

Special Article

## Probing Lymphocyte Biology by Genomic-Scale Gene Expression Analysis

ASH ALIZADEH,<sup>1,4</sup> MICHAEL EISEN,<sup>2,4</sup> DAVID BOTSTEIN,<sup>2</sup> PATRICK O. BROWN,<sup>3,5</sup>  
and LOUIS M. STAUDT<sup>1,5</sup>

Accepted: August 11, 1998

The identity and abundance of mRNA species within a cell dictate, to a large extent, the biological potential of that cell. Although posttranscriptional mechanisms modify protein expression in critical ways, cellular differentiation requires key changes in gene transcription, as evidenced by the potent phenotypes that result from disruption of transcription factor genes in mice. It is now possible to assess the mRNA profile of a cell globally using recently developed genomics techniques. This review focuses on the potential of cDNA microarrays to define gene expression in lymphoid cells, a field which is in its infancy. Examples of cellular activation genes and cytokine inducible genes discovered using this technology are presented but these represent only a taste of the fruit that this new technology will ultimately bear. Gene expression profiles should provide essential new insights into lymphocyte differentiation and activation, the pathogenesis of immune disorders, and the molecular abnormalities in lymphoid malignancies.

**KEY WORDS:** Microarray; gene expression; genome; lymphocyte activation; cytokines.

### TECHNOLOGIES FOR GLOBAL ANALYSIS OF GENE EXPRESSION

In the past several years, a variety of complementary technologies have been developed which can be used to

measure the expression of thousands of genes in parallel (1–10). In various guises, these technologies all involve the deposition of cDNAs or oligonucleotides representing a large number of genes onto a solid support in an ordered array (1, 11–16). A key insight was that these arrays could be hybridized with probes derived from complex mixtures of genes using hybridization methods commonly used in Southern blotting (17). Typically, the total complement of mRNA in a cell is used to create a radioactive or fluorescent probe that is then hybridized to an ordered array of DNA fragments. After washing, the signal bound to each element of the array is quantitated. The signal detected for a given gene on the array is linearly related to the abundance of the gene's mRNA in the starting mixture. Three formats of microarray analysis are currently in use: filter-based arrays of cDNAs (6), arrays of oligonucleotides synthesized on solid supports using photolithography techniques (5, 10), and glass slide microarrays of cDNAs (1–4, 7–9). This review focuses on glass slide microarrays since this technology has many attractive features and is the one we are using in our laboratories.

The first step in creating a cDNA microarray is the definition of the genes that are to be included on the microarray. Most microarrays include both "named" genes, for which full-length cDNAs have been accurately sequenced, and "anonymous" genes, which are defined solely by high-throughput, single-pass sequencing of random cDNA clones to generate expressed sequence tags [ESTs (18)]. Bioinformatic algorithms such as Unigene (19) group cDNA clones with common 3' ends into clusters which tentatively define distinct human genes. An ideal cDNA

<sup>1</sup> Metabolism Branch, National Cancer Institute, Bethesda, Maryland.

<sup>2</sup> Department of Genetics, Stanford University School of Medicine, Stanford, California.

<sup>3</sup> Howard Hughes Medical Institute and Department of Biochemistry, Stanford University School of Medicine, Stanford, California.

<sup>4</sup> These authors contributed equally to this work.

<sup>5</sup> To whom correspondence should be addressed at Metabolism Branch, NCI, Building 10, Room 4N114, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892; or e-mail: pbrown@cmgm.stanford.edu

microarray might therefore contain one representative from each Unigene cluster. In practice, given the current complement of 45,925 Unigene clusters (Unigene release No. 46), most microarrays contain at most one-third of the total Unigene set.

