporal classes of genes induced during yeast sporulation.

One of the most impressive examples of the use of microarrays for bacterial research has been provided by recent work on Caulobactercrescentus Laubet al.,[59] The definition of the cell cycle of C. crescentus by microarray analysis revealed that 572 of 2966 genes (19·3%) were cell-cycle-dependent. Not only were a number of classes of cell-cycle-induced genes identified, but also the proportion dependent upon the global cell cycle regulator CtrA was recognized for the first time. This study led to recognition of the role of 11 novel sensor kinases and 5 new sigma factors. The identification of cascades of gene expression during the Caulobacter cell cycle is an important landmark for bacterial research.

Mammalian gene microarrays have recently been used to study host–pathogen interactions from the viewpoint of the host, by identifying gene expression patterns induced by the presence of a pathogen Manger etal [60]Several in vitro studies have explored the effects of infection on the mRNA expression profile of human cells. The effects of Listeria monocytogenes Cohen et al.,61Salmonella enterica Eckmannet al., [62]and Salmonella typhimurium (Rosenberger et al.[63],) have recently been reviewed by Cummings & Relman [64] .Briefly, these studies reveal a specific host response, which is modulated by different host factors (Rosenberger et al.,[63]). Other groups are using a more complex approach by comparison of the human cellular transcriptional signatures of pathogenic strains carrying well-defined mutations to get a more detailed view of the mechanisms underlying pathogen clearance (Manger etal[60]).

The application of microbial gene expression profiling is only limited by our imagination! Industrial applications of microarrays Gene expression profiling is being used to determine the effects of antibiotics, agrochemicals and pharmaceutical products on different organisms, and is being used in the search for new antimicrobials. Strategies for drug-target validation and the identification of secondary effects have been described previously (Rosamond & Allsop, 2000). Following

determination of the 'expression signature' of a wide range of compounds, the prediction of the mode of action of a novel compound becomes possible, simply on the basis of analysis of the transcriptional changes made by the drug. Large biotech companies are already using this approach to obtain cost-effective information, which avoids large-scale mode-of-action studies Microarrays can also be used to gain clues to gene function through looking at knockout mutants, particularly of predicted regulatory genes. This approach has already been successfully used by Winzeleret al. 65 to follow the growth of pools of 500 yeast knockout mutants under various environmental conditions. Each mutant was tagged with a unique oligonucleotide sequence (a 'molecular barcode') that was detected by hybridization to a custom-built microarray to determine growth conditions when certain mutants were unable to grow. This methodology combined with a massive parallel analysis of mapped mutants (Ross-Macdonald et al.,66 rapid route to determining the function of the FUN genes found in every microbial genome Hinton et al, [67]

Bacteria have been used for decades as sensitive biosensors for mutagenicity Maron etal[68] and this approach has recently been brought up to date.

Minnie Sarwal, Mei-Sze Chua etal,[69] had done a study on DNA microarray profiling which identified the molecular heterogenicity in acute renal allograft rejection.they had done a systematic study of gene-expression patterns in biopsy samples from normal and dysfunctional renal allografts. They found consistent differences among the gene-expression patterns associated with acute rejection, nephrotoxic effects of drugs, chronic allograft nephropathy, and normal kidneys. The gene-expression patterns associated with acute rejection suggested at least three possible distinct subtypes of acute rejection that, although indistinguishable by light microscopy, were marked by differences in immune activation and cellular proliferation. Since the gene-expression patterns pointed to substantial variation in the composition of immune infiltrates, they used immunohistochemical staining to define these subtypes further. This analysis revealed a striking association between dense CD20+ B-cell infiltrates and both clinical glucocorticoid resistance and graft loss. so systematic analysis of gene-expression patterns provides a window on the biology and pathogenesis of renal allograft rejection. Biopsy samples from patients with acute rejection that are indistinguishable on conventional histologic analysis reveal extensive differences in gene expression, which are associated with differences in immunologic and cellular features and clinical course. The presence of dense clusters of B cells in a biopsy sample was strongly associated with severe graft rejection, suggesting a pivotal role of infiltrating B cells in acute rejection.

So As we integrate the power of microarray analyses with our particular research interests, more creative applications are bound to arise.

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