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SEPTEMBER 2003

HHMI
Howard Hughes Medical Institute Bulletin

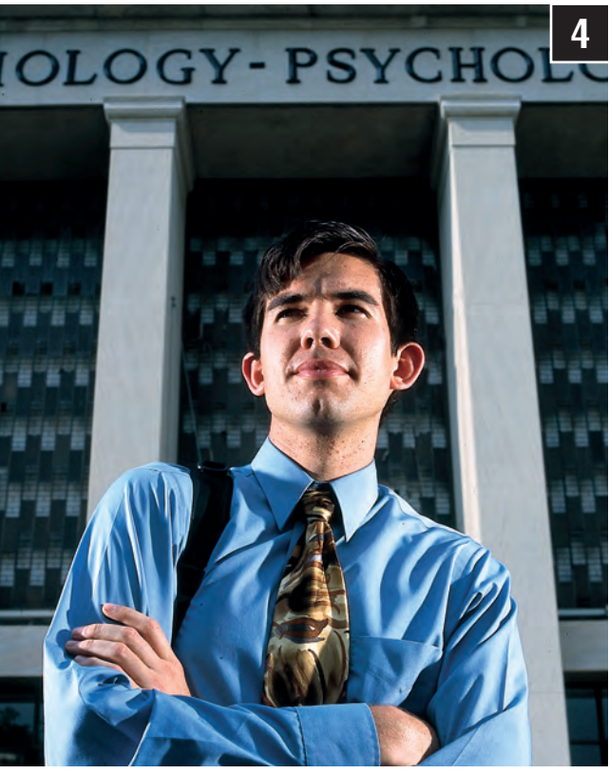
Cholesterol Up Close

It's necessary for life but can also do us harm. So how *does* the body process cholesterol?

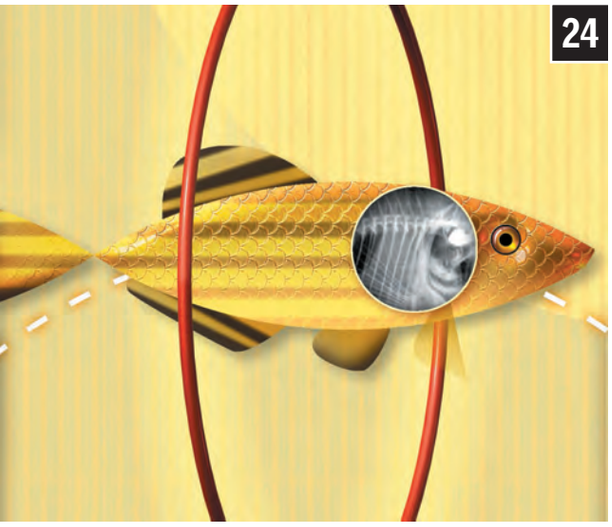
FEATURES

David C. Page (center) and colleagues, announcing the sequencing of the Y chromosome at a June press conference. Accompanying Page were Richard K. Wilson (left), director of the Genome Sequencing Center at Washington University School of Medicine, and Francis S. Collins, director of the National Human Genome Research Institute at the National Institutes of Health.

MAGGIE BARTLETT/NHGRI



D.A. PETERSON



BRYAN LEISTER

PAUL MUHLRAD

DEPARTMENTS

On the Cover: Computer graphic of part of a cholesterol molecule. Image © Alfred Pasieka/Photo Researchers, Inc.

HHMI
Howard Hughes Medical Institute Bulletin

September 2003 ‖ Volume 16 Number 3

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The *Bulletin* is published by the HHMI Office of Communications and Public Affairs.

The Science of Medicine

The girl had been a perfectly healthy child, playing and singing and otherwise acting like a typical toddler—until she turned two, when she stopped making eye contact, shied away from social interactions, ceased to communicate, and started obsessively wringing her hands.

"She made a huge impression on me," says Huda Y. Zoghbi, an HHMI investigator at Baylor College of Medicine, who was in her second year of residency when she saw this patient in 1983. What caused this sudden neurological deterioration? Zoghbi wondered. And why was she normal for so long?

With the help of a handful of affected children and their families, Zoghbi and her collaborating researchers sought answers, ultimately identifying the gene responsible for Rett syndrome, a disorder that occurs almost exclusively in females. Girls with this rare neurodegenerative disorder develop normally for about 6 to 18 months and then start to regress, losing the ability to speak, walk, and use their hands to hold, lift, or even point at things. Zoghbi's search took the better part of 16 years—an odyssey she will describe in December at this year's HHMI Holiday Lectures on Science, which have been titled "Learning from Patients: The Science of Medicine."

Zoghbi will be joined by physician Bert Vogelstein, a cancer biologist and HHMI investigator at the Sidney Kimmel Comprehensive Cancer Center at The Johns Hopkins University School of Medicine. Together they'll discuss how their patients have led them to a deeper understanding of the genetics and biology of human disease and have brought them closer to new methods for early diagnosis, disease prevention, and treatment.

The gene responsible for Rett syndrome, it turns out, encodes a protein whose activity is critical for the normal functioning of nerve cells in the brain. Mutations in this gene disrupt the activity of these neurons early in life.

"I THOUGHT IT WAS IMPORTANT"

Finding the gene for such a sporadic disease (it strikes 1 child in 15,000) was not easy, and Zoghbi didn't have much support from her scientific colleagues along the way. "A lot of people told me I was ridiculous, that I was wasting my time," she says. "After a while, I stopped telling anybody I was working on Rett." But she never gave up. "I had more negative data than you can imagine. But I thought it was important to stick with it." She felt that she could better help her patients at the lab bench than in the clinic. "Seeing these girls was so depressing," she says. "I couldn't handle having to walk in, give the parents the bad news, and walk out."

Vogelstein turned to research for similar reasons. As a physician in the 1970s, one of his first patients was a four-year-old child with leukemia. "Here was this little girl with this disease that we knew almost nothing about, like a disease from outer space," he recalls. "Her father asked good questions, but I didn't have any answers. It was very frustrating."

That feeling of helplessness sent Vogelstein to the laboratory, where he began to study colorectal cancers. These types of cancer provide an attractive target for investigation because tumors in all stages of development are common and can be easily removed for analysis. Colorectal tumors begin as benign polyps—small outgrowths in the lining of the colon. Then, as mutations occur in genes that normally balance the relative rates of cell birth and cell death, the tumors grow larger, their cells eventually breaking away and colonizing other tissues in the body. Genetic analysis of families with a hereditary predisposition to the disease, Vogelstein says, "gets you to the genes quicker."

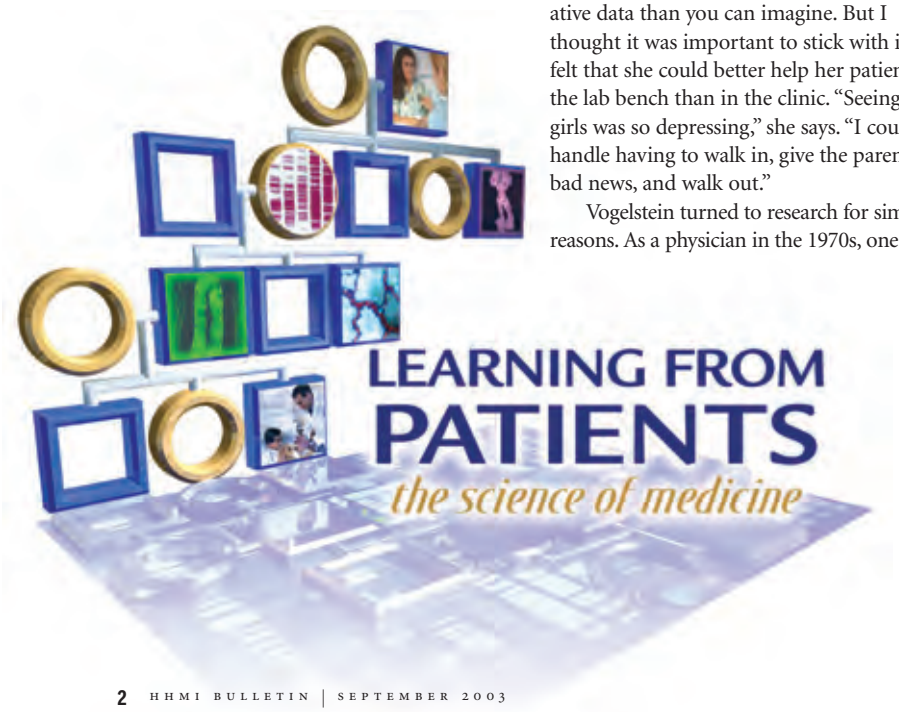
Knowing which genes are involved in a disease can lead to better methods for early diagnosis and improved treatments. Deaths from colon cancer are almost entirely preventable if the tumors are caught early. And in the case of Rett syndrome, identification of susceptible children should allow them to be treated with medications that might stave off the worst of their symptoms—a possibility that Zoghbi is testing in an animal model.

Similarly, cancer researchers hope the identification of cancer genes will lead to novel molecular therapies that prove more effective and less devastating than chemotherapy and radiation. And much the same hope applies to genes that underlie neurodegenerative diseases.

Until then, researchers like Zoghbi and Vogelstein will keep working, remembering who it is they're working for. "I used to pass through the radiation unit to get to my lab," says Vogelstein. "It was filled with patients with incurable cancer. You can't just walk through there every day and see these people and not want to find ways to help."

Zoghbi agrees. "When you see the plight of these patients and their families, how can you quit?" Fortunately for medical science, the interaction is a two-way street. "For me," says Zoghbi, "I wouldn't be where I am without my patients."

