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APPLYING FUNCTIONAL GENOMICS TO NEUROPSYCHOPHARMACOLOGY

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> "The time has come," the Walrus said, "to talk of many things." Lewis Carroll

The time has come indeed. The sequencing of the human genome and the genomes of a number of other species subject to research (1–6) have paved the way for new sorts of studies. Soon researchers will be able to look at the response of every human gene to specific manipulations or developmental events at multiple time points. This will require a new mindset. Researchers will not necessarily be testing specific hypotheses as they have done in the past. Instead, they will rely on the emergence of patterns and systematic features in their data sets (and those of others) to describe the phenomena being examined. Such patterns may hint at functions of collections of genes, the interactions of their products, and their importance in physiologic and pathologic processes. This chapter introduces array technology, discusses the sorts of experiments that can now be done with it, and suggests future advances. Several reviews have already been published on this subject, and the reader should refer to them for additional information (7–15). In addition, university, government, and commercial Web sites are valuable sources of news, background material, reagents, arrays, software, and instrumentation (16–33).

EARLY STUDIES OF GENE EXPRESSION

The human genome is composed of approximately 3 billion DNA nucleotides encoding more than 100,000 genes (16). Each of these genes must be turned on or off in the right cells at the right time for an individual to develop and prosper. The genes that are ultimately expressed in a particular tissue define it. That is, brain is brain and liver is liver because of the particular collections of transcripts found in their respective cells. Brain, however, is extraordinarily heterogeneous. It has been estimated that nearly half of the genes in the genome are expressed there, distributed among the different neuronal and glial populations.

Genes are made of DNA, a nucleic acid polymer that has deoxyribose as its sugar backbone. Each sugar moiety in the chain has a base (adenine, A; cytosine, C; guanine, G; or thymine, T) attached to it. DNA exists as a double-stranded helix. The two antiparallel strands are bound to one another because their sequences are complementary—that is, the opposing bases are held together by hydrogen bonds, A to T and C to G. Similarly, messenger RNA (mRNA), the transcription product of the coding region of each gene, is complementary to the DNA strand from which it was copied and can bind to it. Northern blotting, the first method developed for detecting single mRNA species in a cellular extract, is based on this phenomenon. In this technique, RNA samples are fractionated by agarose gel electrophoresis, and the RNA bands are transferred (blotted) onto nitrocellulose membranes. Single RNA species can then be detected by hybridizing a radiolabeled DNA to the blot that is complementary to the RNA of interest.

In the past, the responses of cells or organisms to environmental cues were studied on a small scale, one gene or pathway at a time. Initially, Northern blotting was used to examine the abundance of specific mRNA species. Subsequently, other methods were chosen because they were simpler and more sensitive, such as reverse-transcriptase polymerase chain reaction (RT-PCR) (34), or because they were more comprehensive, such as SAGE (serial analysis of gene expression) (35,36). These techniques provide useful information, but they are tedious, time-consuming, and expensive to employ.

In the last 5 years, spurred by the availability of large volumes of genomic and cDNA (EST [expressed sequence tag]) sequence data from a variety of organisms, investigators have developed methods to study mRNA profiles in cells and tissues by means of large-scale, high-throughput, parallel methods. In the future, it would be helpful to look at protein and small molecule profiles as well, but the reagents required (panels of antibodies, for example) are difficult to

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assemble and utilize. Even though there is not a one-to-one correspondence between the level of a particular transcript in a cell and that of its translation products, a great deal can be learned by performing mRNA expression profiling.

GENE EXPRESSION ARRAYS

Expression profiling relies on large ordered collections of cDNAs immobilized on glass (microarrays) or synthetic oligonucleotides immobilized on silica wafers or chips (probe arrays). Both of these methods are the conceptual descendants of target nucleic acids immobilized on filters or membranes and detected with complementary radioactive probes. While filter-based systems are commercially available, reasonably priced, and fairly easy to use, it is clear that they will be preempted by glass or chip arrays developed with fluorescent probes. Glass arrays printed on microscope slides are now much cheaper to employ than chips, and many universities and research institutes have already built facilities for printing and probing such arrays. So it is worth discussing the uses to which such arrays have already been put, and the uses to which neuropsychopharmacologists could put them.