Next, a cDNA clone representing each desired gene is obtained for use as a template for polymerase chain reaction (PCR) amplification of the cDNA sequence. Practically, most of the cDNA clones that we are using are derived from the IMAGE cDNA Consortium Project (20) or Cancer Genome Anatomy Project (21). Using PCR primers complementary to the plasmid vector cloning site, the cDNA inserts from each clone are individually amplified in parallel, precipitated, and resuspended at a high concentration in microtiter plates. These amplified cDNA fragments are then "printed" onto a poly-L-lysine-coated glass microscope slide using a custom-built robot. The robot uses slotted metal pins which act like quill pen tips to draw up microliter amounts of DNA fragment. It then deposits nanoliter aliquots of each DNA fragment at defined positions on the glass slide to create a microarray with 100- to 200- $\mu\text{m}$  spacing of the spots. Current robots can print 96 DNA samples on each of 150 slides in roughly 50 min.

Glass slide cDNA microarrays can be used to compare the relative expression of each gene on the microarray in two cell types (Fig. 1A). mRNA from each cell type is used to synthesize first-strand cDNA, but a different fluorescent nucleotide is incorporated into each probe (usually Cy3 and Cy5 fluorochromes are used). The two cDNA probes are then mixed at a high concentration and hybridized to the microarray under conditions that are similar to those used in Southern blotting or *in situ* hybridization. Following washing at a high stringency, the fluorescent probe bound to each spot on the microarray is then quantitated using a scanning confocal microscope coupled with computerized image analysis. In the end, the relative expression of each gene in the two samples is provided by the ratio of the two fluorescent signals, following appropriate normalization and background subtraction.

The relative expression of genes in two samples can be assessed with a high accuracy: gene expression need only differ by twofold between the two cell types to be considered significantly different with 95% confidence. The absolute expression of a gene in a cell cannot be directly derived from the microarray data since hybridization to each microarray element will be differentially influenced by various factors including the gene sequence, secondary structure, and length of the cDNA fragment deposited on the microarray. This is not a serious problem since the relative expression of a gene

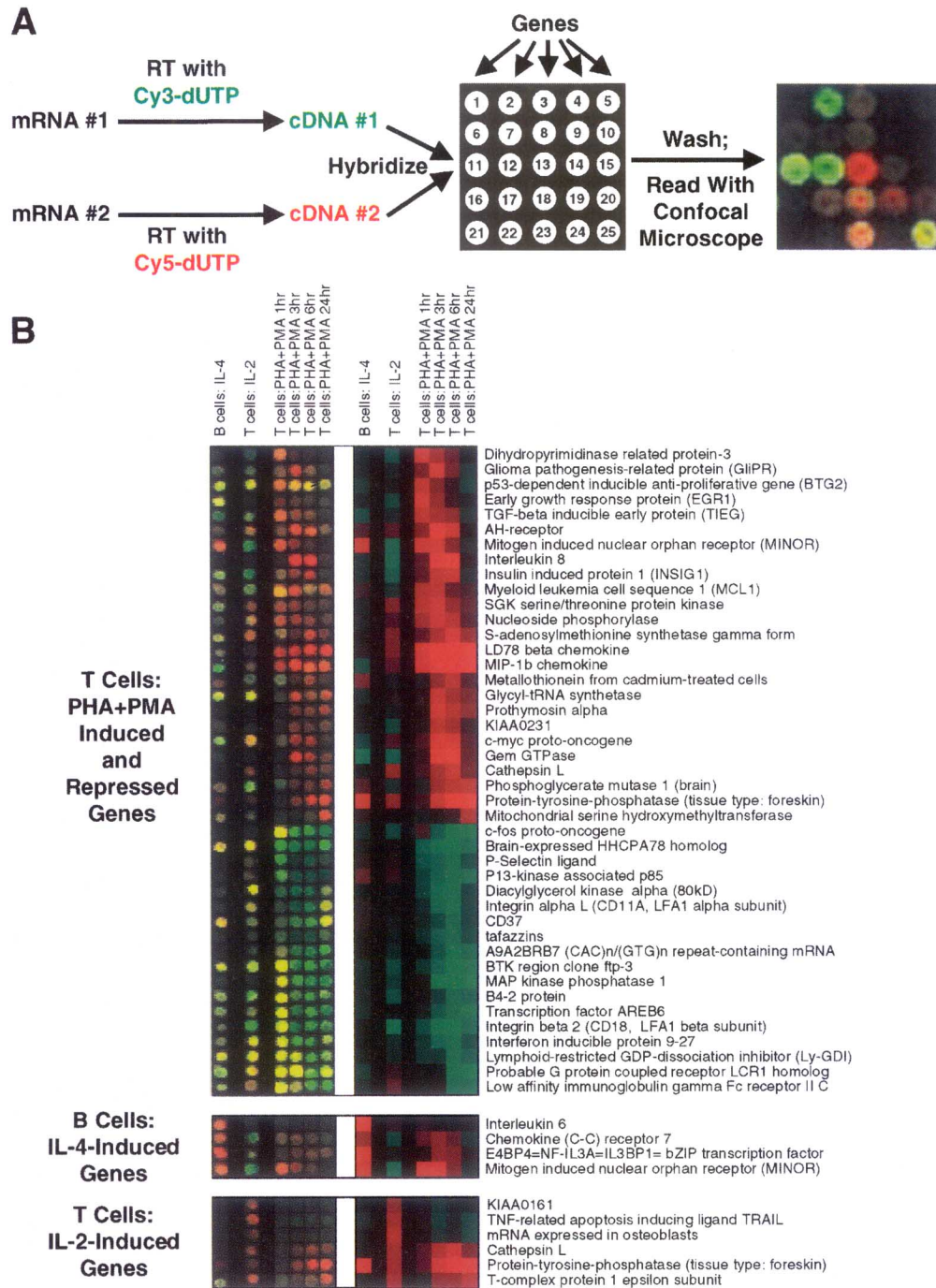
between two cell samples is the more critical parameter in defining the biological differences between cells.

