Patrick Brown wants to share his DNA-array-chip technology with any researchers who are interested. There are plenty of takers.

A N D C H I P S F O R A L L

WHO WILL BE THE NEXT TOUR GUIDE?

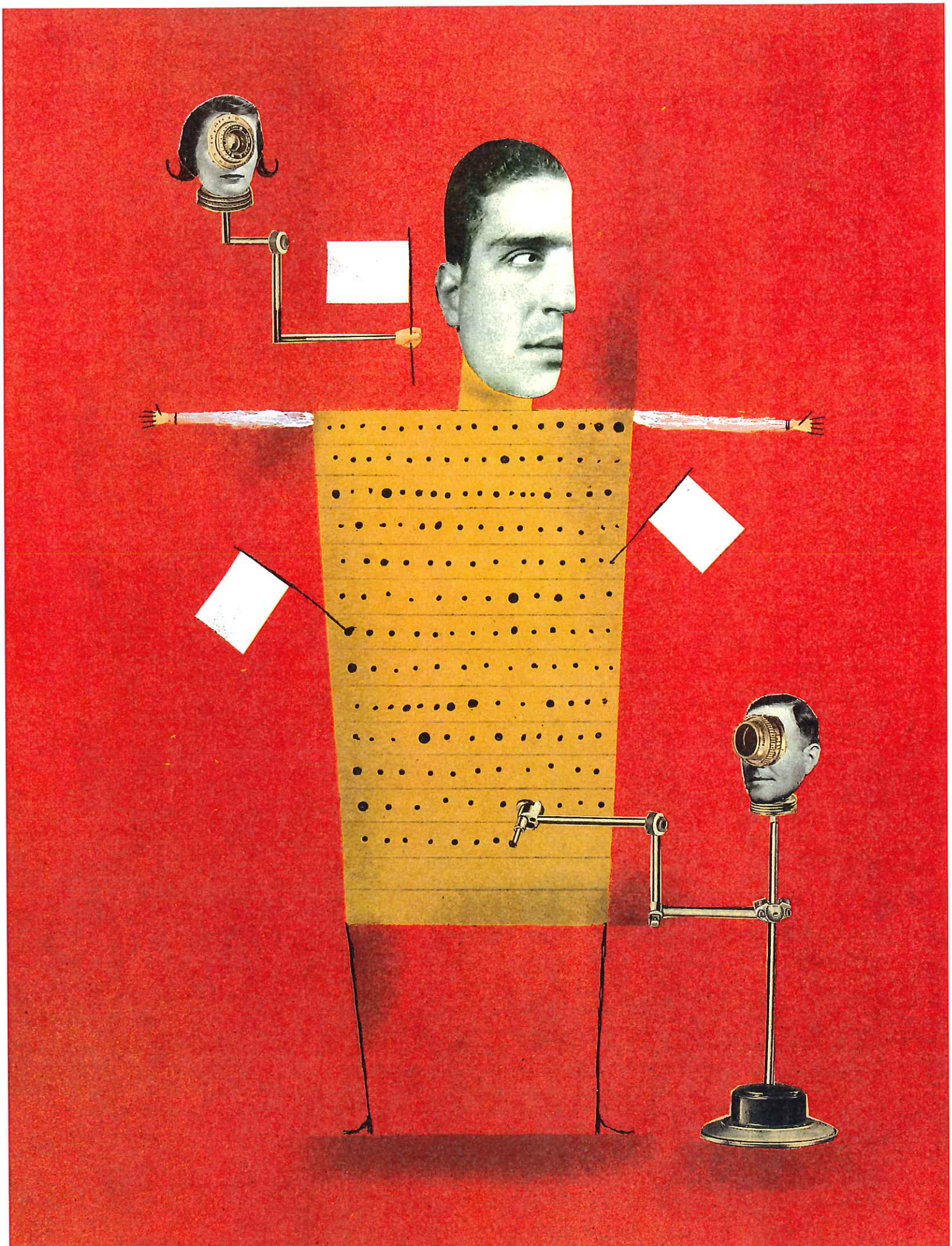
This time, and not for the first time, the honor falls to research associate Linda McAllister, MD, PhD. "You're the third or fourth person today," she says cheerfully, as she leads me off to see the famous Brown laboratory DNA-chip-making machinery. "We really need our own PR person."