Few investigators have used arrays to study brain so far, preferring instead to look at mammalian cell lines and tumors (37–44). In addition, many workers have focused on yeast (45,46) or prokaryotes (47,48) because their genomes are small and have been completely sequenced. Consequently, every protein-encoding gene can be arrayed and examined. This will be true of human and mouse arrays in the not-too-distant future. Meanwhile, experiments can be done with the arrays that are available. These have between a few thousand and a few tens of thousands of elements, and with them we can begin to catalogue genes expressed in regions of the developing and adult nervous system, and to look for alterations in expression patterns associated with pathologic states or physiologic/pharmacologic manipulations (49). Consider, for example, the work that could be done to understand the mechanism(s) of action of selective serotonin reuptake inhibitors (SSRIs) and the reason for their delayed onset of action in depressed patients. As is known, SSRIs increase the availability of serotonin (5-hydroxytryptamine, 5-HT) to presynaptic and postsynaptic receptors, of which there are at least 14 subtypes (50). Among these, 5-HT_{1A} receptors on serotoninergic raphe neurons are thought to play a key role in regulation 5-HT release (51). 5-HT_{1A} agonists, which are used to treat anxiety, inhibit serotonin secretion. Conversely, desensitization of 5-HT_{1A} receptors, which could result from elevated 5-HT levels in the synaptic space following SSRI administration, may have the opposite effect—an increase in 5-HT release by raphe neurons, and chronic stimulation of 5-HT receptors in regions such as the hippocampus, amygdala, and septum. Despite all the research that has been done to date, the identity of the structures and biochemical alterations that are responsible for the antidepressant actions of SSRIs is still moot.

Array experiments will allow investigators to explore the serotoninergic system in a way that is model independent and comprehensive, and the experiments should become easy and cheap enough to perform to permit varying many parameters and comparing many conditions.

Initially, regional responses to a single dose of SSRI at a variety of times in one mouse strain might be examined. Subsequently, mouse strains that differ in their behavioral reactions to SSRIs could be examined; knockout mice known to have altered responses to SSRIs (e.g., 5-HT_{1A} receptor knockouts) could be studied; and drugs that resemble, facilitate, or inhibit the behavioral effects of SSRIs could be investigated. Mice would be better to use for this work than rats as of now because very big mouse arrays are available as are genetically manipulated animals and a variety of well-characterized inbred strains. Unfortunately, mice have small brains, and obtaining samples of minute regions (e.g., raphe nuclei) large enough to make sufficient RNA for labeling is difficult. Help is on the way, though. Better labeling methods, dyes, and detection devices are being developed. In fact, the amount of total RNA needed for an array experiment has already fallen well below 1 μg, and should approach 1 ng shortly.

Each array experiment will let an investigator look simultaneously at thousands of transcripts including those encoding enzymes involved in energy metabolism, receptors, G proteins, second messengers, and ion channels, to name a few. In addition, there will be many species represented on big arrays, the actions of which are unknown. The major task will be to assign them functions (see below).

THE DEVIL IS IN THE DETAILS

Methods for making and probing arrays and analyzing array data have developed quickly. In spite of this, the supply of arrays has not kept up with demand, and demand should increase dramatically if the goal of using arrays is to compare many conditions and then mine the data systematically for patterns of gene expression. Thus, as stated earlier, costly products are unlikely to gain wide acceptance, and glass slide arrays are likely to be most commonly employed. For this reason, I now discuss their production and use.

Large collections of cDNAs and their sequences are now in the public domain. Some sets of cDNAs have been sequence verified and are ideal to use for preparing arrays; others have not been validated and are less useful. To make arrays, plasmid DNA is prepared from gridded sets of clones to be printed, and (typically) the 3′ end of each cDNA is amplified by PCR. The purified PCR products are then spotted using a robotic arrayer. It is possible to fabricate one's own arrayer (18), but many investigators will prefer

to buy an instrument or obtain arrays from core facilities or commercial vendors. Many thousands of 100-μM spots can be printed on a single glass microscope slide (see ref. 10 for details).