#### EARLY VIEWS OF GENE EXPRESSION ON A GENOMIC SCALE

The first eukaryotic genome to be completely sequenced was from the yeast *Saccharomyces cerevisiae*. This genomic sequence set the stage for the first genome-wide study of gene expression using cDNA microarrays (7, 8, 10). Every open reading from *S. cerevisiae* was PCR amplified from yeast genomic DNA and used to create a microarray of 6400 elements. The first experiments have probed the consequences of nutritional shifts on gene expression. Coordinated changes in the expression of genes for metabolic enzymes involved in fermentation and respiration were observed as the yeast metabolized the glucose in the medium. To begin to understand the molecular circuitry underlying these changes, gene expression was compared between wild-type yeast and mutant yeast strains which are deficient in key transcription factor genes involved in this metabolic reprogramming. Several of the gene expression changes that were observed during glucose deprivation could be attributed to changes in the key transcriptional corepressor protein TUP1 (7).

Clearly, the yeast model system provides a tantalizing view of the potential insights that will be obtained with cDNA microarray analysis of gene expression in mammalian systems. Early cDNA microarrays of human genes have consisted of only ~100–1000 elements but have hinted at future riches from larger arrays. These experiments have readily identified genes that are induced or repressed by treatment with phorbol esters, interleukin-1 (IL-1)  $\beta$ , tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), and heat shock (4, 9). A specialized microarray of 96 genes relevant to inflammation was used to study gene expression in rheumatoid arthritis and inflammatory bowel disease (9). Many genes known to be expressed in rheumatoid synovium were detected (e.g., the cytokines TNF $\alpha$ , IL-1, IL-6, and G-CSF and the chemokines IL-8 and RANTES). In addition, the microarrays revealed the expression of several genes that had not been implicated in rheumatoid arthritis, such as the cytokine IL-3, the chemokine Gro $\alpha$ , the IL-1 converting enzyme (ICE), and the human matrix metalloelastase (HME) protease. HME could potentially mediate some of the articular destruction characteristic of this disease.