What they are all flocking to see — the company representatives, research scientists, and clinicians — is a pair of robots squeezed into a tiny room on the fourth floor of Stanford's Beckman Center building. What has brought them here is the astonishing amount of data pouring out of this one room, a space not much bigger than a walk-in closet.

Patrick Brown, MD, PhD, an associate professor of biochemistry and a Howard Hughes Medical Institute associate investigator, uses the robots to create precise arrays of genes on small glass slides — or "chips." Analyzing the chips, also known

BY WILLIAM WELLS

ILLUSTRATIONS BY DAVID PLUNKERT



as microarrays, could provide molecular fingerprints for cancers so that treatments can be individualized. The analysis is already turning up genes involved in various biological processes faster than researchers can study them. "Once you get into these experiments, the opportunities are so huge," says Brown. "It's like being on a frontier or entering an unexplored wilderness."

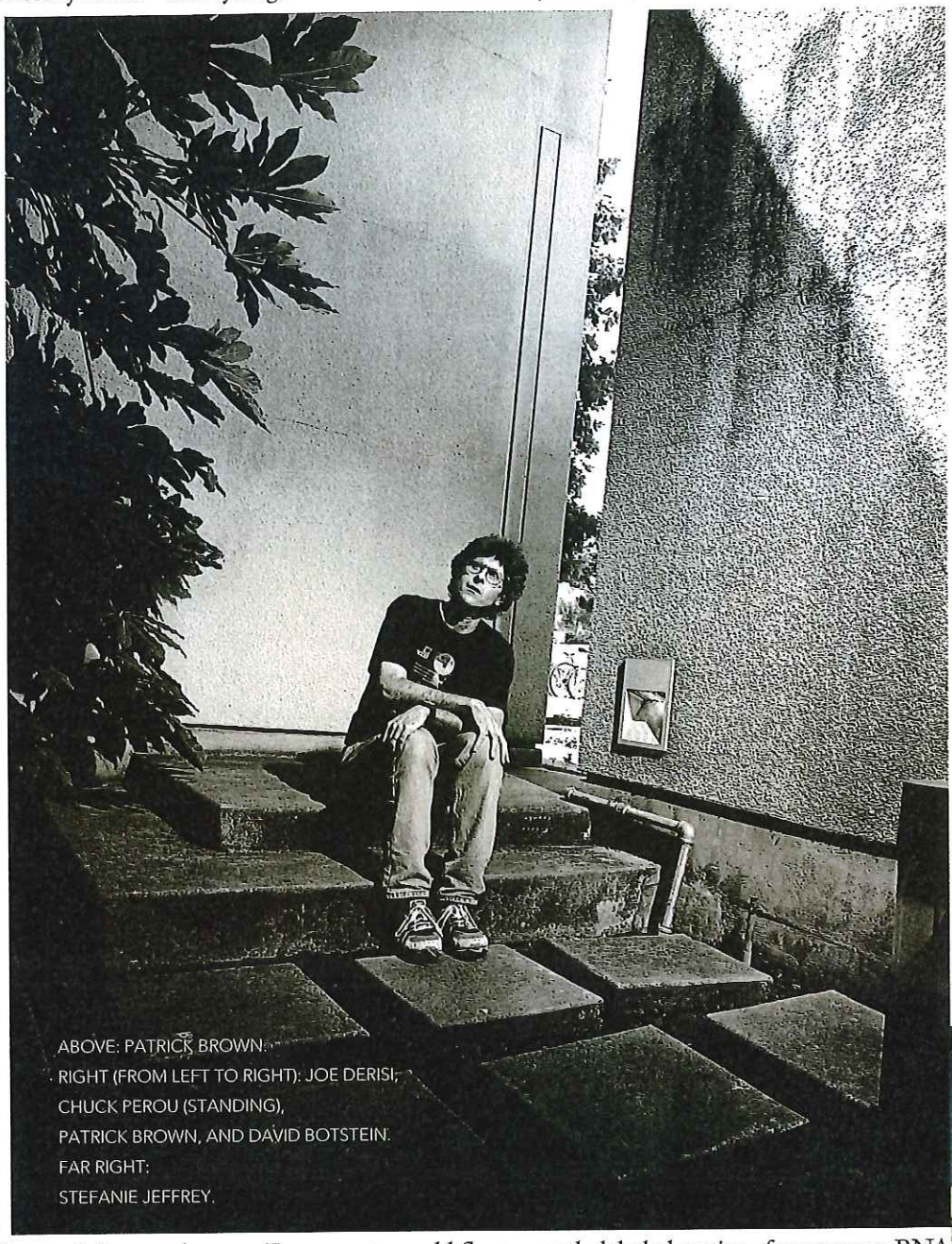
But Brown doesn't want to be alone in that wilderness. "We want to get as many people doing this as possible," says Joe DeRisi, a graduate student in Brown's laboratory. DeRisi has published on the Internet an exhaustive do-it-yourself guide to building an array robot for less than $30,000. In addition to a parts list and construction tips, the Web site features advice on when to take a break for beer hour, and how much fun it is to use the power saw. "With luck," writes DeRisi, "you will finish this part with all your fingers intact."