It is important to realize that array experiments do not permit measurement of the amount of each RNA that is present in a sample. This is because the relationship between the amount of transcript in a mixture and the intensity of the fluorescent spot it produces is a complex one—influenced by labeling efficiency, hybridization and wash conditions, and the sequence, quality, and quantity of the printed DNA. Thus, microarrays are typically employed to measure the relative abundances of RNA species in two or more extracts. To achieve this goal, in a two-sample experiment the RNAs are separately labeled with dyes of different colors, and then the products are mixed and hybridized to the arrayed spots (Fig. 23.1). After washing, the slides are scanned with a "reader" or "scanner" and the intensities of the fluorescent signals produced by the two separate dyes are determined spot by spot. Following background subtraction and "normalization" of the signals from the two channels (see below), a ratio of intensities of the two colors is determined for each spot, and the relative abundance of the two input RNAs can then be estimated. Finally, "clustering" methods are used to sort and display the data.

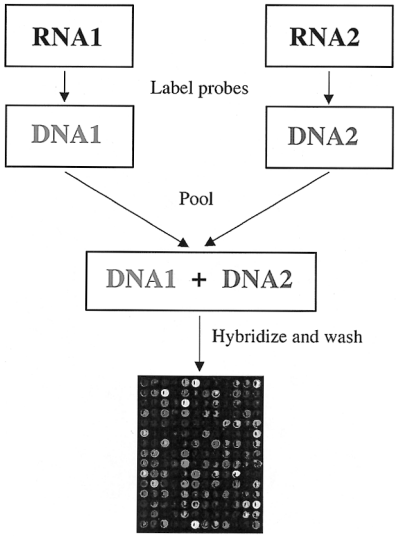

FIGURE 23.1. To perform microarray experiments, RNA is purified from two or more samples of cultured cells or dissected tissues. These RNAs are used to produce labeled probes. In the example given, the dye cy5, which fluoresces red, was used to label probe from sample 1; and the dye cy3, which fluoresces green, was used to label probe from sample 2. The labeled products are mixed and hybridized to the spots on the microarray. Following a wash step, the array is scanned and the signals from the red and green channels are superimposed. If an RNA species is more abundant in sample 1 than 2, the resulting spot will be red; in the reverse case, the spot will be green. When the RNA is equally abundant in the two samples, the spot is yellow. See color version of figure.

Two sorts of experimental paradigms have been defined: type I and type II (42). In the former, two samples are compared to one another; in the latter, multiple samples are compared. To look at multiple samples (e.g., time points, drug doses, developmental ages, brain regions, autopsy specimens), each sample in the set could theoretically be labeled with a different fluorescent dye that could, in turn, be visualized with a different laser. Presently, most commercial readers have only two lasers, but four-color instruments have already appeared on the market. (The number of dyes that it is possible to use for labeling RNA samples is dictated by a reader's ability to resolve the signal from individual dyes and the strength of the signal each dye produces.)

To use a two-color scanner for multiple comparisons, discrete samples must be compared to a reference standard. The ideal standard would have modest amounts of each transcript represented on the array used, because it needs to generate a nonzero denominator for the hybridization ratio. In the case of the mouse, the 17-day embryo and/or the adult brain have been proposed as sources of standard RNA. Pools of cell-line RNAs have been used as standards for human work. It would be useful if a central source of standards existed and if huge batches were prepared.

NORMALIZING RATIOS

Since it is difficult, if not impossible, to measure the amount of RNA used to produce a labeled probe, normalizing the signals from the source RNAs is essential. To do this, a set of "housekeeping genes" is chosen because their transcription is fairly constant across a range of conditions. The ratio of signals from these genes is set to 1. The housekeeping set needs to be defined empirically, and in looking for candidates to include in such a set, few genes have been found that have constant expression levels. When large arrays are used, this is not a problem; hundreds of genes (or the entire set of genes) can be used for "global normalization" (Fig. 23.2). When small arrays are employed, on the other hand, the size and composition of the gene set used for normalization are very important. Just as a reference standard is urgently needed now, a normalization set supplied by a central site would be quite valuable.