—KAREN HOPKIN



CATHERINE NEWTON/RAW SIENNA DIGITAL

PRESIDENT'S LETTER

A Farewell to Friends

PAUL FETTERS

Each issue of the *HHMI Bulletin* gives me an opportunity to reflect on an aspect of the Institute's work and the role we play in the broader scientific community. But in this letter I need to take a moment to reflect on the contributions made by two individuals—Nestor V. Santiago, HHMI's chief investment officer, and James H. Gilliam, Jr., a charter trustee of the Institute. Both men died unexpectedly this past summer. Nestor and Jim shared many attributes, among them a belief in the Institute's mission and a dedication to principle.

Nestor's death of a heart attack on June 12 came without any forewarning, and continues to occupy the daily thoughts of those of us who had the pleasure of knowing him. Beyond being a brilliant investment strategist and a much-loved mentor and friend to his staff and colleagues here at Headquarters, Nestor took special satisfaction in HHMI's accomplishments in biomedical research and science education. He loved our mission, and enjoyed performing his investment magic for such a worthy cause. In this respect, he was similar to many of the HHMI employees I have met at Headquarters and in the field—people who believe they have a great job, and who take personal satisfaction in supporting some of the world's finest medical research and education.

Nestor left us with more than pleasant memories of our many interactions with him. He left the Institute with a team of investment managers and staff who are exceptionally skilled, wonderfully committed, and highly supportive of each other. Nestor also left our endowment in wonderful shape. The seeds he and his colleagues sowed over the past three years have germinated—the HHMI endowment has increased by $1 billion from its low point last year, even though we withdrew over $500 million to fund our programs during that same period.

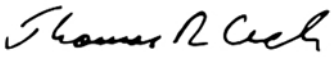
We've done something to honor Nestor's memory. The Nueva Ecija National High School in the Philippines—the school from which Nestor graduated as valedictorian—has struggled to continue its tradition of educational excellence. Some years ago, Nestor and his family created a fund to support key teachers, particularly in science and math. We've now raised over $100,000 from HHMI employees, trustees, and friends—a sum that includes a matching contribution from the Institute—to create a Nestor V. Santiago–HHMI Teacher of Science at Nueva Ecija. It's a way for us to advance a goal that Nestor cared deeply about and to do it within the context of our science education mission.

We had in no way recovered from Nestor's loss when we learned that Trustee Jim Gilliam had died unexpectedly of a heart attack on August 20. As with the loss of Nestor, Jim's death was a blow at both the personal and professional level. Jim had just been at Headquarters for the August trustees meeting and he was his usual vigorous self—smart, incisive, modest, quick with humor. Jim had been appointed to the HHMI board in 1984 by the Delaware court—along with current trustees Frank W. Gay, Hanna H. Gray, and William R. Lummis—and we had hoped to have the benefit of his judicious views for many years to come. He was chairman of the Audit and Compensation Committee, and his wise counsel and conscientious work helped shape the integrity of our business practices.

Jim was always soft-spoken but tenacious in his desire for HHMI to do more to secure better participation of underrepresented minorities in the sciences. These efforts led to our EXROP (Exceptional Research Opportunities) grants program, in which disadvantaged undergraduates from our college and university programs are matched with HHMI investigators for closely mentored research experiences. We've just finished the first year of this program, and already we're hearing back from our scientists about some real success stories. It's certainly too early to declare victory, but we're striving to make EXROP another part of the Jim Gilliam legacy.

The rest of the world knew both Nestor and Jim in different ways: Nestor as a lover of opera, sometime sailor, and adviser to the government of the Philippines; Jim as a highly respected businessman, community leader, philanthropist, and mentor to young lawyers.

Both Nestor and Jim left us far too early. But we say farewell to these remarkable friends knowing that they left a strong legacy—in their contributions to their respective workplaces, in their service to society, in their dedication to their families, and in the many friends whose lives were enriched by the privilege of having known them. Each man left a part of himself in each one of us. I can think of no more fitting way to celebrate their lives than with the knowledge that they will continue to live in our hearts and guide our work for countless days to come.



Thomas R. Cech
President
Howard Hughes Medical Institute

Up Front

Translational Science

The remarkable accomplishments of a student who happens to be deaf.

Bradley N. Buran was contemplating his answers to an early morning midterm exam in eukaryotic cell biology when his beeper began vibrating.

His parents' phone number in Rockville, Maryland, appeared on the display. The telegraphic message: "Call Jack Kent Cooke."

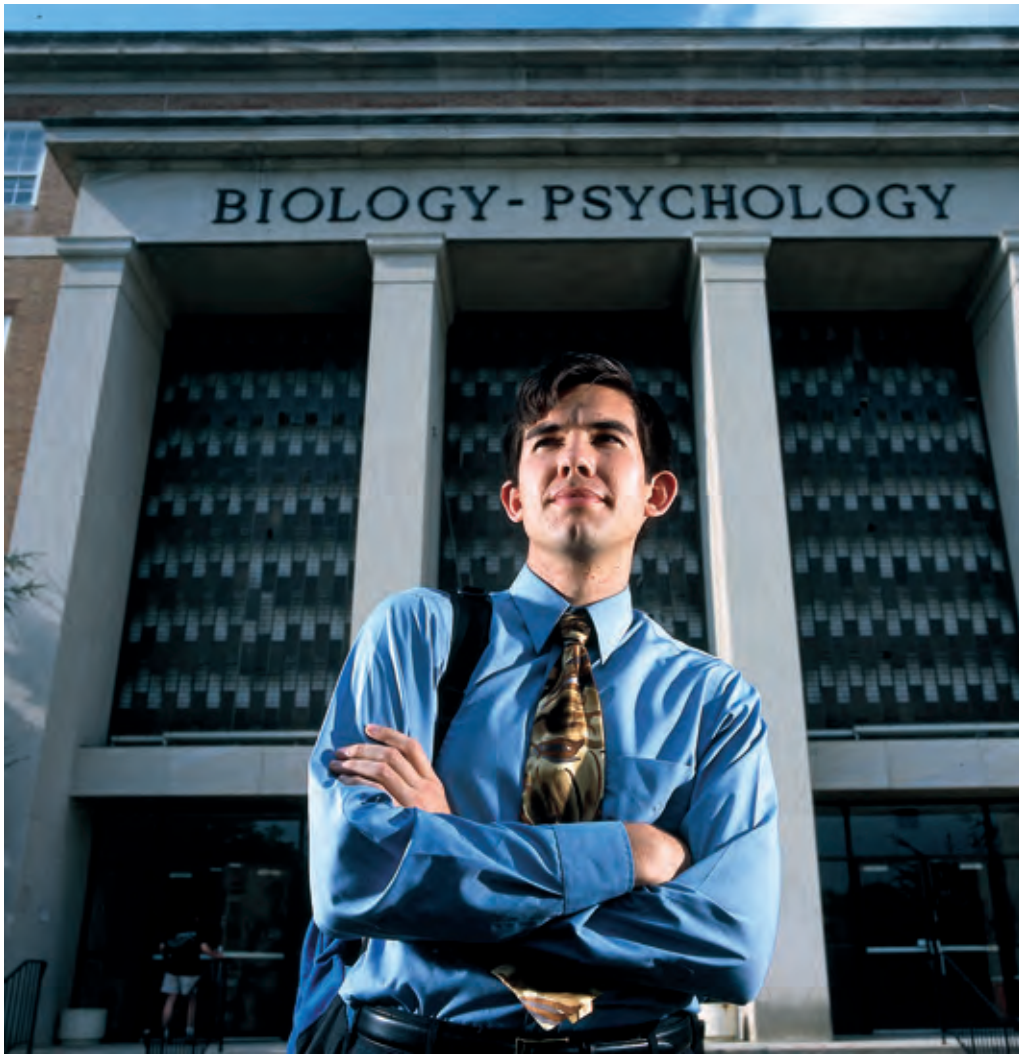
The University of Maryland senior, who is deaf, dialed the number and handed the phone to his cued language transliterator, Susan Arnold. She relayed good news. Buran, 22, an HHMI undergraduate researcher, had won a fiercely competitive Jack Kent Cooke Foundation Scholarship for graduate study. Her charge took the news serenely. "I don't suppose they'd call to tell me I *hadn't* won," he remarked.

Pneumococcal meningitis robbed Buran of his hearing when he was 14 months old, but he has never treated deafness as a handicap. In elementary and middle school, Buran was one of several deaf children, but when he entered a magnet high school, he was the only one. That didn't isolate him for long. "I just taught them sign language," he says with a grin.

Graduating with a 4.0 academic average, Buran applied to the University of Maryland and Cornell University. Both accepted him, and he chose Maryland, where he had won a full scholarship. He was planning a career in architecture, but a freshman biology class, a summer job as a biological technician with the National Park Service, and a subsequent summer doing developmental studies on the inner ear in Doris K. Wu's lab at the National Institute on Deafness and Other Communication Disorders at the National Institutes of Health changed his mind.

When Buran applied to work in Arthur N. Popper's lab at the University of Maryland, the neurobiologist nearly turned him down. "My lab had a full complement of undergraduates already," explains Popper, whose aquatic bioacoustics lab investigates hearing in fish. "Then I realized that, as auditory-system researchers, we could learn a lot from him." So he put the young man to work, "and then I realized that he was extraordinarily bright and creative as well," Popper recalls. Buran joined the lab as an undergraduate researcher in a program supported by an HHMI undergraduate science education grant.