An early use of human microarrays in cancer biology used an 870-gene array to identify gene expression differences between a malignant melanoma cell line and a nontumorigenic variant of this line created by the



**Fig. 1.** (A) Schematic of cDNA microarray analysis of gene expression. (B) cDNA microarray analysis of gene expression changes induced in B lymphocytes by IL-4 treatment, in T lymphocytes by IL-2 treatment, or in T lymphocytes during mitogenic activation by PHA and PMA treatment.

introduction of a normal copy of chromosome 6 containing a putative tumor suppressor gene (3): 1.7% of the genes were expressed at significantly lower levels (>threefold) in the nontumorigenic cell line than in the

malignant cell line and 7.3% of the genes were at significantly higher levels. Among the genes that were more highly expressed in the malignant cell line was a surface protein gene (TRP1/melanoma antigen gp75)

which had previously been shown to be regulated during differentiation of melanoma cell lines *in vitro*. Conversely, a number of genes known to be induced by interferon- $\gamma$  were activated in the nontumorigenic cell line. The biological relationship of these gene expression changes to the putative tumor suppressor gene has not been established. Nevertheless, this study demonstrated that a biological process as complicated as malignant transformation involves changes in the expression of, at most, 10% of the genes. These early experiments are therefore useful guides to the future design of specialized cDNA microarrays that are enriched for genes that are variably expressed under diverse states of cellular activation, differentiation, and transformation.

#### A GLIMPSE OF THE FUTURE FROM LARGE HUMAN cDNA MICROARRAYS

Recent experiments in our laboratories have used human cDNA microarrays that include 10,000 or more of the estimated 60,000–100,000 human genes. One such microarray, manufactured by Synteni, Inc., is composed of 10,000 cDNAs: ~4150 representing “named” human genes and the rest representing distinct “anonymous” genes as defined by Unigene clusters. We have begun an analysis of gene expression in human B and T lymphocytes during cellular activation and/or stimulation with cytokines. This analysis is ongoing but a selection of the early data is given in Fig. 1B. All experiments were performed using human peripheral blood B or T lymphocytes purified to >98% homogeneity by magnetic cell sorting.

In one series of experiments, resting T lymphocytes were induced to enter the cell cycle by combined mitogenic treatment with phytohemagglutinin (PHA) and phorbol myristate acetate (PMA). Mitogenic stimulation of resting human peripheral blood T cells is a classic cell system in which many measurements of gene expression have been made (reviewed in (22, 23). This system is therefore a test case for large-scale human cDNA microarray analysis. mRNA was prepared prior to activation and at 1, 3, 6, and 24 hr after the addition of PHA/PMA. Four separate cDNA microarray experiments were performed. Each microarray was hybridized with a mixture of Cy3-labeled cDNA transcribed from mRNA from the untreated cells (pseudocolored green in Fig. 1B) and Cy5-labeled cDNA transcribed from one of the mRNA samples from activated T cells dye (pseudocolored green in Fig. 1B). In the left panel in Fig. 1B, the actual spots from the four microarrays have been rearranged to present each result for a given gene in a single row. For the sake of comparison with previously pub-

lished experiments, only named genes are shown in Fig. 1B; a large number of anonymously cloned genes were also modulated during mitogenic activation (A. Alizadeh *et al.*, unpublished). The right panel in Fig. 1B is a graphic depiction of the ratio of Cy5 and Cy3 fluorescence for the spots in the left panel: red squares represent high Cy5/Cy3 ratios and indicate induced genes, whereas green squares represent low Cy5/Cy3 ratios and indicate repressed genes. Black squares represent genes whose expression changed by less than 1.5-fold at the indicated time. The most highly induced gene was IL-8 (51-fold) and the most highly repressed gene was MAP kinase phosphatase-1 (20-fold).