"Really, the technology doesn't require an engineer to put the robot together," says Brown. Other researchers agree. Already at least 12 other research centers have or are building a robot to create DNA-array chips, Brown says.

Here's how the microarrays work: Every one of the thousands of dots in a given array is a different gene, each with a designated place. To make the arrays, the robot dips a metal tip into a store of DNA, stamps the DNA that sticks to the tip onto a glass microscope slide, and repeats the process on several hundred slides for each of several thousand genes. For some organisms, such as yeast and a few bacteria, DNA sequencers have spelled out the DNA of the entire gene set — or genome. This knowledge base has allowed DeRisi and postdoctoral fellow Vishy Iyer, PhD, to put together a chip with virtually all of the 6,215 genes of brewer's yeast on it. Another chip has 10,000 human genes.

In September, the U.S. Patent and Trademark Office issued a patent for the Brown lab's method and machinery for making microarrays. (For licensing information, contact Stanford's Office of Technology Licensing). Their use of the microarray is described in scientific detail by DeRisi, Iyer and Brown in the October 24, 1997, issue of *Science*.

Once a microarray is made, the members of Brown's laboratory add fluorescently labeled copies of messenger RNA (mRNA) and let them stick to the DNA spots. Messenger RNAs are the read-out from genes and serve as templates later used to create proteins — which do the work of the cell. These mRNA copies, called cDNA, will stick only to the genes from which they were produced (or 'expressed'), so the amount of cDNA on each spot is a measure of how much that gene is turned on. The researchers can measure how much cDNA adheres to each spot because the cDNA glows — a result of the fluorescent building blocks of various colors added



ABOVE: PATRICK BROWN.
RIGHT (FROM LEFT TO RIGHT): JOE DERISI, CHUCK PEROU (STANDING), PATRICK BROWN, AND DAVID BOTSTEIN.
FAR RIGHT: STEFANIE JEFFREY.

"Having a comprehensive list of conditions and places where a gene is expressed moves you a long way toward understanding what a gene does."

"Once you get into these experiments, the opportunities are so huge. It's like being on a frontier or entering an unexplored wilderness."

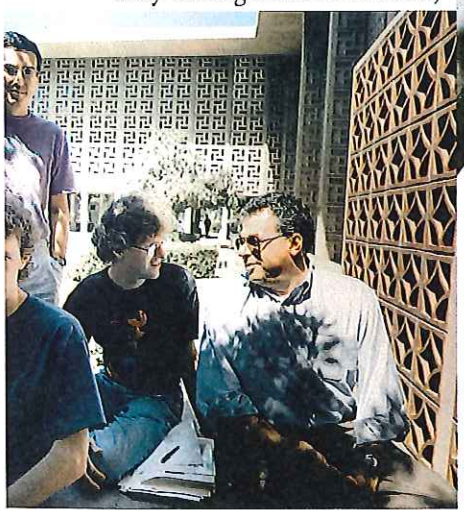
PABLO SERRANO: LEFT AND MIDDLE; ROBERT OLDING: RIGHT

during the cDNA's synthesis.