Learning to dissect the ears of deep-sea fish was a challenge for Buran and his mentor, graduate student Xiaohong Deng. "I can only visually focus on one thing at a time," Buran explains, so he had to either watch Arnold transliterate Deng's instructions or look through the microscope at the specimen. "I couldn't talk and have him work with the specimen at the same time," says Deng, "and it was especially difficult for me because



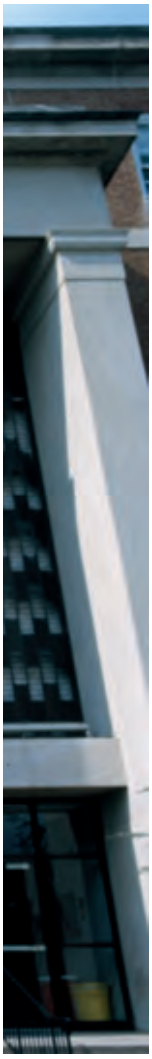
Refusing to treat his deafness as a handicap, Bradley Buran graduated from the University of Maryland with high honors.

Bradley Buran has worked with cued language transliterator Susan Arnold (right) since elementary school.

I am not a native English speaker." Also, the transliterator wasn't always there. "Brad did a lot of lipreading and talking-on-paper with people in the lab," Deng recalls. "We were all amazed that after some time, we were communicating very well."

Arnold was one of several cued language transliterators working for the Montgomery County (Maryland) Public Schools when Buran entered first grade. She transliterated for him through elementary, middle, and high school, then went to work for the University of Maryland where Buran was enrolled. Now employed by Language Matters, Inc., a Cambridge, Maryland, firm that contracts with the university, Arnold is working toward national certification as a cued language transliterator.

Cued Speech uses cues to represent the vowels and consonants of a language, as opposed to American Sign Language (ASL), in which the signs represent words or concepts. Buran points out that although he knows ASL as well, it doesn't have signs for many of the complex scientific terms.



D.A. PETERSON (2)

Macaroni and critiques

At a lunchtime lab meeting, Buran practices his honors-thesis presentation on structural variations of the inner ear of two types of deep-sea eels. Plastic refrigerator containers of macaroni in marinara sauce, pasta salad, and chunks of fish over rice litter the table as the undergraduate shows his slides and explains his findings.

The group is giving him a hard time.

"One of the goals of this research is to understand the evolution of the development of the inner ear," says Buran. "Is this really an evolutionary question, or is it an ecological question?" asks graduate student Michaela Meyer. "Ecological," Buran concedes. "I really can't say anything about the evolution of the ear from these data." Michael Smith, a postdoctoral research associate, wants to know the undergraduate's hypothesis. "I didn't start with a hypothesis," he replies. "This was a descriptive study." His mentor, Deng, suggests that he consider reworking his conclusions. "You had a bunch of stuff on your conclusion slide that you never talked about," she points out.

The comments are made kindly, and Buran takes them like a pro. "It's good feedback, and it shows they were paying attention," he says. "Actually, they went easy on me. I've seen much tougher critiques."

A week later, with his lab colleagues as a silent cheering section in the audience, the undergraduate presented his revised thesis to an approving committee, not only passing, but earning a B.S. degree with high honors in physiology and neurobiology.

In addition to attending classes and working in the lab during his senior year at Maryland, Buran taught an undergraduate honors seminar on how to do research. Half of the students were arts or business majors, "but the underlying principles of research are the same," says Buran. "They need to learn to develop a question and a strategy for approaching that question, to collect and analyze data, and to present it in a way that can be understood. It's basically just the scientific method."

As his students file in, Buran notices one of them giving the week's assignment a last-minute read. "I think I'll tell them to put the book away and take out a sheet of paper and a pen," he says. "They could use a good scare."

Work as an advocate

A Rhodes Scholar finalist, Buran has been accepted into a graduate program in speech and hearing bioscience and technology, part of the Division of Health Sciences and Technology, run jointly by Harvard University and the Massachusetts Institute of Technology. But, as much as he enjoyed his research experience, Buran doesn't think the life of a research scientist is for him. "I want to do something more applied after I get my Ph.D.," he says, "something that will combine research and working with Deaf people. I plan to get certified as a cued language instructor, and I want to work as an advocate for Deaf people and Cued Speech."

Like his scientific research, Buran's fascination with biocultural anthropology—in which he earned a second bachelor's degree—is related to his concern for the quality of life of people who cannot hear. Committed to helping hearing people understand the Deaf culture (with the word "deaf" intentionally capitalized), he founded a sign-language club at the University of Maryland, where hearing students can learn and practice. Buran also cofounded a journal, *Maryland Essays in Human Biodiversity*, with his hearing roommate Soroush Rais-Bahrami.

In the journal's first issue, the undergraduate authored a provocative essay exploring "deafness as a cultural construct rather than a pathological disorder," in which he suggests that deafness may be within the range of normal human variation. Buran writes, "To the Deaf, the cochlear implant represents another attempt at sociocultural genocide. When a deaf person obtains an implant, they are essentially rejecting their deafness as something normal and abandoning their identity as a Deaf person."

Several years ago, Buran himself received a cochlear implant, which enables him to hear environmental sounds such as a car horn or a telephone ringing, although he cannot understand human speech. "I really didn't consider the issues as much as I do now," he admits. "But I'm glad I got it. I see it as one more tool to help me interact with hearing society. It doesn't 'cure' deafness, but it does give me more information about the world around me." Disagreeing with the more radical Deaf, Buran believes it is all right for parents to get a cochlear implant for a child, "as long as they also raise that child as a Deaf person, with Cued Speech and sign language, aware of their culture and all their options."

He plans to continue editing *Maryland Essays in Human Biodiversity*. "I think it's really important for all kinds of people to have a forum where they can explore and discuss what makes us different and what makes us the same," he says.

— JENNIFER BOETH DONOVAN

Killing the Messenger

Miniscule machines shred genetic memos.

Messenger RNAs (mRNAs) have essential but abbreviated lives. The stringy molecules relay working orders from the genes to the protein-making factories of living cells. Once their genetic instructions are carried out, the messengers themselves have outlived their usefulness, and they're targeted for destruction.

Killing the messenger may well be essential to a cell's vitality. HHMI investigator Roy Parker says that destroying mRNAs is one of the quickest, surest ways for a cell to revise its fundamental purpose and moment-by-moment agenda.

Parker and Ujwal Sheth, a doctoral student in Parker's lab at the University of Arizona, recently discovered where used-up mRNAs are destroyed. They are disassembled and digested piece by piece by subcellular particles that Sheth and Parker named "P bodies." The two researchers published their findings in the May 2, 2003, issue of *Science*. Behind the scientific paper is a story of dogged work in the lab—and a little good fortune.

Death in the cell

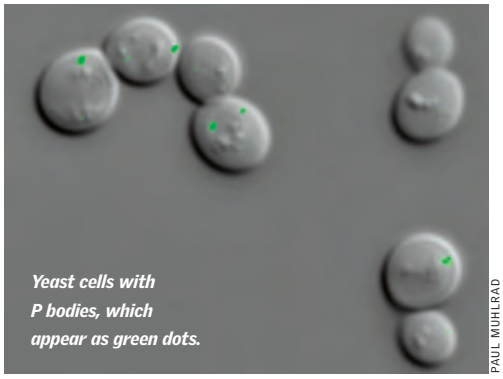
Throughout its life, a cell constantly transcribes the master instructions archived in the DNA into temporary memos in the form of mRNAs. The instructions become translated into proteins, which form the equivalent of the cell's bricks, cables, motors, and generators. Once the proteins have been made, the cell destroys the mRNAs.

Cells also have a surveillance system for searching out and expunging mRNA memos that contain certain types of mutations called nonsense codons. These memos erroneously instruct the protein-building machinery to halt production midway through the assembly of proteins. The partially formed protein molecules that result can wreak considerable havoc for the cell, possibly even killing the organism. "It's good to catch those mRNAs early in life and destroy them rapidly," Sheth says.

Using common baker's yeast, a single-celled fungus, as its study model, Parker's team has untangled the chain of events resulting in the demolition and death of an mRNA: First, the "tail" gets trimmed from the back end of the long-chained molecule. Next, a protective chemical cap is removed from the front end. With the two end features removed, the rest of the molecule gets digested piecemeal, from front to back.

Parker and colleagues had previously identified and characterized several of the enzymes that drive this process. But it was unclear where in the cell mRNAs were actually destroyed. Biologists had thought that mRNA destruction happened throughout the soupy broth of the cell. In the course of their research, Sheth and Parker discovered where the destruction really takes place.

Sheth was especially interested in an enzyme named Dhh1p, which triggers the mRNA-decapping reaction, and wanted to pinpoint the protein's position in yeast cells. To visualize the protein, she borrowed a gene from a bioluminescent jellyfish and spliced it to the gene encoding her decapping protein. When she inserted the patchwork gene into yeast cells, the cells produced many copies of the decapping protein, each tethered to its own glowing green beacon. Peering at her genetically engineered yeast through a fluorescence microscope, Sheth saw that every cell contained several small green dots. The glowing Dhh1p molecules literally illuminated their place in the cells.



Yeast cells with P bodies, which appear as green dots.

PAUL MUHLRAD

She then examined other proteins involved in mRNA degradation and found that five of them, those involved in the decapping and digestion steps, also illuminated the same spots as Dhh1p. From these and other findings, it appeared to Sheth as if mRNA destruction might occur in discrete particles rather than throughout the cell. But she still needed proof.

The clincher came when Sheth caught the particles red-handed in the act of destroying—or at least *trying* to destroy—mRNA. This time she devised an experiment to shine light on the mRNA rather than the protein. First, Sheth armed her mRNA with

JEFF TOPPING

Roy Parker (foreground) and his colleague Ujwal Sheth discovered the subcellular particles that destroy used-up messenger RNA.

degradation "barriers"—sequences that formed tight kinks in the RNA chain, making it practically impossible for the digestion enzyme to drive through and chew apart the molecule. The barriers ensured that the end of the mRNA (that is, everything past the kinks) was shielded from destruction.

