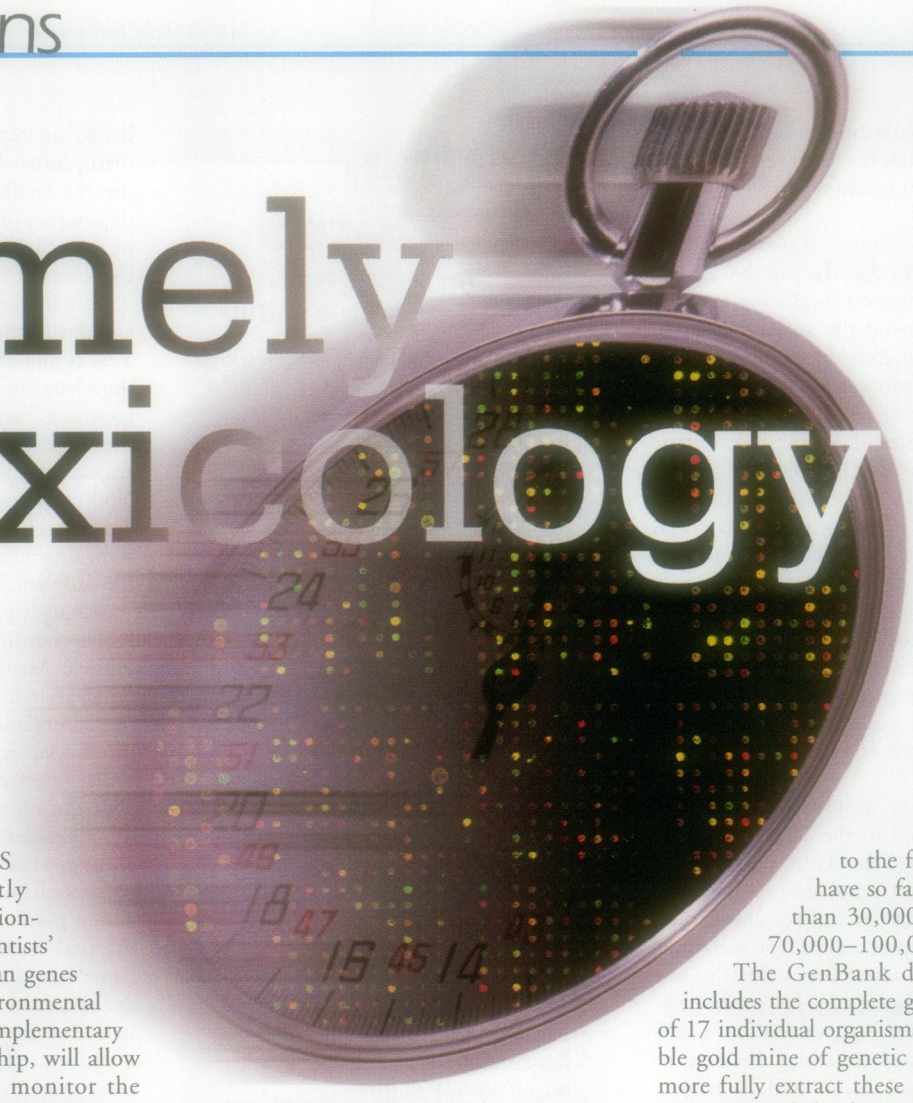
Timely Toxicology



A group of NIEHS researchers recently developed a revolutionary tool that will boost scientists' ability to analyze how human genes change in response to environmental agents. The ToxChip, a complementary DNA (cDNA) microarray chip, will allow life sciences researchers to monitor the expression levels of thousands of different genes at a time, thereby condensing months of painstaking laboratory tasks into a day's work. For toxicology researchers in particular, this tool is an important development because it promises a more effective way to identify environmental hazards and their effects on DNA. The ToxChip could transform the way toxicologists approach environmental problems.

NIEHS scientific director J. Carl Barrett, Cynthia Afshari, director of the NIEHS cDNA Microarray Laboratory, and postdoctoral fellow Emile F. Nuwaysir, working with Jeff Trent and colleagues at the National Human Genome Research Institute, pioneered the ToxChip technique for studying toxicology. According to the scientists, the cloned cDNA microarrays measure gene expression, which in turn serves as a highly sensitive and informative marker for toxicity. The ToxChip allows greater sensitivity in detecting the effects of harmful compounds, the researchers say. At the same time, it will significantly trim the amount of time needed to gauge how these compounds affect biological systems.