QUALITY CONTROL

While we have methods to assess the quality of DNA sequence data, for example, there is no generally accepted method for establishing the quality of an array study. In spite of this, there are some controls that can be built into an array. As noted earlier, scientists are arraying DNAs generated by PCR from plasmid templates. It is highly desirable to use sequence-verified cDNA sets. Amplifying these with

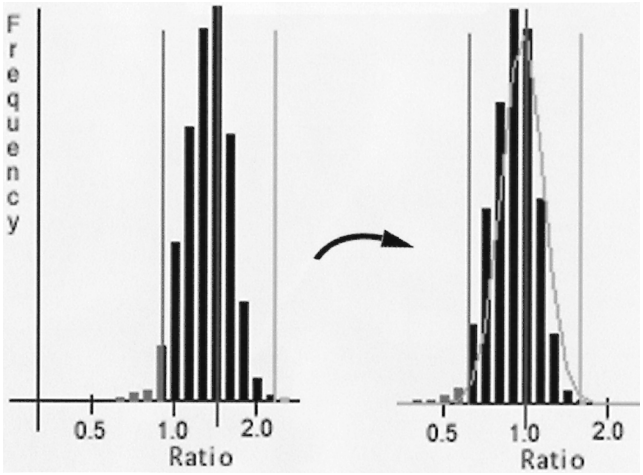

FIGURE 23.2. Normalization. Most genes, especially those with ''housekeeping'' functions, do not change very much from one experimental condition to another. For this reason, housekeeping genes, or the entire collection of genes on a large array, can be used to set the ratio of signals from the two color channels to 1 as shown here. See color version of figure.

specific primers would confirm the identity of each clone, but this is expensive. Assuming we can obtain a reasonably well-validated clone set, the cheapest way to produce the 1.5- to 2.0-kilobase (kb) DNAs for printing is to use vector primers, and to analyze the products on agarose gels. The resulting cDNAs will include T-tails of varying lengths, and repeat sequences. Hybridization of labeled probe to these sequences is prevented by addition of a blocking solution containing oligo (dA) 20-mers, yeast transfer RNA (tRNA), and (for human probes) Cot-1 DNA to the probe solution. To show that the blocking was successful, a number of negative controls should be included among the samples arrayed: spotting buffer, Cot-1 and human genomic DNA, plasmid DNA, oligo (dT), and oligo (dA).

It is useful to array targets for nonmammalian transcripts and to ''spike'' the samples with the corresponding polyA-tailed RNAs. These RNAs can be added in different concentrations to crude tissue extracts, total RNA samples, or purified polyA-plus RNA to determine extraction efficiencies and detection sensitivities. It would be a mistake to imagine that the added RNA standards can be used to generate figures for absolute amounts of RNA in samples for the reasons given earlier. They should be used exclusively for quality control.

An additional set of spots that have been found useful to array are ''landing lights.'' These DNAs, which are printed at regular intervals, are used for orientation.

SMALL SAMPLES, FALSE NEGATIVES, AND FALSE POSITIVES

Failure to detect transcripts or changes in transcripts could result from low-quality arrays or poor labeling methods. Over time, the methods used to make and probe arrays should improve, and false negatives will grow less important. Presently, we can detect RNAs with an abundance of about 1:300,000 in a complex sample. This translates into a few copies per cell if one is studying a homogeneous cell line. Seeing increases in rare transcripts under these circumstances should be simple, but measuring decreases will difficult if not impossible when one can barely detect a weak signal in the first place. Since brain samples are much more heterogeneous than cell lines, the problem of detecting rare mRNAs is even harder. For this reason, it may be necessary to isolate neuronal populations from brain sections by microdissection or to collect single neurons by laser capture methods to enrich and study rare, cell-specific transcripts. To take full advantage of these dissection techniques, methods will have to be developed for isolating and labeling picogram quantities of RNA. (One million cells yield about 5 to 10 μg of total RNA.) It is important to note that labeling methods have to preserve the heterogeneity and relative abundances of the RNAs in the samples to be studied. Care must be taken if PCR is used in the labeling procedure to avoid biasing the sample. Novel labeling methods can be tested using arrays and serially diluted RNA templates.

At present investigators use Northern blotting, the TaqMan system, or *in situ* hybridization histochemistry to weed out false-positive responses of selected mRNAs. When arrays are no longer limiting, this could be accomplished by studying replicate samples, but the argument could be made that one should focus on variations in collections of genes instead of single ones, and that looking at many conditions once may be more powerful than looking at the same condition many times.