Sheth predicted that the synthetic mRNAs would ultimately find their way to the proposed destruction particles, where they would become snarled because their structures would prevent them from being completely degraded. With further genetic engineering, Sheth rendered her synthetic mRNA molecule visible by tagging it with the glowing green jellyfish protein.

When she set the elaborate molecular sting operation in motion under the microscope, Sheth saw the tagged mRNA molecules trapped, as predicted, in the midst of their own demise, illuminating the same spots where she had previously witnessed the degradation proteins. This evidence was the smoking gun for mRNA destruction particles.

Parker and Sheth named these newly discovered yeast structures "P bodies," for processing bodies. They're betting that similar structures recently found in human cells serve the same purpose.

Luck in the lab

Clever as Sheth's experiments were, they were helped by a smidgen of good luck. The day her genetically engineered yeast cells were finally ready, she couldn't wait to put them under the microscope and take a peek. But to her frustration, the lab's resident microscope authority, who planned to help with the experiment, was out sick that day. Unfamiliar with the established lab procedure—a detailed regimen of treating the cells with chemicals to preserve their internal structures—Sheth went ahead anyway, simply washing the cells in water and putting a drop of the suspension on a microscope slide. "And when we looked, we saw these dots in the cells!" she gleefully recalls.

The fact that Sheth couldn't find the spots in experiments later on when using the "proper" protocol led her to realize that she had made a fortunate decision to wash the yeast cells in water before putting them under the microscope. It turns out, Parker says, that this was key to seeing the P bodies: The water removed certain nutrients from the yeast, making the P bodies grow larger, possibly by stressing the cells and causing them to go into mRNA decay overdrive. The P bodies are still there in nonstressed cells, but they're so inconspicuous that it's easy to miss them.

P bodies may have a more subtle and complex role than just wiping out mRNA. Some of the proteins contained in yeast P bodies are also found, in other organisms, in specialized granules that store mRNA for future use. Sheth and Parker suspect that P bodies may be able to act similarly. In other words, an mRNA molecule entering a P body may not be automatically condemned to immediate death. Instead, the molecule may stay there for the long term, with the possibility for early release in case it is needed again in the future. Of this stay of execution, Sheth and Parker only half joke that while the P in "P body" officially stands for "processing," it might more aptly stand for "purgatory."

Why does the cell use P bodies to destroy mRNAs? It is too soon to know the answer. For mRNA degradation, organization may simply enhance efficiency or add an element of control. P bodies may not be important for mRNA degradation per se, Parker speculates, as much as to prevent other things from happening to the mRNA that is destined for destruction, such as being translated to protein. "The more we come to understand cells," Parker says, "the more we realize that things are organized. We don't understand yet what the significance of the organization is."

—PAUL MUHLRAD

Walking the Jungles and Deserts of Chromosome 7

Searching for meaning among the genes.

Clinical geneticists lately have been making their way to Stephen W. Scherer's small office at The Centre for Applied Genomics, located in the research area of Toronto's Hospital for Sick Children (affectionately known as "Sick Kids"). They know that Scherer, an HHMI international research scholar and director of the center, plays an important role in the Human Genome Project—particularly regarding the detailed mapping of chromosome 7, which figures in numerous genetic diseases—and they need his help in using the project's results to counsel parents and would-be parents.

President Bill Clinton said that the Human Genome Project created "the most important, most wondrous map ever produced by humankind." But the clinicians seeking out Scherer had difficulty reading the map—its databases were designed by molecular biologists for other molecular biologists. Scherer believed there had to be a way to make the project more accessible.

Scherer took what he says was his first "walk along chromosome 7" in the late 1980s when, as a graduate student, he worked in the laboratory of the Sick Kids' team that identified the *CFTR* gene, mutations of which cause cystic fibrosis. Over the years, Scherer has identified other disease genes on chromosome 7, including the Sonic Hedgehog gene, which causes holoprosencephaly, a disorder marked by developmental defects in the face and brain, and those involved in Williams syndrome, which results in a range of medical and developmental problems. In a paper first published online in *Science* in April 2003, Scherer and colleagues presented the sequence of chromosome 7 in conjunction with a database that linked no fewer than 440 places on the chromosome to specific diseases. (A map of the chromosome—well, 99.4% of it—was also published in the July 10, 2003, issue of *Nature* by a team that includes HHMI investigator Sean R. Eddy of the Washington University School of Medicine.)

Of chromosome 7's many quirks, Stephen Scherer wonders, "What was evolution thinking about when it was designing this real estate?"

EVAN DION

Figuring out how the chromosome was put together was like solving a jigsaw puzzle with 158 million pieces, says Scherer, and it had other features that also confounded researchers' efforts. The chromosome has gene jungles, for example—gene-rich regions where some genes overlap—and like all chromosomes, it has gene deserts, vast stretches where there are no apparent genes. "What was evolution thinking about when it was designing this real estate?" he wonders.

Fortunately, the Chromosome 7 Project team had an advantage unique among participants in the publicly funded Human Genome Project—a deal with Celera Corporation, the project's private-sector competitor, to use its chromosome 7 databases. As Scherer explains it, the Celera data had more jigsaw-puzzle pieces, while the public consortium put together the pieces it had in a more complete way. "So in fact the two projects were very complementary," says Scherer, and by combining the data from the public and private sectors, "we had essentially all the jigsaw pieces."

Simply fitting the pieces together was just the first part of the Chromosome 7 Project, however. The challenge now is to figure out just what these pieces mean—what the genes do. Back in the 1980s, when grad-student Scherer first "walked" along the chromosome, the only way to identify the cystic fibrosis gene, for example, was to first study the genetic makeup of cystic fibrosis patients and then hunt along the chromosome's hundreds of millions of nucleotides to see whether there was a small difference between a cystic fibrosis patient's genome and that of an illness-free child. To do that, one had to walk carefully and slowly indeed.

Breakpoints

These days, the process has been reversed. The Chromosome 7 Project has identified regions of the chromosome sequence, called breakpoints, that are especially fragile and can be interrupted. Other genes have their chemical orders reversed, as if they were chemical dyslexics. Still others have genes on different parts of the chromosome than might normally be expected. Thus, now researchers first identify the breakpoint or other mutation, and then put the call out to the medical and research communities to report diseases and symptoms of people who possess these genetic markers.

More than 90 scientists in 10 countries have responded by sending this kind of information to the project. "We collected, worldwide, [data on] as many patients as we could with chromosome 7 rearrangements. They could be deletions or translocations or inversions that had defined clinical conditions," says Scherer.

The result can be seen on the Internet at *www.chr7.org*. The site is organized so that a user can click on any point or gene on the chromosome. It then lists the diseases and conditions associated with mutations of that gene.

During the first week the site was up, it received 14,000 hits. "So a lot of people are looking for this information," says Scherer. The site has also already added a hundred rearrangements and breakpoints to the 440 listed in April. "People keep sending us e-mails saying 'you missed this gene' or 'we found a new gene,' " says Scherer. "This [effort] is going through the roof."

—ED UNGAR

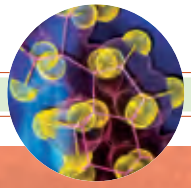
Rapid Response to SARS

Just as Scherer's team was working to publish results of the Chromosome 7 Project in the journal *Science* and on the project's Web site, severe acute respiratory syndrome (SARS) struck Toronto's hospital system. Scherer had to move the staff of his applied genomics center and draw up contingency plans to relocate its facilities. Because the center is situated in the hospital's research wing, some distance away from patient areas, its researchers were allowed to return relatively soon. This proved fortuitous, because the genome center staff was able to work around the clock to quickly sequence and share with their colleagues the first piece of the Toronto form of the virus suspected of causing SARS.

Toronto shared with Hong Kong the dubious distinction of being an epicenter of a SARS outbreak. But there was also a silver lining—Scherer's colleague and mentor, the chief investigator of the cystic fibrosis gene project at Sick Kids, is Lap-Chee Tsui, now head of the University of Hong Kong. (At the time of the cystic fibrosis project, Tsui too was an HHMI international research scholar.) Scherer's team sent essential scientific reagents and information not available in Hong Kong to the university by courier and e-mail. "So what would have otherwise taken weeks for them to get, they were getting in less than 36 hours," says Scherer. "We also helped them analyze the sequence of the Hong Kong variant, so it was kind of important that we were actually here."

LUIS ENRIQUE ASCUI/GETTY IMAGES

Wearing masks to help contain the spread of SARS, passengers from Hong Kong arrive at Singapore's Changi Airport this past April.