The first gratifying aspect of these results was that many of the genes that were observed to be induced are well known to be involved in the transition from G0 to G1 in the cell cycle [e.g., early growth response protein 1 (EGR1), Gem GTPase, and *c-myc*]. Additionally, a number of the most induced genes were chemokines which are known to be transcriptionally induced in T cells following mitogenic stimulation (e.g., LD78, MIP-1 $\beta$ , and IL-8). Some genes had been shown previously to be activated in other cell systems but not during T cell mitogenesis (e.g., insulin-induced protein 1). Indeed, these and other cDNA microarray experiments have shown that some genes are highly responsive to a panoply of agents, whereas most other genes show very stable expression levels. The cDNA microarray data in Fig. 1B illustrate beautifully the ordered progression of gene expression changes that take place during T cell mitogenesis: *c-fos* and dihydropyrimidinase-related protein-3 are representative of the immediate early class of genes that were induced within 1 hr, *c-myc* is characteristic of the delayed early genes that were induced only after 3 hr, and the nuclear-encoded mitochondrial serine hydroxymethyltransferase gene is an example of a gene that was expressed at high levels only after 1 day of T cell activation. An interesting gene not known previously to be induced during lymphocyte activation is the aryl hydrocarbon receptor (AH-receptor). The AH-receptor is a basic helix–loop–helix transcription factor that mediates signaling by toxins such as dioxin. Dioxin is known to be lymphotoxic, and the fact that the AH-receptor is highly induced during T cell activation suggests that activated T cells may be preferentially targeted by dioxin.

Less is known about genes that are repressed during T cell activation. Our cDNA microarray analysis revealed a large number of such genes (Fig. 1B). Three genes encoding adhesion molecules were coordinated repressed during T cell activation (selectin P ligand, integrin  $\alpha$ -L (LFA-1  $\alpha$  subunit; CD11A) and integrin  $\beta$ 2

[LFA-1  $\beta$  subunit; CD18]. All of these genes encode proteins that are involved in attachment of leukocytes to endothelium and extravasation into tissues. These genes were found to be maximally repressed at 3 and 6 hr following activation and then return to near-resting levels after 24 hr. This suggests that a window of time exists early during T cell activation in which the trafficking of the cell is inhibited. A second repressed gene that may illuminate T cell physiology is AREB, a transcription factor which represses the IL-2 promoter. Down-regulation of AREB may be necessary to allow for maximal IL-2 production in activated T cells.

Much work remains to determine the significance of these gene expression changes to normal T cell function. In particular, it will be important to investigate the changes in gene expression that accompany more physiologic activation of T cells in the context of antigen presenting cells and costimulation. The power of cDNA microarray data can also be extended by comparison of multiple microarray experiments involving different cell systems. For example, some but not all of the genes activated or repressed during mitogenic T cell activation are similarly modulated during serum stimulation of fibroblasts (Iyer *et al.*, unpublished results). Those genes which are selectively modulated during T cell activation are less likely to be involved in stereotypic events in cell cycle progression and more likely to mediate lymphoid-specific functions.

Despite the voluminous literature demonstrating myriad functional consequences of cytokine stimulation, the analysis of cytokine target genes is just beginning. Figure 1 presents a preliminary analysis of IL-4 target genes in B cells and IL-2 target genes in T cells. Human peripheral blood B cells were stimulated for 24 hr by cross-linking the surface immunoglobulin receptor with anti-IgM antibodies in the presence or absence of IL-4. Some IL-4-induced genes (chemokine C-C receptor 7, E4BP4, and MINOR) were also strongly or weakly induced during mitogenic activation of T cells. In contrast, the IL-6 gene was responsive only to IL-4 stimulation. The IL-4 receptor is known to transduce signals to the nucleus by two parallel pathways, one involving the Stat6 transcription factor and the other mediated by the signaling molecules IRS-1 and IRS-2. IRS-1 and IRS-2 signaling feeds into the MAP kinase signaling cascade, which is also strongly activated during mitogenic stimulation of T cells. Conversely, the Stat6 signaling pathway appears to be quite dedicated to IL-4 signaling. Based on these considerations, the cDNA microarray data suggest that IL-4 may activate IL-6 expression via Stat6 signaling, whereas the other IL-4-induced genes may be downstream of IRS signaling.

A particularly intriguing IL-4-induced gene was E4BP4, a B-ZIP transcription factor gene also known as NF-IL3A (24, 25). E4BP4 transactivates the IL-3 gene and is itself induced by IL-3 (25, 26). The ability of IL-3 to prevent programmed cell death in certain IL-3-dependent cell lines requires E4BP4 (26). Since IL-4 also has antiapoptotic activity in B lymphocytes (27, 28), it is conceivable that IL-4 induction of E4BP4 may play a role in its ability to prevent apoptosis.