BIOINFORMATICS

Analyzing the earliest, small-scale, array experiments was simply a matter of listing the names of transcripts that appeared to increase or decrease from control levels (Fig. 23.3). As the sizes of arrays increased and labeling methods improved, new algorithms had to be developed that ''clustered'' the hundreds or even thousands of expression changes found in a typical experiment. Clustering methods permit the classification of genes on the basis of similarities or differences in their patterns of expression across multiple experiments (52,53). The output is usually in tabular form. Experimental conditions are listed across the top of a table, and names of genes listed along the side. The response of each gene in each experimental condition is color coded—one color (red) indicating an increase and another (green) a decrease vs. a standard signal (Fig. 23.4). The eye can readily detect patterns in complex images of the sort described, and groups of genes can be identified that parallel one another. Commonly, such genes function in concert.

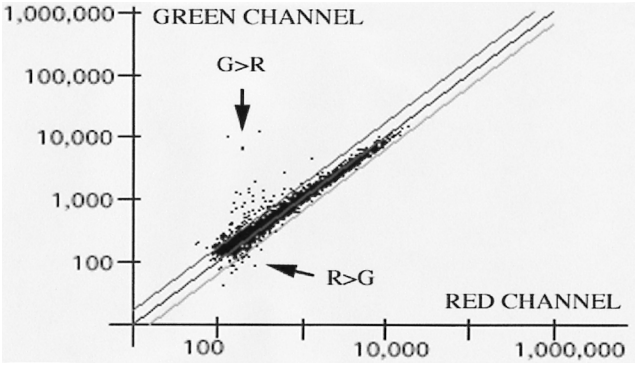

FIGURE 23.3. After normalization, a scatter plot shows that most genes fall in a ratio = 1 space. *Arrows* above and below this space point at genes with altered expression. See color version of figure.

In the hypothetical SSRI study described above, one group of genes may be increased or decreased in the raphe nuclei following chronic, but not acute, treatment with drug, and a different collection of genes is altered in areas innervated by raphe neurons such as the hippocampus. Different sets of genes might respond to SSRI treatment when behaviorally responsive mouse strains are compared to unresponsive ones, and the pattern of gene expression is different in knockout animals that do not respond to SSRIs as compared with animals that do. Each additional experiment may narrow (or broaden) the list of genes of interest.

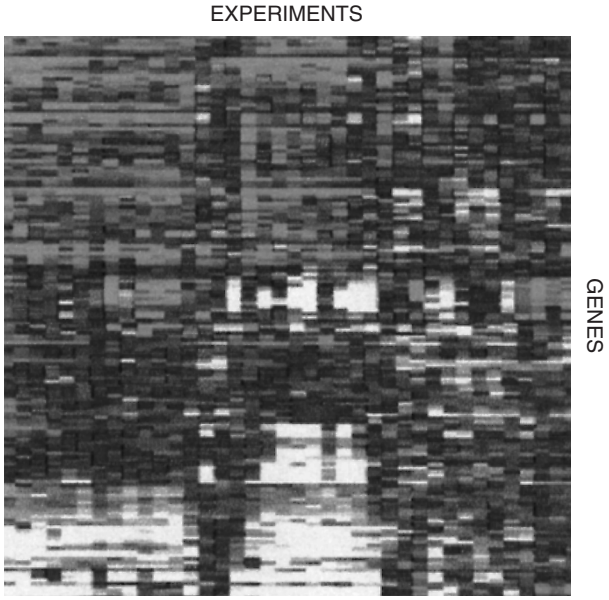

FIGURE 23.4. Clustering. The clustering algorithm has sorted the genes that were studied in a series of experiments according to similarities in their patterns of expression. By convention, red indicates an increase from the standard used, and green a decrease. The collections of genes that are moving up or down in parallel can be readily seen. See color version of figure.

There are ways of evaluating data that are not completely model-independent. Gene responses can be imposed on metabolic charts or on maps of chromosomes. In the former case, increases or decreases in the utilization of certain pathways can be detected; in the latter, deletions or changes in copy number may be recognized, or strong positional candidates identified.