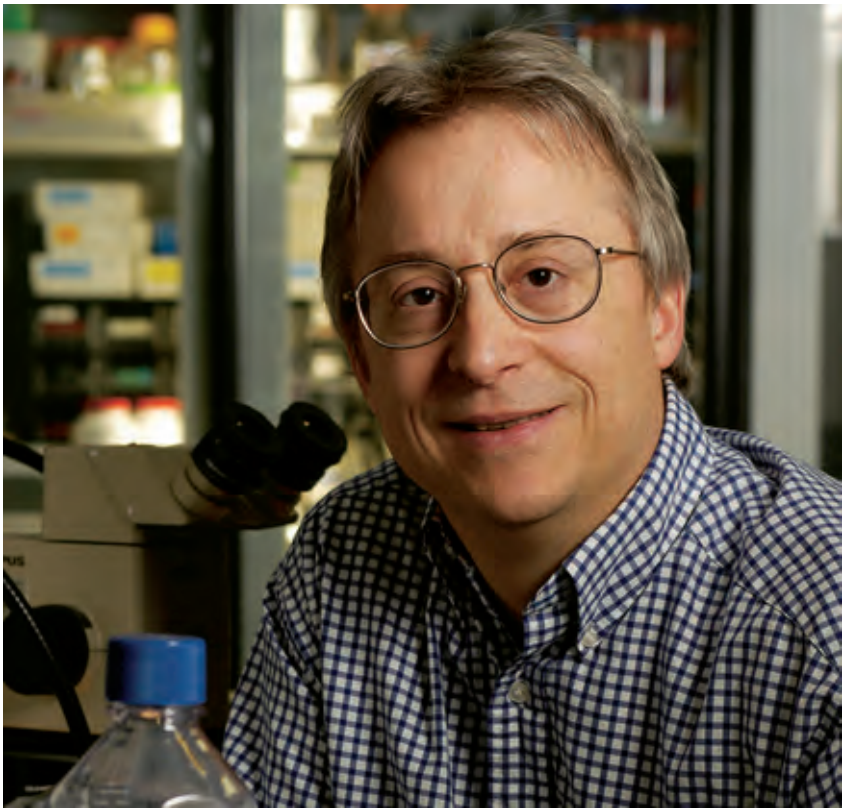


Cholesterol Up Close

Cholesterol is contradictory. On the one hand, the substance is a vital part of cell membranes, and essential for life. At the same time, an overabundance of cholesterol-carrying low-density lipoprotein—the infamous LDL—can lead to atherosclerosis and other diseases.

To help understand how the human body manages this seeming paradox, researchers look at cholesterol up close, at the cellular level. Matthew Scott, an HHMI investigator at Stanford University School of Medicine, says that "the cell has developed elegant control systems to handle cholesterol because it has such a profound impact on the properties of the cell."

In this series of articles, we take a closer look at some of the cell's elegant systems for processing cholesterol. In a related account, two Nobel Prize winners summarize the history of cholesterol research.



BILL DENISON

Cholesterol plays a very important role in organizing the cell membrane, Philip Beachy says.

Signal to Signal

Cholesterol molecules shed light on how cells communicate. By Karyn Hede

Most of us are at least a little concerned about cholesterol, but for some scientists, it's an obsession. Investigators who study cholesterol closely—deep inside the cell—are finding that it has some rather fascinating properties.

"It turns out cholesterol actually has a very important role in organizing the cell membrane," says Philip A. Beachy, an HHMI investigator at The Johns Hopkins University School of Medicine. "This is a huge area of cell biology right now, and cholesterol is an important player."

HHMI investigator Matthew P. Scott, a biologist at Stanford University School of Medicine, believes cholesterol can shed light on how cells communicate. In what he calls an "exquisite interplay," cells send signals to each other as they multiply—transmitting information that determines a cell's ultimate function and destiny. Thus, "cholesterol is arising as a very interesting molecule for signaling processes, in addition to its known role in affecting membrane properties," Scott says.

MYSTERIOUS PROTEIN

Scott, who had been studying the signaling proteins that direct body patterning during embryonic development in the fruit fly *Drosophila* and in mice, found himself drawn into the study of human disease when NPC1, one of two proteins behind a devastating disorder called Type C Niemann-Pick disease (NPC), turned out to resemble Patched, a protein that regulates development.

"When the NPC1-Patched connection was found, a new opportunity arose to learn about development from cholesterol metabolism and vice versa," says Scott. NPC1 normally helps transport excess cholesterol out of the cell. But if the protein is disrupted, as in NPC, transport processes inside the cell fail, high levels of cholesterol accumulate, and certain cells in the brain and other tissues are damaged or killed. The unfortunate few who are born with the disease rarely live past childhood, because basic bodily functions deteriorate progressively and there is no known treatment.

"The cell has developed elegant control systems to handle cholesterol because it has such a profound impact on the properties of the cell," says Scott. "But much remains to be learned about the roles of cholesterol in cell properties and signaling mechanisms. The NPC proteins are providing a way to try to understand the components of the cholesterol-processing pathway."

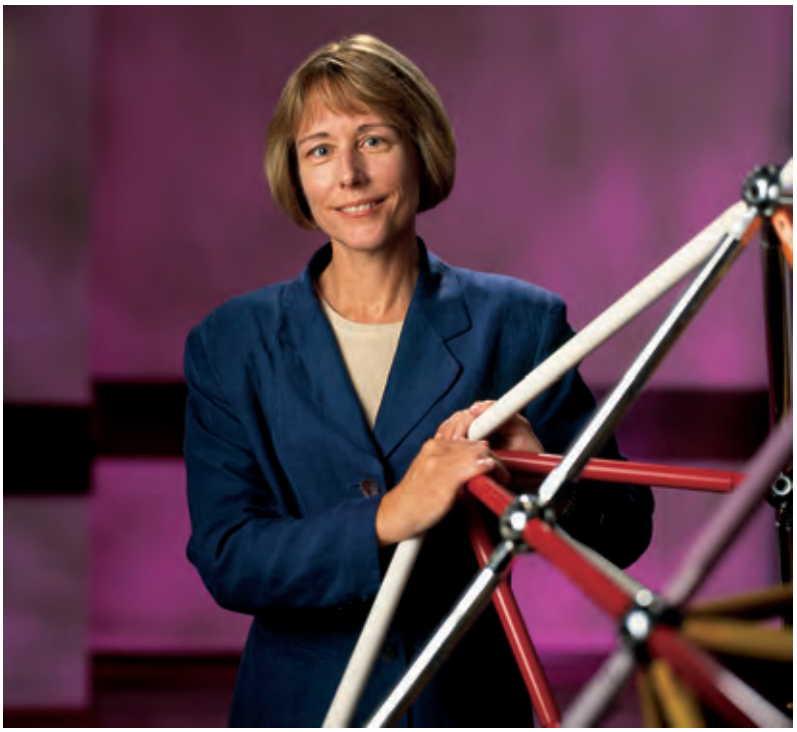
Two articles published in the March 4, 2003, issue of the *Proceedings of the National Academy of Sciences* (*PNAS*) describe the structure and cholesterol-binding properties of NPC2, the second protein that can cause NPC, which was discovered three years ago by Peter Lobel, professor of pharmacology at Robert Wood Johnson Medical School–University of Medicine and Dentistry of New Jersey (UMDNJ). NPC2 is responsible for about 5 percent of NPC cases, but apart from the fact that it can bind cholesterol, little else has been reported about it until now. Lobel recruited HHMI investigator Ann M. Stock, also at UMDNJ, to determine the crystal structure of the protein, and together the two labs reported a protein structure with unexpected features.

Other proteins known to bind cholesterol contain a large cavity. The interior of the cavity is hydrophobic, which means that it repels water, but it attracts highly insoluble molecules such as cholesterol. The hydrophobic interior of NPC2 lacks a large cavity, or pocket, that could accommodate a large molecule such as cholesterol, but it contains several small cavities. "What was a surprise to us when we determined the structure was that NPC2 lacked an obvious binding pocket," says Stock. "The structure of NPC2 is basically a sandwich of two beta sheets with an intriguing pore leading into the protein's interior. When we inspected the structure, it became obvious that this could be the binding site, but it is too small to accommodate the cholesterol molecule. The NPC2 protein must change its shape when it binds cholesterol."

Confirmation of their hypothesis came at the annual meeting of the Ara Parseghian Medical Research Foundation in 2002, when Scott and M.D.-Ph.D. student Dennis C. Ko reported results of their genetic studies of NPC2, which are now published in *PNAS* back-to-back with Stock and Lobel's work. (The Parseghian Foundation was established by the legendary Notre Dame football coach, whose three grandchildren were born with NPC. The foundation funds Lobel, Scott, and Stock's work, and its annual meeting brings together dozens of scientists whose work is focused on the disease.)

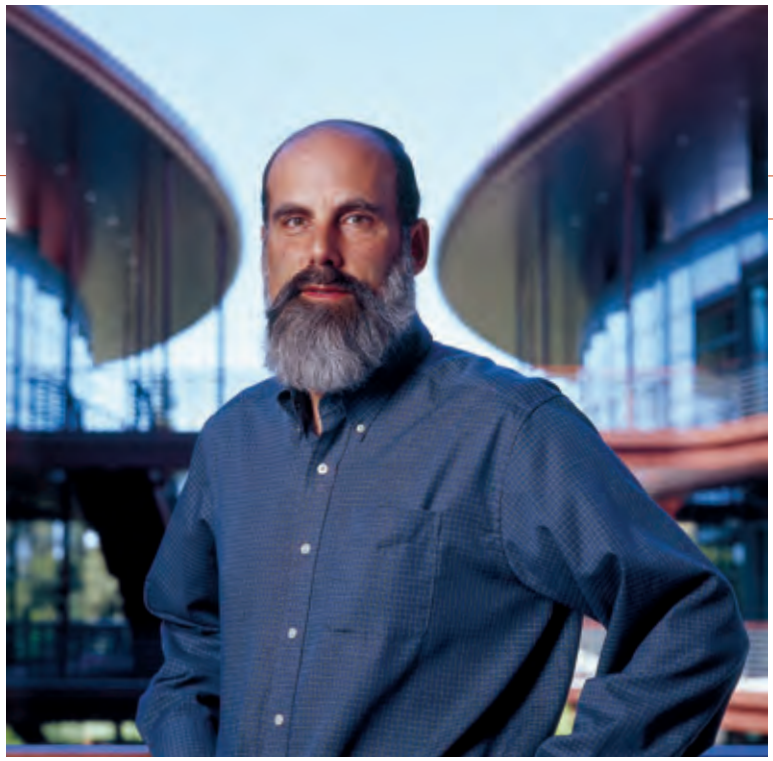
By studying evolutionarily conserved regions among NPC2 genes in several species, Ko identified and mutated several key amino acids in the protein. He then tested their ability to bind cholesterol and to reverse cholesterol buildup in cells that lack NPC2 function. "What we found were three mutations unable to bind cholesterol and also unable to rescue NPC2-deficient cells," Ko says.