Two conditions are tested. In one experiment, DeRisi tested mRNA from yeast cells grown in sugar (labeled green) against mRNA from yeast cells grown without sugar (labeled red). He added the two sets of mRNA to a single grid, and each mRNA strand in these two sets stuck to the dot corresponding to its gene. DeRisi measured the color of the dots. For genes that turn on when sugar is taken away, there is more red mRNA than green mRNA, and the spots are red. Green dots represent genes that are more active when sugar is around. Happily, the green dots include genes that already are known to be used for turning sugar into energy.

That kind of correlation is the key to expression analysis. "Genes tend to be expressed when their function is important, and not just in all places at all times," says Brown. "Having a comprehensive list of conditions and places where a gene is expressed moves you a long way toward understanding what a gene does."

The same logic can be applied to almost any imaginable problem (*see sidebar, page 28*) because so many biological questions can be rephrased in terms of how two sets of mRNA differ. If you want to know how a human heart forms, you could sample mRNA from the site of the developing heart before, during and after the heart has formed and then look for the differences. If a gene is turned on only during heart formation, the protein made by the gene is probably either a part of the heart, or it may play a role in orchestrating the construction of a heart. Having picked this special subset of genes from the tens of thousands of genes in the genome, heart researchers can get busy working out exactly how those genes work.



"For a huge number of properties of gene sequences you can design an experiment that surveys the whole genome with respect to that property," says Brown. "It's so much fun; it's really a blast."

Within Brown's laboratory, the experiments range from tracking the changes as cells start growing to heal a wound to cataloging the ups and downs of gene expression as yeast forms weather-resistant spores. Many of the other projects are collaborations with clinicians.

"It's really hard to find common ground for collaboration with clinicians, much harder than you might expect," says Brown. "This technology turns out to be a really nice bridge for such collaboration."

One of those clinicians is Stefanie Jeffrey, MD, an assistant professor of surgery and chief of breast surgery at Stanford. As she explains, she is no stranger to the chip's reputation: "At a recent breast cancer meeting, Richard Klausner, the head of the National Cancer Institute, put up a slide of the Brown lab's work and said, 'This is the future of cancer research in this country.' "

THE BASIS OF THIS HOPE IS ANOTHER DECEPTIVELY SIMPLE CONCEPT. "When two biological samples have differences that are significant enough that we care about them, it is a sure thing that there will be corresponding differences in gene expression," says Brown. "That means the pattern of gene expression provides a marker for those differences. It provides a huge wealth of information you can use for diagnostics."

"When we look at a tumor under the microscope, it is only a very rough method of predicting what the clinical outcome will be," says Jeffrey. But the diversity of gene expression patterns means that there should be a unique pattern for those cancers that spread or don't spread, that are aggressive or nonaggressive, that respond to surgery or don't, or that are killed or not killed by chemotherapy drugs.

Once these correlations are made, treatments can be individualized and successes will rely less on trial and error. "You don't want to test chemotherapy agents on patients," says Brown. "You want to look at the cancer in the absence of a treatment and look at markers that tell you which treatments will be effective."