INTERPRETING EXPERIMENTS

Recent studies of changes in gene expression in yeast associated with nutritional and environmental stresses, the cell cycle, or genetic manipulations are examples of well planned and executed surveys (45,46,54–56). All of the 6,200 known and predicted protein-coding genes in the yeast genome were arrayed on a single microscope slide, and sufficient numbers of cells were grown to make ample amounts of RNA for labeling. Each experiment gave a richly detailed picture of molecular responses to a physiologic process or perturbation.

Unfortunately, the brain is much more difficult to examine than yeast. It varies with age, and is composed of hundreds of different sorts of cells that express, in aggregate, as many as half of the genes in the genome in a highly regulated manner. To determine the properties of single populations of cells in the context of the intact structure will be difficult, but perhaps not impossible. Initially, it would make sense to identify all the genes expressed in the developing and adult nervous system. This, in fact, is one goal of the Brain Molecular Anatomy Project (BMAP) (57). After this goal is achieved, the regional and cellular localization of "brain genes" will be determined.

In addition to cataloging the transcripts in the brain, it would be helpful to look at the reactions of isolated populations of neurons or glia to specific signals or environmental alterations—e.g., oxidative stress, excitotoxins, neurotransmitters, hormones, and drugs. Some responses may be of a global nature—increases or decreases in energy metabolism or protein biosynthesis—while others may be quite specific to the cell studied or the agent administered.

The availability of transcript maps and collections of "expression motifs" should help us interpret some of the changes observed in human or mouse brain samples. For example, if it were possible to examine the responses of isolated raphe neurons to SSRI treatment *in vitro*, it might be easier to recognize similar responses in tissue samples. Bear in mind, however, that the majority of arrayed genes discovered by sequencing the human genome and large collections of cDNAs are of unknown function. Structural motifs may hint at the function of some gene products (58), but the role of most will remain an enigma. Expression studies may help solve this problem because genes with similar expression profiles often have related jobs. Functional proteomic work will be useful as well. The combination of

two-dimensional gel electrophoresis, ultrasensitive detection methods, and mass spectroscopic analyses will permit researchers to map protein species to specific organelles or macromolecular complexes (59). It is important to remember that most proteins in a cell do not exist or act in isolation. Components of metabolic pathways and regulatory cascades reside in protein communities. Interactions between members of such communities can be detected with yeast two-hybrid methods, and researchers have already begun to examine protein–protein interactions in simple organisms on a genome-wide basis (60–62). Furthermore, yeast "*n*-hybrid" methods have been developed that permit one to look at protein–DNA and protein–RNA interactions (63). Finally, it is worth mentioning that large collections of mice are being produced with random mutations in their genomes. The goal of "saturation mutagenesis" projects is to use multiple screens to identify animals with interesting phenotypes. The goal of investigators who are making insertional mutations in embryonic stem (ES) cell lines, on the other hand, is to determine the insertion site of each cell produced so that knockout animals can be made on demand.

In the future, in analyzing the results of array experiments, the field will benefit from work on animals, proteomics, and earlier expression studies too—but only if everyone adheres to standard formats in archiving and annotating data.

ANNOTATING EXPERIMENTS

Presently, there are no standards for annotation, but efforts are under way to solve this problem. To create useful and searchable archives, all features of each experiment will have to be described in a standard way using an explicit and unambiguous, "controlled" vocabulary. Some of the required vocabulary already exists. For example, DNAs spotted onto an array can be given and linked to identifiers in public databases. (Unfortunately, different databases sometimes use different identifiers for the same gene. Consequently, the sequence of each DNA on an array should be specified.) Drugs used in array experiments can be referred to by Merck Index number (64), organisms can be described using names in the taxonomy database (65), and mouse strains and mutants can be named according to established rules and guidelines (66). Much of the language needed to describe array experiments has not been standardized, however, and for now databases will have to contain many free-form text fields.

For studies of autopsy samples from psychiatric patients, a good deal of specialized information should be provided. The patient's age at death, gender, diagnosis, genotype (if available), cause of death, postmortem interval, pathology, toxicology screening results, and medication/drug abuse history should be given. The brain region dissected, dissection method used, side of the brain sampled, specimen weight, microdissection procedure and cells selected, RNA extraction method and quality, and labeling method are all essential fields as well. It would be useful to have quality scores for some of these—e.g., the diagnosis and sample condition. Short of a quality score for diagnosis, a detailed description of the key elements of the patient's clinical and laboratory findings should be made available.