The three amino acids that affect cholesterol binding when mutated are located in the same hydrophobic region of the NPC2 protein that, based on their structural work, Stock and Lobel suggested would bind cholesterol. However, although the convergence of the structural and genetic studies strengthens the case of NPC2 as a cholesterol-binding protein, "exactly what's happening isn't clear," says Stock. "Cholesterol is very insoluble, so it makes some sense that it does not exist in a free state to any significant extent. It may be passed from protein to protein inside the cell. It is logical to hypothesize that cholesterol binding by NPC2 somehow facilitates delivery of cholesterol for export out of the cell. But it is a relatively unexplored field, and there are presently far more questions than answers."



MARC BRYAN-BROWN

Ann Stock worked to determine the crystal structure of the protein behind the devastating disorder Type C Niemann-Pick disease.



ROBERT CARDIN

Matthew Scott, a biologist at Stanford University School of Medicine, believes cholesterol can shed light on how cells communicate.

PATCHED AND SMOOTHENED

Answers may come from the study of NPC1's evolutionary relative Patched and its binding partners Hedgehog and Smoothened, a trio of proteins that constitute a pathway for regulating basic body patterning during embryonic development. When Hedgehog, a secreted signaling molecule, binds to Patched on the cell surface, it releases Smoothened to transmit signals to the nucleus for eventual activation of a plethora of genes that direct cell specialization. It turns out that cholesterol is a crucial piece of this pathway.

In 1996, a seminal *Science* paper from the laboratory of Philip Beachy at Johns Hopkins showed that the Hedgehog signal is formed through an unusual autocatalytic process that involves cleavage of a precursor protein into two parts and the attachment of a cholesterol molecule to the signaling portion of the molecule.

"It was a bolt from the blue," says Beachy. "The specific modification of a protein by cholesterol is truly unique."

Beachy speculates that cholesterol's insolubility may have been advantageous during evolution to restrict the movement of the nascent differentiation signal. "You can't give an accurate accounting of what evolution was doing, but it may have been advantageous at the dawn of multicellularity to restrict Hedgehog's effects to the cell right next door," says Beachy. "Then you could have three cell types: the one that makes it, the one right next door, and the one just a little farther away. Maybe evolution co-opted this autoprocessing domain to add cholesterol and restrict the range of Hedgehog signaling."

Since his discovery of cholesterol's role in development, Beachy too has been drawn into the study of human genetic disease. He became intrigued by a family of human syndromes, the most well-known being Smith-Lemli-Opitz syndrome (SLOS), caused by a defect in the final step of cholesterol synthesis. Lack of cholesterol in these diseases leads to birth defects—such as brain and facial malformations, reduced branching of the lungs, and defects in the development of the nervous system—that resemble those associated with loss of Hedgehog signaling.

"Naturally, we thought what's probably happening in these patients is that there is not enough cholesterol, Hedgehog isn't getting made properly, and so there's a defect in the signaling," says Beachy. However, after using a mouse model of SLOS in a series of experiments, Beachy's team ruled out Hedgehog processing as the culprit. Instead, the scientists discovered that reduced cholesterol levels affect the cell's ability to *respond* to Hedgehog signals.

In a series of studies using mutant forms of Patched and Smoothened proteins in cholesterol-depleted cells, the scientists narrowed the effect to Smoothened's inability to undergo conformational change from the inactive to active form. In a paper published in the April 2003 issue of *Nature Genetics*, they showed that the level of cholesterol in the cell membrane in SLOS patients is too low to support the critical transition. "The state of the membrane must be such that Smoothened can make this transition from inactive to active, and cholesterol seems to be a key part of that," says Beachy.

But a mystery remains. The genetic and biochemical studies, says Beachy, suggest a missing piece of the puzzle—a regulator in between Patched and Smoothened—even though no such player is known to act between the two proteins.

In an article in the August 22, 2002, issue of *Nature*, Beachy and colleagues reported that Patched does not directly suppress Smoothened, as had previously been suggested. Instead, Patched may transport a small molecule, whose identity is not yet known, that affects Smoothened activity. Beachy notes that Patched and NPC1 are both related to a family of bacterial transporters called the resistance-nodulation-division proteins. Another related protein, Dispatched, is responsible for releasing the Hedgehog signal from the cell—perhaps, speculates Beachy, by releasing the cholesterol component that binds Hedgehog to the cell membrane.

"We've been studying how Patched regulates Smoothened, and we think that Patched may transport a small molecule that then regulates Smoothened," says Beachy. "That may be what's in between Patched and Smoothened. But we don't know its identity yet."

What is known is that the Hedgehog signal itself and possibly other pathway components associate with cholesterol-rich domains of the cell membrane, known as rafts, which have been implicated in several cellular processes, including signal transduction and vesicle traffic—the very process that is defective in NPC disease.

"We were all taught 20 years ago that membranes were just this sort of sea of lipids with proteins floating in them," says Beachy. "Well, it's not so. Parts of membranes are much more ordered." In fact, lipid rafts have emerged as a crucial element of many signal-transduction pathways, most prominently in white-blood-cell formation, immune-cell activation, and neuron signaling. H

Floppy Loops and Flexible Tails

Exploring the gateway that processes "bad" cholesterol. By Renee Twombly

On the street, LDL (low-density lipoprotein) is known as bad cholesterol. While cholesterol itself is essential for life—it's a vital part of cell membranes—an overabundance of LDL in the blood can help lead to atherosclerosis and other diseases.

To better understand how the body normally regulates the buildup of bad cholesterol, researchers at the University of Texas Southwestern Medical Center at Dallas are exploring the gateway created by the LDL receptor, which helps regulate levels of cholesterol in the blood. HHMI investigator Johann Deisenhofer, who shared the Nobel Prize in Chemistry in 1988, leads the team.

Deisenhofer and colleagues wanted to know how the LDL receptor lets go of LDL after snatching it from the bloodstream and dragging it inside a cell, so that the cholesterol transported by the LDL can be used by the cell.

To learn more about this receptor's structure and functions, the researchers decided to use x-ray crystallography to make a three-dimensional image of the LDL receptor. They therefore had to grow crystals

REID HORN

Johann Deisenhofer, who shared a Nobel Prize in 1988, and colleague Gabrielle Rudenko used x-ray crystallography to make a 3-D image of the LDL receptor.

of the LDL receptor—a task that took Gabrielle Rudenko, an instructor of biochemistry who heads the team in Deisenhofer's lab, no less than six years. X-ray crystallography requires that proteins with the same shape be neatly stacked together into a crystal. When x-rays are beamed at the crystal, electrons diffract the x-rays, which creates a pattern that is used to reveal the protein's atomic structure. But this was incredibly difficult, Rudenko says, because the receptor "is inherently very flexible, with lots of floppy loops and flexible tails."

Rudenko finally found the right chemical conditions to produce a baker's dozen of identical receptors and then took a "snapshot." What she and Deisenhofer saw, and published December 20, 2002 in *Science*, is a 3-D picture of what the researchers think the receptor looks like after releasing LDL.

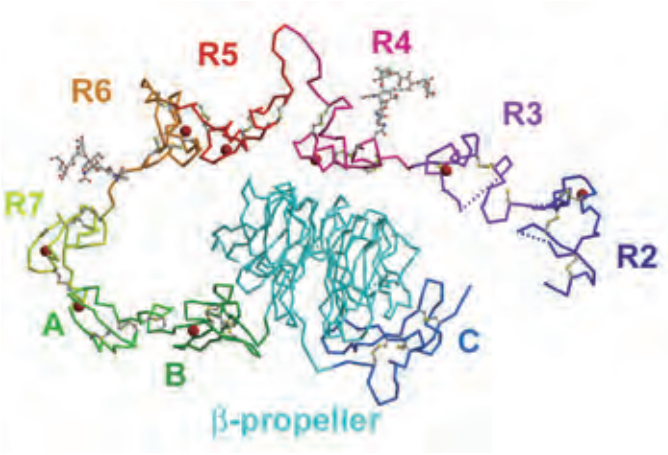
At neutral pH (like the pH in blood), the LDL receptor binds LDL on the cell surface. In this state, the receptor is likely to look "long and floppy and all of the modules are aligned like a long string of beads," Rudenko says.

However, once internalized in the cell, in a compartment with acidic pH, the LDL receptor seems to snap shut, releasing LDL again. In this state, it acts something like a folding cellphone that can be snapped together to shield its buttons; the receptor similarly doubles over on itself to cover its binding domains. The receptor is then effectively closed, and the LDL is free to be taken apart elsewhere for use by the cell. The receptor recycles back to the cell surface, ready for new duty.

"You want receptors to bind tightly, but then let go of their cargo at the right moment. The system around the LDL receptor does this by decreasing pH and generating an internal competition for the ligand-binding site," Deisenhofer says. "I would expect to see this kind of action in a lot of receptors that transport molecules into the cell."

Deisenhofer's new findings relate to some of his earlier research on how statin drugs work to reduce cholesterol. As reported earlier in *Science* (May 11, 2001), Deisenhofer and colleagues used x-ray crystallography to show how six different statin compounds—such as atorvastatin and simvastatin—inhibit the liver enzyme HMG-CoA reductase, which catalyzes a key step in cholesterol production.