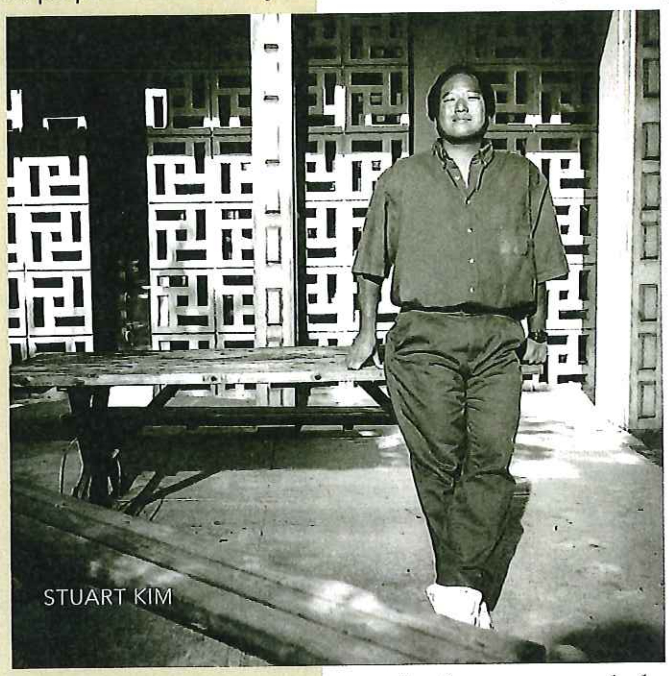
To make the correlations, Jeffrey collects breast cancer biopsies. Postdoctoral fellow Chuck Perou, PhD, tests which genes are turned on in each cancer using a chip with 10,000 human genes (out of the approximately 80,000 human genes). Perou then tries to find common expression patterns for those cancers that responded to surgery or to particular

The lowly worm gets high-tech treatment in an effort to reveal clues about development of cells both healthy and cancerous.

WORM ON A CHIP

Developmental biologist Stuart Kim, PhD, describes microarrays as "revolutionary" and "the future" because they get at problems that classical genetics has largely failed to open up. "Many processes in the cell work together and are very difficult to pick apart," he says. "They are like a spider web — everything is interwoven and interlinked, and it's hard to ascribe a function to a single string. The chips look at many genes at once, so they have the potential to get at these questions."

Kim, an associate professor at Stanford University School of Medicine, is building a worm chip. Four people in his lab are busy creating a microarray (*see main story*) spotted with each of the worm *Caenorhabditis elegans'* 13,000 genes. They aim to finish the chip by the end of the year.


STUART KIM

Kim has already been studying his two favorite problems using chips dotted with just 1,200 of the worm's genes. The first problem is determining how egg and sperm cells differ from all other cells. These cell characteristics must be conferred by the expression of certain genes, and Kim is determined to find those genes with the help of the chip.

The second problem starts with a specific gene called *ras*, but ends with a web of effects that results in cancer. "We want to find out why — when the *ras* pathway gets activated — does the cell grow," says Kim. "It's unclear how you get from the master regulators such as *ras* to cell growth and cancer."

"We've never been able to look at the whole thing before," he continues. "This is the first time you can look at all the genes in parallel. Now you can say, 'This is everything that is involved in cell growth. This is the whole story.' "

Kim will measure all changes that occur when *ras* is turned on and then subdivide and make sense of that list by measuring the changes resulting from only one of the cancer-related processes. Those processes include a decrease in cell stickiness (cancer cells must lose their stickiness to spread through the body), a decreased propensity of the cells to commit suicide (cancer cells turn off this self-defense mechanism), and an increase in the rate of cell growth.

While he is doing his own chip experiments, Kim will also be working on the experiments that others ask him to do. "I predict that for two years I can more than fulfill the needs of the entire worm field," he says. "Two technicians could do all of the chips that the worm community needs and probably the work equivalent to that of 100 people working outside using other methods." Eventually Kim expects to be superseded. "Two years from now," he says, "if it's as big as I think it will be, there will be machines for making gene chips popping up everywhere." — WW

drugs. These patterns can then be used to predict, in advance, the likelihood of success for other patients. Jeffrey says she and her gene-expression colleagues predict that future breast cancer therapies will be designed according to specific patterns of gene expression.

A similar analysis can be applied to any type of cancer and even to susceptibility or treatment outcomes for other diseases. Genetics professor David Botstein, PhD, is a principal investigator with Brown on a National Institutes of Health (NIH) grant to investigate expression analysis for many types of cancer, including the breast cancer study, a lymphoma study with NCI scientists and a leukemia study with the Southwest Oncology Group. The grant also covers a collaboration with the NCI to look at 60 groups of cells that have been grown from tumors. These cell lines have been tested by the NCI for their sensitivity to thousands of different chemicals, including established and experimental cancer drugs. Postdoctoral fellow Doug Ross, MD, PhD, is now analyzing the cells' expression profiles. "Once we do statistics with the drug database, we hope to find patterns of gene expression that correlate with patterns of drug sensitivity," says Ross.