The NIEHS team hopes that investigators will soon be able to use the ToxChip to determine the relative safety of natural or synthesized compounds for human exposure and to study how chemical mixtures interact. Further down the road, the ToxChip may be useful in helping to prevent diseases caused by environmental exposures—besides detecting toxic responses in human tissues and other organism models such as mice, rats, and yeast, these microarrays could be used to speed clinical trials for potential new drugs and even suggest optimal dosages for treatment.

The collective work of the NIEHS group and other researchers around the United States who are conducting related studies has engendered a new scientific subdiscipline called toxicogenomics. This blend of toxicology and genomics makes use of genomics resources (namely, the huge GenBank database of DNA sequence information that has been compiled over the last decade or so) to advance one main goal: identifying potential human and environmental toxicants and their modes of action.

"There has been a revolution in the economy of scale," says Nuwaysir, referring to the fact that scientists have so far identified more than 30,000 of the possible 70,000–100,000 total genes. The GenBank database, which includes the complete genomic sequence of 17 individual organisms, boasts a veritable gold mine of genetic information. To more fully extract these riches, however, scientists needed a faster and farther-reaching tool. Afshari and Nuwaysir put their heads together to tailor available microarray chip technology to achieve parallel monitoring of gene expression in an application for toxicology. Traditional methods—including Northern blotting, RNase protection assays, S1 nuclease analysis, plaque hybridization, and slot blots—are tedious and time-consuming. Using these methods, an investigator typically studies one gene at a time. The sheer amount of genetic information available to be analyzed today renders these methods inappropriate and possibly obsolete. The ToxChip, however, can monitor expression levels for thousands of genes simultaneously. "What's novel about this technique is its scale," Nuwaysir asserts. "And it can be applied to almost any question in biology."

Decoding Mechanisms of Response

Toxicity almost always alters gene expression, either directly or indirectly. With the ToxChip, researchers can quickly and methodically run experiments to analyze the characteristic and specific pattern of gene expression caused by a particular toxicant. The result is a toxicant "signature,"

or a common set of changes in gene expression caused by a certain class of toxicant. Once these various signatures are established, researchers can compare them to the gene expression profiles caused by unknown agents in the same model system. When a match is found, a presumed mechanism of action is assigned to the toxicant being tested.

The ToxChip contains genes that exhibit changes resulting from what Nuwaysir refers to as "very basic, classic, well-studied agents." These include oxidant stressors, which are important from a pathological standpoint; dioxin and dioxin-like compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a known carcinogen; peroxisome proliferators; and environmental estrogens. "We look for characteristic changes, then see if the unknowns line up, if there is a subset that matches. It's a way to predict a mechanism or mode of action," he explains.

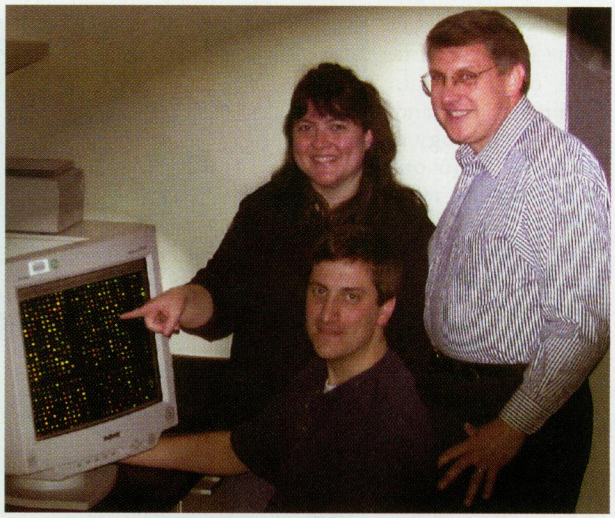
"We trick normal cellular machinery into doing our work for us," Nuwaysir says, referring to the manner in which the complementary nucleic acids from each of the two cDNA groups (from a normal cell and tumor cell, for example) present on the chip will adhere to one another. "Basically, we're exploiting what nature has already perfected," he adds. "It's like two people finding each other in a crowded bus terminal." Each of the minuscule dots on the glass chip contains concentrated copies of the same genetic material, so they function as "magnets," attracting the complementary pieces of DNA floating around in solution on the chip.

How the ToxChip Works

ToxChip version 1.0 features a subarray of clones of 2,100 human genes, which were selected because they are involved in basic cellular processes and are proven to respond to different types of toxic injury. Large quantities of each gene clone are produced through polymerase chain reaction. Using high-speed robotics, microarrays of up to 10,000 clones are etched onto a glass substrate, or microchip.

When it's time to test a particular toxicant, fluorescent nucleotide probes are generated from control and test RNA samples through the single-round reverse transcription reaction process. The control and test cloned cDNA groups are tagged with different fluorescent nucleotides, one group with green and the other with red. Then, the cDNA groups produced from the control and test populations are commingled and hybridized onto the chip under a glass coverslip. During hybridization, each cDNA in solution binds specifically to the cDNA on the chip that is its perfect complement.