The scientists are now working to understand how alterations in the amino acid sequence in critical regions of the LDL receptor might cause familial hypercholesterolemia, a common inherited disease marked by high cholesterol levels, atherosclerosis, and increased risk of a heart attack early in life. H



Like a String of Beads

Schematic of the extracellular domain of the LDL receptor as it is seen at acidic pH in the crystal structure. The ligand binding region (R2–R7) is folded back over the rest of the molecule, and the epitopes R4 and R5, important sites for binding LDL, are buried against the β-propeller, rendering them inaccessible.

A New Angle on Cholesterol

In the fight against heart disease, researchers have their eye on a protein called LXR that may be a master switch for cholesterol. By Robert Kuska

Marketed under brand names such as Lipitor and Zocor, the statin drugs help many patients keep their cholesterol in check. But this standard treatment doesn't work for everyone.

"People are still dying [from heart disease] even though they're on cholesterol-lowering drugs," says Helen H. Hobbs, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas and director of the Dallas Heart Disease Prevention Project, a study of heart disease in a population of 6,000 individuals. "Until Americans decide to give up their hamburgers and French fries, we have to figure out how to interrupt this disease in other ways."

Searching for new approaches, scientists are looking at the problem of cholesterol from a different angle. Rather than focusing on therapies that target production of cholesterol, which is essentially what statins do, researchers are concentrating on the way the body gets rid of surplus cholesterol—and getting promising results.

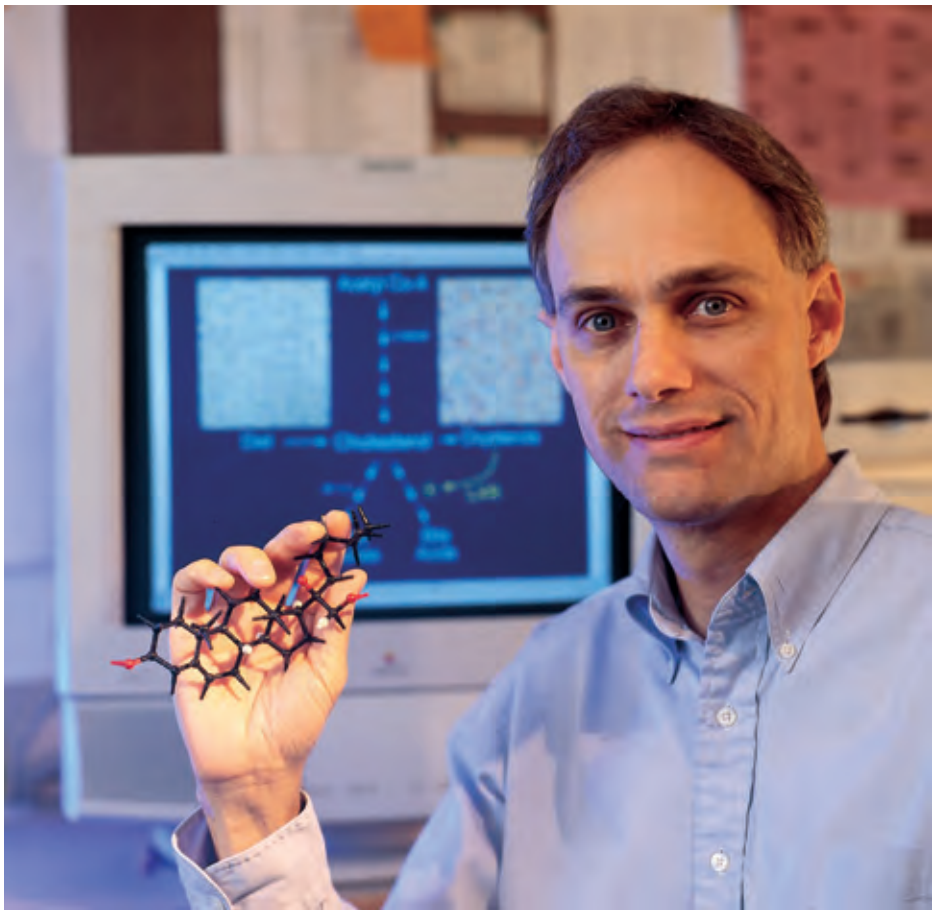
An important breakthrough came not long ago when researchers identified a unique protein, called LXR (for liver X receptor), which appears to serve as a cellular "master switch" for removing excess cholesterol. A logical next step: Try to develop synthetic compounds that might control this intriguing switch—and in turn control the leading culprit in heart disease.

Cholesterol's Bad Rap

Despite its rotten reputation in the doctor's office, cholesterol is essential to human health. Cells insert cholesterol into their membranes to help control which substances enter and leave the cells. Cholesterol also is the sole precursor of steroid hormones such as testosterone and estradiol, certain vitamins, and the bile acids in the liver.

How much cholesterol we need varies from person to person. Researchers estimate that a 155-pound male has about 100 grams of cholesterol in his body. Each day, the liver and other tissues produce 600 to 900 milligrams of cholesterol to meet the body's routine daily demands for the substance. We get into trouble when we have an excess of this tough-to-metabolize substance—which the body cannot entirely get rid of.

The liver, being the only organ equipped to break down cholesterol efficiently, serves as a centralized treatment plant. However, it's



DOUG HANDEL

David Mangelsdorf studies which genes and proteins are involved in the cholesterol-transport process.

limited by the countervailing effects of two distinct types of lipoprotein, the form in which cholesterol is carried in the blood. High-density lipoprotein (HDL), the so-called "good" cholesterol, picks up globules of cholesterol that cells have pumped to their outer membranes as if they have dropped off a bag of garbage at the curb. The HDLs then transport the cholesterol to the liver, where it is broken down in the bile. But low-density lipoprotein (LDL), or "bad" cholesterol, functions in an opposite fashion, bringing cholesterol from the liver into the body's cells.

The effects are not surprising. "Studies tell us that high levels of LDL and low levels of HDL are associated with atherosclerosis," says Hobbs. "Conversely, we know that if you lower plasma levels of LDL you have a profound [preventive] effect on the development of heart disease and a profound effect on reducing the incidence of coronary events in people with established heart disease."

REVERSE TRANSPORT

Scientists have long hoped to define precisely how cells pump the excess cholesterol to their membranes—a process that's commonly called "reverse transport"—for pickup and disposal. That knowledge could pave the way for the design of drugs to manipulate the process. (Statins, the current drugs of choice, largely target an enzyme involved in the body's own production of cholesterol, a completely different cellular process than reverse transport.) About four years ago, a team of scientists provided an important first clue to the reverse-transport puzzle when they showed that a protein called ABCA1 transfers cholesterol from the cell membrane to HDL. This raised an obvious next question: What activates ABCA1?

The answer came soon thereafter from the laboratory of David J. Mangelsdorf, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas. As a postdoc, Mangelsdorf had cloned the genes of a dozen previously unknown protein receptors in the cell nucleus. Because the functions of these assumed hormone-binding proteins were unknown, scientists referred to them as "orphan nuclear receptors." Later, he discovered that one of these orphans—LXR—bound cholesterol metabolites.

Given that most nuclear receptors, once saturated with their activating ligand, signal for the transcription of specific genes, Mangelsdorf followed up with a series of experiments to determine which genes LXR mobilizes; the hope was that this information would lead to genes and proteins directly involved in the cholesterol-transport process. He observed that when mice were given small molecules that stimulated the LXR receptor, their production of ABCA1 was markedly increased. Although the details have yet to be fully worked out, this result suggests that an LXR-stimulating drug would increase cholesterol transport and boost HDL levels, both beneficial effects.

Actually, as Mangelsdorf and colleagues would show, this was just the tip of the LXR iceberg. In these same mice, the scientists noted a prominent decrease in cholesterol absorption. The explanation was that LXR signals ABCA1 to pump out the cholesterol in the intestine, thereby preventing it from being absorbed into the blood. Though this hypothesis has not been confirmed, it suggests that LXR has the potential to limit the absorption of dietary cholesterol, another greatly desired effect.

In the liver, the data are equally striking. Mangelsdorf reported that LXR activates a gene whose protein plays a key role in the synthesis of bile acids, meaning a ramping up of cholesterol degradation—another positive effect. There is also evidence that LXR activates other genes that transport cholesterol into the bile.

Then there is LXR's beneficial effect on macrophages, a type of white blood cell that ingests foreign material. Peter Tontonoz, an HHMI investigator at the University of California, Los Angeles, and colleagues have published a series of articles showing that LXR aids in the efflux of cholesterol from macrophages, presumably by activating ABCA1 proteins. This discovery has major implications because cholesterol-laden macrophages contribute to the formation of foam cells, a fundamental component of artery-clogging plaques. In addition, the group has

TOM KELLER

Peter Tontonoz thinks LXR-modulating drugs and statins could be combined in the fight aganist cholesterol.

reported that LXR induces macrophages to produce apolipoprotein E, a plasma protein that has a protective effect against atherosclerosis.

COMPLEX INTERPLAY

These and additional studies show that LXR plays a key role in reverse transport and in ancillary processes as well. They also suggest that the pharmaceutical company that corners the LXR market could stand to benefit handsomely.

There seems to be a big obstacle, however. Mangelsdorf has shown that LXR wears a second metabolic hat: It regulates the metabolism of triglycerides, the body's store of fatty acids that constitute a major energy source. This has raised fears that although LXR-targeted synthetic drugs might flush out the extra cholesterol, they might also cause blood fatty acid levels to spike, which can lead to health problems such as pancreatitis.