THE AIM IS TO BE IN A POSITION TO SAY WITH ASSURANCE THAT A CERTAIN EXPRESSION PATTERN INDICATES THAT DRUG "X" SHOULD BE USED.

"It's not obvious how you get from 10,000 spots to these kinds of statements," says Botstein. That job has fallen to postdoctoral fellow Mike Eisen, PhD, who has developed software to crunch the vast collection of data coming out of each experiment. Eisen will soon make his methods public, and then the software will be available to any who are interested.

The cancer projects are tantalizing fishing expeditions, representing a new way of exploring biology. Most molecular biologists work on a specific protein involved in a specific process. Then, if they want to branch out, they might hazard a guess that a related protein is also involved and test their hunch. But the arrays allow Brown to study the whole process at once and see what turns up.

"I like to do projects with a large discovery component rather than hypothesis-testing projects," Brown says. "There is so little of nature that we understand in enough detail to ask specific questions about and so much of nature that we don't have a clue about. In the past, people have been forced to do the hypothesis-driven experiments because they didn't have the resources to do the discovery projects. You couldn't search the genome."

"I think most of the big breakthroughs in biology are from serendipitous observations made while intending to look at something else," Brown says. "These DNA-chip experiments

PABLO SERRANO

Microarrays probably won't increase 'genetic discrimination' by health insurers. But they are likely to increase treatment restrictions for some people.

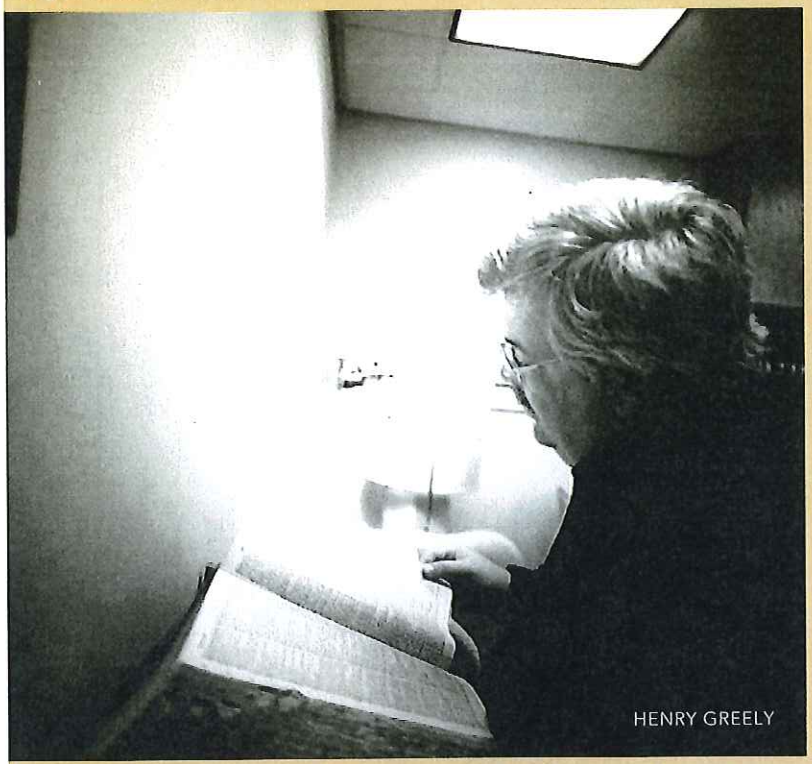
DOES THE CHIP HAVE A DARK SIDE?

A gene expression profile provided by a DNA-array chip should predict susceptibility to diseases, both genetic and infectious. Will health insurers leap on this new technology, using it to exclude all but the healthiest and cheapest customers?

HANK GREELY THINKS NOT. "Many people fear that health insurers will use current genetic technologies to discriminate. That possibility does exist, but its extent is often exaggerated," says Greely, JD, professor of law and, by courtesy, of genetics. "Chip technologies may change medicine, but they seem unlikely to increase 'genetic discrimination' in health insurance."