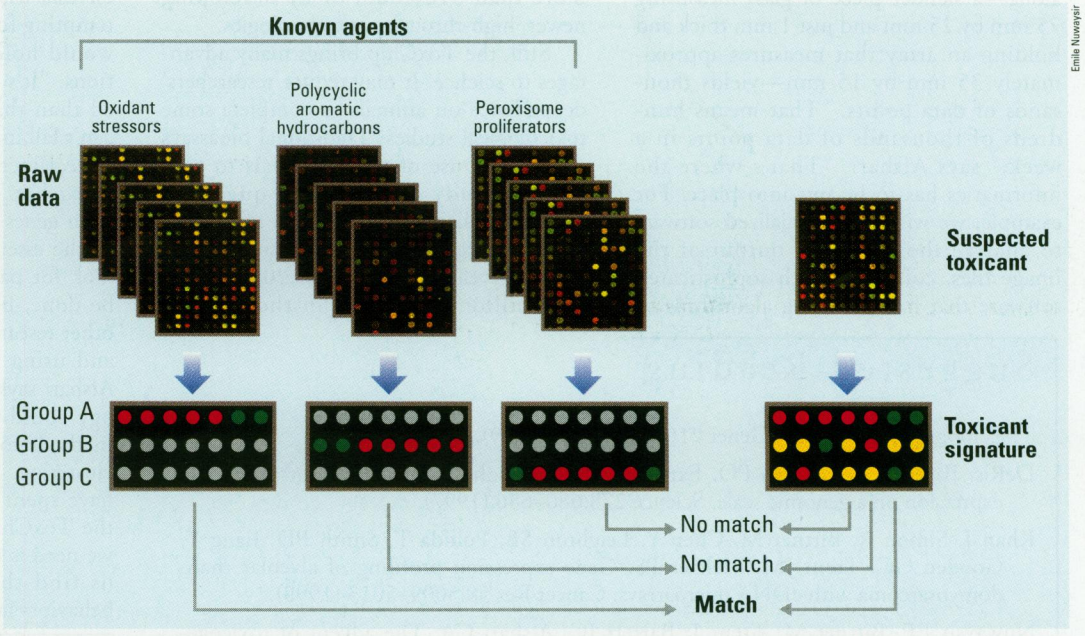
Next, a scanning confocal microscope with a motorized stage and lasers to excite the fluors detects the fluorescent signal. Lasers—first red, then green—pass beneath the chip, revealing the matches between the control and test cDNA groups. Attached to the microscope is a computer equipped with custom digital image analysis software, which sifts through the data and determines the ratio of the two different fluors (red to green) at each cDNA feature on the chip. According to Nuwaysir, green intensity signals expression of the gene in the control sample, while red intensity signals expression of the gene in the treated, or test, sample. "The color of one particular gene does not necessarily indicate toxicity," he says. "However, the color pattern for many genes can be associated with a toxic condition."



A microarray of toxicologists. Emile Nuwaysir, Cynthia Afshari, and J. Carl Barrett (clockwise from bottom) examine gene expression data collected using the ToxChip.

Afshari, Nuwaysir, and their colleagues are working on version 2.0 of the ToxChip, which would organize 12,000 individual genes on one chip. Other chips in the works will study other model systems, such as rats, mice, frogs, and yeast, all of which could eventually be employed in toxicology studies.

While the NIEHS lab has been developing the ToxChip specifically for use in toxicological studies, other scientists have been working on similar technologies to meet other goals. "This whole approach is very generic," says Kevin Becker, head of

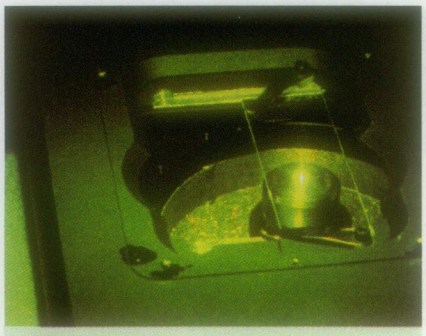


Red light, green light. Using ToxChip, gene expression data are derived from exposure of model systems to known toxicants (Group A, B, and C genes). These data are compared to a set of gene expression changes elicited by a suspected toxicant. If the characteristics match, a putative mechanism of action can be assigned to the unknown agent.

the DNA Array Unit at the National Institute on Aging in Baltimore, Maryland. "The technology is the same as in our chip," he adds, referring to the DNA microarray chips he and his colleagues have been developing, but, he says, "Our [chip] is more disease-focused."