To identify IL-2 target genes, peripheral blood T cells were stimulated for 72 hr with PHA, washed, and then incubated with IL-2 for an additional 16 hr. Some IL-2-responsive genes were also induced by mitogenic activation of T cells. A trivial explanation for this observation could be that mitogenic activation of T cells induces IL-2, which can then alter gene expression. Other genes seem to be specifically induced by IL-2, including the TNF-related apoptosis inducing ligand TRAIL. It has been shown previously that T cells simultaneously treated with both concanavalin A and IL-2 up-regulate TRAIL expression (29). The cDNA microarray data suggest that TRAIL is induced by IL-2 alone.

#### THE FUTURE OF GLOBAL ANALYSIS OF GENE EXPRESSION IN THE IMMUNE SYSTEM

Virtually every corner of immunological research will benefit from cDNA microarray analysis of gene expression. One present limitation in such an analysis is that the available microarrays, large as they are, are incomplete. Ultimately, every open reading frame from the human (and mouse) genome will be deposited on microarrays and we will truly have a genomewide view of gene expression. At present, however, many human genes have yet to be identified as evidenced by the continued increase in the number of Unigene clusters resulting from ongoing EST sequencing. Furthermore, current technology limits the number of cDNAs that can be deposited on a single glass microscope slide to ~15,000. Currently, therefore, there is considerable value in specialized microarrays that focus on genes preferentially expressed in a given cell type or which are of functional importance to a particular biological process.

Recently, we have devised the so-called "Lymphochip" cDNA microarray, a 13,000-element array that is enriched for genes of importance to the immune system. One starting point for the Lymphochip was a normalized cDNA library prepared from germinal center B lymphocytes. The Cancer Genome Anatomy Project has obtained 47,662 cDNA sequences from this library. These sequences fall into 10,665 Unigene clusters. Roughly

12% of these Unigene clusters represent novel human genes that have only been observed in the germinal center library. These novel cDNAs are all included on the Lymphochip, as are a number of cDNAs that were more frequently observed in the germinal center library than in other cDNA libraries. In addition, we have included a variety of lymphocyte activation genes and cytokine-responsive genes discovered during the first generation of experiments reviewed above. Finally, roughly 3000 named genes of functional importance to lymphocytes or to oncogenesis have been included.

We are currently using the Lymphochip to address a number of questions related to lymphocyte development and activation, in lymphoid deficiency disorders and in lymphoid malignancy. cDNA microarrays are ideal tools to understand the programs of gene expression that accompany differentiation of lymphocytes in response to antigenic stimulation or cytokines. Indeed, the rich literature defining precise functional stages of differentiation of lymphocytes *in vivo* and *in vitro* makes the immune system an attractive model system in which to view the global changes in gene expression that accompany differentiation.

In immune-mediated disorders, gene expression analysis may reveal previously unsuspected molecular heterogeneity among patients who carry the same diagnosis. This heterogeneity may confound attempts to map the genes responsible for these disorders. Even within families with one affected individual, it may be valuable to analyze gene expression in the lymphocytes of the unaffected relatives. If the immune disorder has a multigenic basis, the relatives may have a subset of the pathogenic alleles that is insufficient to produce frank disease but will nevertheless be manifest as gene expression abnormalities in lymphocytes. Thus, gene expression profiling can be viewed as an elaborate "phenotype" which may be important in discovering all the genes responsible for a multigenic disease. In some cases, the underlying genomic lesions in immune-mediated diseases may reside in the regulatory regions, rather than the coding regions, of genes. As regulatory elements can often be difficult to identify, standard approaches to the identification of the causative mutations may be aided by knowledge of the gene expression abnormalities in immune cells from these patients.