Annotating array experiments can be tedious and time-consuming. I am not suggesting that the recommendations above should all be implemented immediately. This would hinder progress. On the other hand, the field would benefit from well-annotated work, and the sooner guidelines are agreed to and implemented, the better. If this chapter serves one useful purpose, it would be to promote this agenda.

REFERENCES

1. *http://www.ncbi.nlm.nih.gov/genome/guide/*.
2. *http://www.ncbi.nlm.nih.gov/UniGene/Hs.stats.shtml*.
3. *http://www.ncbi.nlm.nih.gov/dbEST/index.html*.
4. *http://www.celera.com/celerascience/index.cfm*.
5. *http://www.tigr.org/*.
6. *http://genome.rtc.riken.go.jp/*.
7. Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. *Nature Genet* 1999;21(1 suppl):33–37.
8. Brownstein MJ, Trent JM, Boguski MS. Functional genomics. *Trends Guide Bioinformatics* 1998;27–29.
9. Duggan DJ, Bittner M, Chen Y, et al. Expression profiling using cDNA microarrays. *Nature Genet* 1999;21(1 suppl):10–14.
10. Eisen MB, Brown PO. DNA arrays for analysis of gene expression. *Methods Enzymol* 1999;303:179–205.
11. Ferea TL, Brown PO. Observing the living genome. *Curr Opin Genet Dev* 1999;9(6):715–722.
12. Kao CM. Functional genomic technologies: creating new paradigms for fundamental and applied biology. *Biotechnol Prog* 1999;15(3):304–311.
13. Schena M, Shalon D, Davis RW, et al. Quantitative monitoring of gene expression patterns with a complementary DNA microarray [see comments]. *Science* 1995;270(5235):467–470.
14. Schena M, Heller RA, Theriault TP, et al. Microarrays: biotechnology's discovery platform for functional genomics [see comments]. *Trends Biotechnol* 1998;16(7):301–306.
15. Shalon D, Smith SJ, Brown PO. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Res* 1996;6(7):639–645.
16. *http://www.incyte.com/*.
17. *http://www.nhgri.nih.gov/DIR/LCG/15K/HTML/*.
18. *http://cmgm.stanford.edu/pbrown/*.
19. *http://w95vcl.neuro.chop.edu/vcheung/*.
20. *http://sequence.aecom.yu.edu/bioinf/funcgenomic.html*.
21. *http://chroma.mbt.washington.edu/mod www/*.
22. *http://www.affymetrix.com/*.
23. *http://www.biodiscovery.com/*.
24. *http://tagc.univ-mrs.fr/*.
25. *http://www.genscan.com/*.
26. *http://www.genomicsolutions.com/products/bio/index.html*.
27. *http://www.intelligentbio.com/*.
28. *http://www.interactiva.de/vlab/xna.html*.
29. *http://www.apbiotech.com/application/microarray/default.htm*.
30. *http://www.packardinst.com/*.
31. *http://www.ultranet.com/~radius/*.