Weighing the Pros and Cons

Generating vast quantities of toxicogenomic data quickly is one thing. Sorting and analyzing it once it's in hand is another matter entirely. One must decide exactly what to do with the information or determine the next question that needs to be asked. Both Afshari and Nuwaysir concede that these tasks can be extremely difficult. The technique produces a staggering volume of raw data for scientists to observe. One chip alone—a slender piece of glass measuring 75 mm by 25 mm and just 1 mm thick and holding an array that measures approximately 35 mm by 15 mm—yields thousands of data points. "That means hundreds of thousands of data points in a week," says Afshari. "That's where the informatics has to be put into place. For example, we will need specialized software to analyze the computer output of the image files, combined with sophisticated software that has clustering algorithms to allow us to determine patterns of gene expression across doses or time points."



Steve McCaw, Image Associates

Gene Categories on ToxChip*

- Apoptosis
- Cell cycle control
- Cytochromes
- Dioxin/polycyclic aromatic hydrocarbon responsive
- DNA replication and repair
- Estrogen responsive
- Heat shock proteins
- Housekeeping
- Kinases
- Oncogenes and tumor suppressor genes
- Oxidative stress and redox homeostasis
- P450s
- Peroxisome proliferator responsive
- Phosphatases
- Receptors
- Transcription factors

* Some genes may not be represented in their respective categories or may be represented in multiple categories.

The technology has a few other hurdles to leap, as well. First, it requires significant amounts of the DNA that is spotted onto the chip and the RNA used to probe the chip. Second, it can be difficult to distinguish between two gene family members that have very similar DNA sequences. Third, one machine can scan only 8–12 chips in any given workday, so even though output is high (as measured in data gleaned from each chip), the actual throughput (the number of chips scanned) is low. That's a disadvantage that can be overcome only by equipping the lab with additional expensive scanning machines, which cost anywhere from $40,000 to more than $100,000, or by developing newer, high-throughput technologies.

Still, the ToxChip brings many advantages to science. It may reduce researchers' dependence on animals to complete some toxicological studies. Traditional bioassays require the use of many animals to adequately study one research question. Animal studies also require lots of time. A rodent cancer bioassay, for example, may last four years, expending 1,200 animals and millions of dollars in the process. While the ToxChip won't replace animal studies entirely, it could give valuable information to help a researcher do a better job of tailoring a bioassay to the toxicological agent being studied, potentially reducing the amount of money, time, and animals necessary to complete it.

Screening with the ToxChip, when used to complement a bioassay, may also enable researchers to lower toxicant dosages in studies to a level that more closely resembles typical human exposure levels. Microarrays may help researchers explore the connection between acute and chronic toxicity and identify presumed secondary effects by looking at the relationship between the length of exposure and the gene expression profiles generated by that toxicant. This could mean shorter bioassays, more realistic test dosages, and considerable cost savings when compared with typical assays.

Perhaps its chief advantage is that the ToxChip can help give scientists a sense of the bigger picture. "It allows researchers to see the organization of the genome and its response to a disease state or chemical insult," Nuwaysir says. Until now, researchers have been limited to studying one gene at a time, one gel at a time, one week at a time in a serial process that proceeds at an often-slow, inexorable pace. With the advent of this newest microarray chip technology, one researcher can analyze 10,000 genes in one day.

Becker says the technology "allows for the simultaneous analysis of many, many things." In the past, he explains, when scientists discovered a new gene, it was tempting for them to believe that one gene would hold the answers to many questions. "It's usually much more complicated than that," he says. "Now researchers can't fall into that trap."

A larger genetic perspective should help researchers understand patterns of responses in genes to toxic agents. As Afshari sees it, the essential work of all biologists is to look for patterns. Much work remains to be done, both in the NIEHS lab and in other research labs dedicated to developing and using the technology. For instance, Afshari says, researchers using the technology could benefit from better statistical methods for pattern recognition and gene clustering. She and her NIEHS colleagues have spent the last two years fine-tuning the ToxChip. Now, she concludes, "what we need is computers and software to help us find the patterns that show where behaviors fall together."

Jennifer F. Medlin

Suggested Reading

The chipping forecast. Nat Genet 21(suppl):1–60 (1999).

DeRisi JL, Iyer VR, Brown PO. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278:680–686 (1997).

Khan J, Simon R, Bittner M, Chen Y, Leighton SB, Pohida T, Smith PD, Jiang Y, Gooden GC, Trent JM, Meltzer PS. Gene expression profiling of alveolar rhabdomyosarcoma with cDNA microarrays. Cancer Res 58:5009–5013 (1998).

Nuwaysir EF, Bittner M, Trent J, Barrett JC, Afshari CA. The advent of toxicogenomics. Mol Carcinog (in press).