Global analysis of gene expression promises to make especially important contributions to the understanding of lymphoid malignancies. Despite the great success in identifying genes altered by chromosomal translocations and deletions in cancer, much less is known about the downstream consequences of these genomic abnormalities on the biology of the cell. For example, gene

expression analysis may reveal the downstream targets of important transcription factors such as BCL-6, which are frequently translocated in lymphoid malignancies but also play key roles in lymphoid development (30). Gene expression patterns may reveal subtypes of lymphomas or leukemias that have a distinct biology and distinct responsiveness to therapy. By coupling cDNA microarray analysis with clinical trials, it should ultimately be possible to predict which patients will respond to a particular treatment. This stratification should lead to more appropriate, individualized choices of therapeutic modalities.

Finally, it is conceivable that gene expression patterns in peripheral blood cells may be useful as sentinels of occult disease processes such as inflammation, infection, and malignancy (31). By virtue of the inherent recirculation of blood cells, it is conceivable that a signature of an underlying disease process may be evident in the gene expression profile of peripheral leukocytes. Similarly, gene expression in peripheral blood cells may show a telltale fingerprint of unnoticed exposure to environmental insults. Many pollutants such as dioxin produce their toxic effects on cells by activating transcription factors and modulating gene expression. Clearly, much work in the next few years will be required to evaluate the soundness of these proposals. Historically, though, new instruments of scientific measurement have often produced new views of the world. Genomic-scale analysis of gene expression promises to provide a new perspective on biology and medicine.

#### ACKNOWLEDGMENTS

The authors thank Larry Wahl, NIDR, NIH, for help in purification of human lymphocytes; Deval Lashkari and Dari Shalon, Synteni, Inc., for their assistance in providing cDNA microarrays for this work; and Vishy Iyer, Doug Ross, and Ed Cheng for sequencing. This work is supported in part by funds from the Howard Hughes Medical Institute and by a grant from the NHGRI to P.O.B. P.O.B. is an associate investigator of the Howard Hughes Medical Institute. A.A. was a Howard Hughes Medical Institute Research Scholar at the National Institutes of Health during this work.

#### REFERENCES

1. Schena M, Shalon D, Davis RW, Brown PO: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:467-470, 1995
2. Shalon D, Smith SJ, Brown PO: A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Res* 6:639-645, 1996

3. DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM: Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* 14:457–460, 1996
4. Schena M, Shalon D, Heller R, Chai A, Brown PO, Davis RW: Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. *Proc Natl Acad Sci USA* 93:10614–10619, 1996
5. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, Brown EL: Expression monitoring by hybridization to high-density oligonucleotide arrays [In Process Citation]. *Nat Biotechnol* 14:1675–1680, 1996
6. Pietu G, Alibert O, Guichard V, Lamy B, Bois F, Leroy E, Mariage-Sampson R, Houllatte R, Soularue P, Auffray C: Novel gene transcripts preferentially expressed in human muscles revealed by quantitative hybridization of a high density cDNA array. *Genome Res* 6:492–503, 1996
7. DeRisi JL, Iyer VR, Brown PO: Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–686, 1997
8. Lashkari DA, DeRisi JL, McCusker JH, Namath AF, Gentile C, Hwang SY, Brown PO, Davis RW: Yeast microarrays for genome wide parallel genetic and gene expression analysis. *Proc Natl Acad Sci USA* 94:13057–13062, 1997
9. Heller RA, Schena M, Chai A, Shalon D, Bedilion T, Gilmore J, Woolley DE, Davis RW: Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. *Proc Natl Acad Sci USA* 94:2150–2155, 1997
10. Wodicka L, Dong H, Mittmann M, Ho MH, Lockhart DJ: Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nat Biotechnol* 15:1359–1367, 1997
11. Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D: Light-directed, spatially addressable parallel chemical synthesis. *Science* 251:767–773, 1991
12. Southern EM, Maskos U, Elder JK: Analyzing and comparing nucleic acid sequences by hybridization to arrays of oligonucleotides: Evaluation using experimental models. *Genomics* 13:1008–1017, 1992
13. Fodor SP, Rava RP, Huang XC, Pease AC, Holmes CP, Adams CL: Multiplexed biochemical assays with biological chips. *Nature* 364:555–556, 1993
14. Maskos U, Southern EM: A study of oligonucleotide reassociation using large arrays of oligonucleotides synthesised on a glass support. *Nucleic Acids Res* 21:4663–4669, 1993
15. Southern EM, Case-Green SC, Elder JK, Johnson M, Mir KU, Wang L, Williams JC: Arrays of complementary oligonucleotides for analysing the hybridisation behaviour of nucleic acids. *Nucleic Acids Res* 22:1368–1373, 1994
16. Lipshutz RJ, Morris D, Chee M, Hubbell E, Kozal MJ, Shah N, Shen N, Yang R, Fodor SP: Using oligonucleotide probe arrays to access genetic diversity. *Biotechniques* 19:442–447, 1995
17. Southern EM: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–517, 1975
18. Adams MD, Dubnick M, Kerlavage AR, Moreno R, Kelley JM, Utterback TR, Nagle JW, Fields C, Venter JC: Sequence identification of 2,375 human brain genes. *Nature* 355:632–634, 1992
19. Schuler GD: Pieces of the puzzle: Expressed sequence tags and the catalog of human genes. *J Mol Med* 75:694–698, 1997
20. Lennon G, Auffray C, Polymeropoulos M, Soares MB: The I.M.A.G.E. Consortium: An integrated molecular analysis of genomes and their expression. *Genomics* 33:151–152, 1996
21. Strausberg RL, Dahl CA, Klausner RD: New opportunities for uncovering the molecular basis of cancer. *Nat Genet* 15 Spec No: 415–416, 1997
22. Kelly K, Siebenlist U: Immediate-early genes induced by antigen receptor stimulation. *Curr Opin Immunol* 7:327–332, 1995
23. Ullman KS, Northrop JP, Verweij CL, Crabtree GR: Transmission of signals from the T lymphocyte antigen receptor to the genes responsible for cell proliferation and immune function: The missing link. *Annu Rev Immunol* 8:421–452, 1990
24. Chen WJ, Lewis KS, Chandra G, Cogswell JP, Stinnett SW, Kadwell SH, Gray JG: Characterization of human E4BP4, a phosphorylated bZIP factor. *Biochim Biophys Acta* 1264:388–396, 1995
25. Zhang W, Zhang J, Kornuc M, Kwan K, Frank R, Nimer SD: Molecular cloning and characterization of NF-IL3A, a transcriptional activator of the human interleukin-3 promoter. *Mol Cell Biol* 15:6055–6063, 1995
26. Ikushima S, Inukai T, Inaba T, Nimer SD, Cleveland JL, Look AT: Pivotal role for the NFIL3/E4BP4 transcription factor in interleukin 3-mediated survival of pro-B lymphocytes. *Proc Natl Acad Sci USA* 94:2609–2614, 1997
27. Illera VA, Perandones CE, Stunz LL, Mower DA, Jr., Ashman RF: Apoptosis in splenic B lymphocytes. Regulation by protein kinase C and IL-4. *J Immunol* 151:2965–2973, 1993
28. Panayiotidis P, Ganeshaguru K, Jabbar SA, Hoffbrand AV: Interleukin-4 inhibits apoptotic cell death and loss of the bcl-2 protein in B-chronic lymphocytic leukaemia cells in vitro. *Br J Haematol* 85:439–445, 1993
29. Screaton GR, Mongkolsapaya J, Xu XN, Cowper AE, McMichael AJ, Bell JI: TRICK2, a new alternatively spliced receptor that transduces the cytotoxic signal from TRAIL. *Curr Biol* 7:693–696, 1997
30. Staudt LM, Dent AL, Shaffer AL, Yu X: Regulation of lymphocyte cell fate decisions and lymphomagenesis by BCL-6. *Int J Immunol* (in press), 1998
31. Brown PO, Hartwell L: Genomics and human disease—Variations on variation. *Nat Genet* 18:91–93, 1998