32. *http://www.resgen.com/*.
33. *http://www.stratagene.com/gc/gene.htm*.
34. Berchtold MW. A simple method for direct cloning and sequencing cDNA by the use of a single specific oligonucleotide and oligo(dT) in a polymerase chain reaction (PCR). *Nucleic Acids Res* 1989;17(1):453.
35. *http://www.ncbi.nlm.nih.gov/sage/*.
36. Velculescu VE, Zhang L, Vogelstein B, et al. Serial analysis of gene expression [see comments]. *Science* 1995;270(5235): 484–487.
37. Afshari CA, Nuwaysir EF, Barrett JC. Application of complementary DNA microarray technology to carcinogen identification, toxicology, and drug safety evaluation. *Cancer Res* 1999; 59(19):4759–4760.
38. MacLachlan TK, Somasundaram K, Sgagias M, et al. BRCA1 effects on the cell cycle and the DNA damage response are linked to altered gene expression. *J Biol Chem* 2000;275(4):2777–2785.
39. Duan Z, Feller AJ, Penson RT, et al. Discovery of differentially expressed genes associated with paclitaxel resistance using cDNA array technology: analysis of interleukin (IL) 6, IL-8, and monocyte chemotactic protein 1 in the paclitaxel-resistant phenotype. *Clin Cancer Res* 1999;5(11):3445–3453.
40. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling [see comments]. *Nature* 2000;403(6769):503–511.
41. Perou CM, Jeffrey SS, van de Rijn M, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci USA* 1999;96(16):9212–9217.
42. Geiss GK, Bumgarner RE, An MC, et al. Large-scale monitoring of host cell gene expression during HIV-1 infection using cDNA microarrays. *Virology* 2000;266(1):8–16.
43. Ross DT, Scherf U, Eisen MB, et al. Systematic variation in gene expression patterns in human cancer cell lines [see comments]. *Nature Genet* 2000;24(3):227–235.
44. DeRisi J, Penland L, Brown PO, et al. Use of a cDNA microarray to analyse gene expression patterns in human cancer [see comments]. *Nature Genet* 1996;14(4):457–460.
45. Nacht M, Ferguson AT, Zhang W, et al. Combining serial analysis of gene expression and array technologies to identify genes differentially expressed in breast cancer. *Cancer Res* 1999;59(21): 5464–5470.
46. Spellman PT, Sherlock G, Zhang MQ, et al. Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell* 1998; 9(12):3273–3297.
47. Tao H, Bausch C, Richmond C, et al. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. *J Bacteriol* 1999;181(20):6425–6440.
48. Hayward RE, Derisi JL, Alfadhli S, et al. Shotgun DNA microarrays and stage-specific gene expression in *Plasmodium falciparum* malaria. *Mol Microbiol* 2000;35(1):6–14.
49. Tassone F, Lucas R, Slavov D, et al. Gene expression relevant to Down syndrome: problems and approaches [In Process Citation]. *J Neural Transm Suppl* 1999;57:179–195.
50. Julius D. Serotonin receptor knockouts: a moody subject [comment]. *Proc Natl Acad Sci USA* 1998;95(26):15153–15154.
51. De Vry J. 5-HT1A receptor agonists: recent developments and controversial issues. *Psychopharmacology (Berl)* 1995;121(1): 1–26.
52. Eisen MB, Spellman PT, Brown PO, et al. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 1998;95(25):14863–14868.
53. Zhang MQ. Large-scale gene expression data analysis: a new challenge to computational biologists [published erratum appears in *Genome Res* 1999;9(11):1156]. *Genome Res* 1999;9(8):681–688.
54. DeRisi JL, Iyer VR, Brown PO. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 1997;278(5338):680–686.
55. Ferea TL, Botstein D, Brown PO, et al. Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proc Natl Acad Sci USA* 1999;96(17):9721–9726.
56. Lashkari DA, DeRisi JL, McCusker JH, et al. Yeast microarrays for genome wide parallel genetic and gene expression analysis. *Proc Natl Acad Sci USA* 1997;94(24):13057–13062.
57. *http://grants.nih.gov/grants/guide/notice-files/not98–052.html*.
58. Orengo CA, Todd AE, Thornton JM. From protein structure to function. *Curr Opin Struct Biol* 1999;9(3):374–382.
59. Blackstock WP, Weir MP. Proteomics: quantitative and physical mapping of cellular proteins. *Trends Biotechnol* 1999;17(3): 121–127.
60. Bartel PL, Roecklein JA, SenGupta D, et al. A protein linkage map of Escherichia coli bacteriophage T7. *Nature Genet* 1996; 12(1):72–77.
61. Fromont-Racine M, Rain JC, Legrain P. Toward a functional analysis of the yeast genome through exhaustive two-hybrid screens [see comments]. *Nature Genet* 1997;16(3):277–282.
62. Ito T, Tashiro K, Muta S, et al. Toward a protein-protein interaction map of the budding yeast: a comprehensive system to examine two-hybrid interactions in all possible combinations between the yeast proteins. *Proc Natl Acad Sci USA* 2000;97(3): 1143–1147.
63. Vidal M, Legrain P. Yeast forward and reverse 'n'-hybrid systems. *Nucleic Acids Res* 1999;27(4):919–929.
64. *http://library.dialog.com/bluesheets/html/bl0304.html#BI*.
65. *http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/*.
66. *http://www.informatics.jax.org/mgihome/nomen/table.shtml*.

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