Greely is co-director of the Program in Genomics, Ethics and Society at Stanford's Center for Biomedical Ethics. The center's two yearlong working groups have issued reports on genetic testing for breast cancer and Alzheimer's disease; the third report will be on the ethical implications of DNA-array-chip technology.

What is at stake with DNA-array chips is the detection — and then exclusion from insurance coverage — of pre-existing conditions (including future events such as diabetes complications). The 1996 Kennedy/Kassenbaum Act restricted non-coverage of pre-existing conditions to 12 months per lifetime for those with major employers. Those employed by small businesses and the self-employed are not protected.



HENRY GREELY

Microarrays should make the detection process cheaper, but they may not change the insurance picture too much. If a genetic variation has a clear and powerful effect on a person's health, tests are likely to be done with or without chips, says Greely. "Chips should make it feasible to test for genetic variations with weaker associations with illness — and therefore of less interest to insurers," he says.

For example, insurers are unlikely to start testing men for the breast cancer gene *BRCA2*. Men with a mutant *BRCA2* gene have a 15- to 20-fold higher risk of getting male breast cancer. But this condition is so rare that the increased susceptibility adds less than a five percent lifetime risk.

"If you are an insurer, do you care about a five percent lifetime risk for male breast cancer?" asks Greely. "I don't think so. A lot of genetic information is just not going to be very dramatic."

"You are talking about smaller variations in both a person's health and, from the insurer's perspective, a person's predicted health costs. For much genetic information, those variations will be so small that it won't be worthwhile for insurers to bother with them," he says. "But the information could still be medically useful. If so, privacy issues are going to be more important because there will be more genetic data used in medical care."

Greely says that microarrays probably will increase treatment restrictions for specific individuals. "One person's prostate operation may be turned down because his gene expression pattern reveals he is in a group less likely to respond well to the operation," he says. "This certainly sounds like discrimination, but to those in medicine it is nothing new. It's called a treatment decision."

"Right now insurers by contract must provide 'medically necessary' care," says Greely. "When you push that term hard enough no one really knows what that means. Chips add personalized information and so may add to that set of dilemmas."

One of the DNA-array chip's boons — that it can bring more individualized treatments and drugs (*see main article*) — raises another potential problem. "To the extent that you individualize therapies, you may make the markets too small to be worthwhile," says Greely. The solution may be special incentives for development of drugs with small markets. Such a system is already in place in the United States, for drugs targeted at rare diseases.

Perhaps the most unexpected effect of the chips may be a positive one — to take medicine beyond an outmoded view of race. "Because some diseases are more or less common in different ethnic groups, medicine today uses the traditional concept of race even though, biologically, it is meaningless," Greely says.

A higher incidence of a disease in one group may arise because that group shares a culture (such as a preference for certain foods), because it shares a common genetic heritage, or some combination of the two. But to the extent these ethnic variations are based on genetic variations, they reflect only differences in percentage. "Thirty percent of white Americans may have a particular genetic variation as opposed to 60 percent of Asian-Americans and 10 percent of African-Americans. Right now, skin color serves as an extremely inaccurate marker for the genetic variation. But when you can get to the gene itself you can get past skin color — and 'race' — entirely." — WW

Numerous groups at Stanford are using Patrick Brown's microarrays.

APPLYING DNA-ARRAY CHIPS TO THEIR PET PROBLEMS

■ David Hogness, PhD, a professor of developmental biology, is sampling messenger RNA (mRNA) as a larva metamorphoses into an adult fly and adding the samples to a fly chip to see which genes are turned on and off. Many of these changes in genetic expression are driven by a single chemical called ecdysone, so he is also comparing the mRNA from normal larvae and larvae treated with ecdysone to identify all genes turned on by ecdysone. Patrick Hurban, PhD, a former postdoctoral fellow (now a research scientist at Paradigm Genetics Inc. in North Carolina), and two others in the Hogness lab used a chip with about 5,000 of the 12,000 to 15,000 genes of the fruit fly *Drosophila melanogaster*. "We thought we might need an army to do this sort of genome work," he says, "but we've got a lot of information with just the three of us."

■ Peter Sarnow, PhD, an associate professor of microbiology and immunology, and Gregg Johannes, PhD, a postdoctoral fellow, are using the chip with ten thousand human genes (*see main story*) to help investigate a trick used by poliovirus. After infecting a cell, the virus turns off translation of all but a few of the cell's mRNAs, but keeps translation of its own mRNA on. The handful of cellular mRNAs that do stay on seem to code for proteins involved in the proliferation of the cell; they are therefore the types of proteins that are likely to be turned on in cancer. Sarnow and Johannes can get a list of all these potential cancer genes by using the chip to reveal mRNA present on the cell's translation "machines" (the polyribosomes) before and after infection and then comparing its distribution.

■ Gary Schoolnik, MD, chief of the Division of Infectious Diseases and Geographic Medicine, and graduate student Michael Wilson are seeking new antibiotic drug targets for the treatment of tuberculosis. In collaboration with the Brown lab they have prepared a DNA-array chip carrying all of the roughly 4,000 genes of the bacterium that causes TB. Many antibiotics kill pathogenic bacteria by inhibiting enzymes involved in the pathways that produce proteins essential for the bacteria's survival. Using the chip, Schoolnik and colleagues have discovered that the inhibition of one of these pathway enzymes changes the expression of genes that code for other enzymes in the pathway — including some enzymes that had not been previously identified.

"Because the pathway produces an essential end product, we believe that the inhibition of one or more of the newly discovered pathway enzymes will prove lethal to the bacterium. So, these pathway enzymes become novel targets for the pharmaceutical industry's drug discovery process," he says. — WW

are designed so that we deliberately make those accidental observations."

It is exciting to find that a new gene is involved in a process, but simply discovering which old familiar genes are active during a process can tell researchers a lot. From the yeast experiment, for example, DeRisi found that he could tell which metabolic pathways in the cell were active — turning food into energy and energy into new cells. "No one has been able to do that before, because no one has looked at more than about five genes at a time," he says.

"It's like watching a movie of the cell, but you're watching ten thousand different things," says postdoc Iyer. "You can make a good guess of what the cell is doing in a given physiological setting."

BUT SOMETIMES THE DISCOVERIES — PLUCKED FROM THE CHIP WITHOUT AN ATTENDANT HYPOTHESIS — DON'T MAKE TOO MUCH SENSE. "A lot of the time you'll do an experiment and you'll get a weird result," says Brown. "That's a statement about how little we know about biology. The result is real. It's highly likely that there has been evolutionary selection for that pattern of expression. It's our job to work out what the nature of that selection is."

The sheer number of changes is also daunting. When the sugar ran out in DeRisi's yeast experiment, over 700 genes were turned up and over 1,000 genes were turned down. "A third of the genome becomes re-programmed," says DeRisi. "That is a major lifestyle change for the yeast. That tells you how complex the system really is."

The best way to make sense of that complexity is to group genes. The groups are based on several characteristics: genes that double in output versus those that quadruple in output; genes that are turned on early or late; genes that respond to changes in temperature, acidity, or both. Some of those groups can be recognized in subsequent experiments, so every new experiment makes just a little more sense. As word about the potential for using DNA-array chips spreads, the number of these experiments is increasing rapidly.

Several companies are marketing methods for taking advantage of microarrays and similar technologies, including Affymetrix Inc. (Santa Clara, Calif.), Molecular Dynamics Inc. (Sunnyvale, Calif.), Protogene Laboratories Inc. (Palo Alto, Calif.), and Synteni Inc. (Fremont, Calif.), a spin-off from Brown's laboratory (in which Brown has no stake). And though disputes over ownership of the rights for the technologies are arising, Brown remains intent on making the instructions for the microarray-generating robot widely available, he says.

Brown and Botstein feel that the top priority is to see that no one researcher or institution has control over the microarray technology, they say.

"We're trying very hard to disseminate this technology," says Botstein. "This is a big deal. Everybody understands the opportunity here, and it's our responsibility to make the best of it." **SM**

PABLO SERRANO