"Perhaps the LXR linkage of the two is a way to coordinate the balance between triglycerides and cholesterol," speculates Ronald M. Evans, an HHMI investigator at The Salk Institute for Biological Studies. "They are both packaged and trafficked around in the LDL and VLDL [very low density lipoprotein] particles, which constitute the major delivery systems for lipids throughout the body. So it may not be inappropriate, biologically, for them to be linked. Interestingly, internalization of these particles and their lipids is controlled by another set of nuclear receptors, termed PPARs" (see article on page 17). A mentor to both Mangelsdorf and Tontonoz, Evans is the father of "reverse endocrinology," an approach that led to Mangelsdorf's discovery of LXR.

Many scientists say they are cautiously optimistic that the problem of balance will one day be solved. But first they have to fill in the blanks in the molecular chain of events that activate LXR. "Cholesterol homeostasis does not occur in a vacuum," says Evans. "The physical links between cholesterol and triglycerides in lipoprotein particles reflect a more global coordination in which other nuclear receptors, along with LXR, control the ebb and flow of metabolic energy. Understanding the logic of this molecular circuit is key."

Referring to the many cellular signals, or pathways, activated by LXR, Mangelsdorf notes that "a signaling network resembles the arms of an octopus, all branching out in various directions but all feeding back in some way to one central thing. The question is: What is that central thing? If answered, simplicity will emerge from the complexity."

Meanwhile, researchers have learned the identity of the target genes that LXRs regulate and that are responsible for the undesired increase in fatty acids and triglycerides. According to Mangelsdorf, the ability of LXRs to target both "good" genes (that lower cholesterol) and "bad" genes (that raise fatty acids and triglycerides) may be exploited by the development of new drugs that selectively activate expression of only the good genes.

He notes, moreover, that the idea of selectively activating a receptor is not without precedent. Cancer researchers have done it for years with tamoxifen, which selectively binds to the estrogen receptor to treat or prevent breast cancer. "The fact that tamoxifen, raloxifene, and estrogen have different activities in different tissues is important," Mangelsdorf says. "It suggests that by giving a selective modulator that's tissue-specific, it might be possible to dial out the bad effects and keep the good effects."

Tontonoz adds that because LXR-modulating drugs and statins target different cellular pathways, they conceivably could be combined to provide a one-two punch to clear out excess cholesterol. He acknowledges, though, that work has only just begun on LXR and the application of its cholesterol-lowering capabilities. "There's a complex interplay between diet, environment, immune responses, and genetic predispositions that will require a lot of investigation," Tontonoz says. "Right now, we're at the point where we can look at individual pathways, but the bigger picture is how all of the pieces fit together—why different people exposed to those same pathways have different responses."

Using the metaphor that the body is a factory, it could be said that the statin drugs fight cholesterol on the assembly line, while LXR helps take it out with the trash. If researchers can ultimately craft therapeutics that adapt LXR's approach, this different angle on controlling cholesterol may be a key to solving the pervasive problem of heart disease.

Searching for the Fat Switch

Can we control fat by controlling metabolism?

By Karen F. Schmidt

Like our earliest ancestors, we metabolize food to prepare for both feast and famine. The body stores energy as fat when food is plentiful and burns it later when food is scarce. But we're only starting to learn the details of how that transition occurs.

A key molecular switch appears to regulate the critical balance between fat storage and burning—a finding that could lead to treatments for obesity, cardiovascular disease, and diabetes. Ronald M. Evans, an HHMI investigator at The Salk Institute for Biological Studies in California, has identified this "fat switch" as PPARδ, a nuclear receptor in the family known as peroxisome-proliferator-activated receptors. (Nuclear receptors are activated by hormonal fats—fat derivatives that regulate cellular function much as hormones do—which trigger them to turn on specific genes.) Two other PPARs were already known to play a role in fat metabolism. Indeed, drugs activating these two receptors are used to treat hyperlipidemia—a condition characterized by elevated levels of lipids, including cholesterol, in the bloodstream—and type 2 diabetes. But the function of PPARδ remained a mystery, largely because no ligand had been found to activate it.

Evan's group got around this problem by creating transgenic mice with PPARδ receptors that were permanently activated in fat cells. In the April 18, 2003, issue of *Cell*, his team describes its experiments and findings on PPARδ over seven years. Remarkably, young mice with activated PPARδ weighed 20 percent less than normal counterparts on the same diet, and by 1 year of age, they were 35 percent lighter. Moreover, the transgenic mice were protected against weight gain on a high-calorie, high-fat diet, while normal mice became obese. In addition, in 2001, Glaxo researchers discovered a chemical to activate PPARδ. Evans's group gave the chemical to obese mice that ate all the time because of a defect in leptin, an appetite regulator, and the mice lost weight. "When you activate this fat switch, you increase fat burning," concludes Evans.

MISHA GRAVENOR

Investigator Ronald Evans identified a key molecular switch that appears to regulate fat storage and burning.

These results raise the possibility of a new approach to obesity treatment—"losing weight by controlling metabolism, rather than behavior," says Evans—which has pharmaceutical companies such as Lilly and GlaxoSmithKline eagerly searching for drugs to activate PPARδ. "The exciting thing about nuclear receptors such as PPARs is that we know ligands for them can be drugs and there's never a problem with absorption in the body," notes Mitchell A. Lazar, director of the Penn Diabetes Center at the University of Pennsylvania School of Medicine.

Lazar, who also studies PPARs, nevertheless cautions that there are many remaining questions about PPARδ. For example, does tilting the balance toward fat burning raise body temperature? Does PPARδ, which is ubiquitous throughout the body, play different roles in different tissues? Would a ligand for PPARδ activate the other PPARs as well?

As he pursues answers to these and related questions, Evans is optimistic about ultimate success—though with possible qualifications. "I am convinced that we can activate fat metabolism in people," he says. "The issue now is whether it will show a favorable safety profile over a long period of time." H

Cholesterol: A Century of Research

Nearly 100 years of research on atherosclerosis has taken us from merely recognizing the disease to understanding its cause and producing an effective therapeutic approach.

By Joseph L. Goldstein and Michael S. Brown

Cholesterol is essential for the functioning of all human organs, but it is nevertheless the cause of coronary heart disease—a condition that is responsible for more than one-third of all deaths in the Western world. It is in fact the number one killer in the United States and in other industrialized nations.

Over the course of nearly a century of investigation, researchers have developed four lines of evidence—experimental, genetic, epidemiologic, and therapeutic—that irrefutably established the causal connection between cholesterol-carrying low-density lipoprotein (LDL) and atherosclerosis. Building on that knowledge, scientists have been successful in developing an effective course of therapy—the statin drugs.

Few other major diseases have been subject to such intensive and ultimately fruitful research. Here, briefly, is how the history of cholesterol research unfolded.

THE EXPERIMENTAL EVIDENCE

The first hint that cholesterol was related to atherosclerosis goes back to 1910, when the German chemist Adolph Windaus reported that atherosclerotic plaques from aortas of human subjects contained 20- to 26-fold higher concentrations of cholesterol than did normal aortas. Three years later, the Russian pathologist Nikolai Anitschov fed pure cholesterol to rabbits, which produced marked hypercholesterolemia and severe atherosclerosis of the aorta. This was the first experimental production of atherosclerosis, and Anitschov's experiment has been repeated many thousands of times ever since in virtually every animal species from pigeons to humans.

Windaus and Anitschov studied aortic plaques rather than the coronary artery plaques that are responsible for heart attacks. Aortic plaques in humans had been noted by 19th-century pathologists, who believed that coronary artery plaques were rare; they also believed that when a thrombotic occlusion of an atherosclerotic plaque did occur in a coronary artery, it was always a fatal event. This view persisted until 1918, when the syndrome of nonfatal myocardial infarction was recognized by James Herrick, a Chicago clinician, who made the first use of the electrocardiograph to diagnose heart attacks in patients who presented with crushing chest pain. Herrick provided the first clear demonstration that thrombosis of a coronary artery was not always fatal and that coronary heart disease was responsible for the acute chest pain that had been previously ascribed to all kinds of causes, from indigestion to apoplexy.

Joseph L. Goldstein and Michael S. Brown shared the 1985 Nobel Prize in Physiology or Medicine for their research on the mechanism underlying cholesterol metabolism.

THE GENETIC EVIDENCE

The genetic connection between cholesterol and heart attacks was first made in 1938 by Norwegian clinician Carl Müller, who described several large families in which high blood-cholesterol levels and premature heart attacks together were an inherited trait. The genetic understanding of this syndrome, which came to be known as familial hypercholesterolemia (FH), was greatly advanced 25 years later by the astute observations of Lebanese clinician Avedis K. Khachadurian, who delineated two clinically distinct forms of FH in inbred families—the *homozygous* form, in which affected individuals manifest severe hypercholesterolemia at birth (with plasma cholesterol levels of about 800 mg/dl) and heart attacks that occur as early as 5 years of age, and the *heterozygous* form, characterized by levels in the 300– to 400–mg/dl range and premature heart attacks that occur typically between 35 and 60 years of age. The incidence of heart attacks in children with homozygous FH provided strong genetic evidence that hypercholesterolemia alone can produce atherosclerosis.

The mounting clinical interest in cholesterol led to an intense effort in the 1950s to determine the process by which cholesterol was synthesized in the body. Most of the crucial steps in this complex pathway, involving 30 enzymatic reactions, were worked out by four biochemists—Konrad E. Bloch, Feodor Lynen, John Cornforth, and George Popják—in a triumph of technical virtuosity that combined organic

REID HORN

Nobel Prize winners Joseph Goldstein (left) and Michael Brown provided the first molecular link between LDL cholesterol and atherosclerosis.

chemistry, enzymology, and one of the earliest uses of radioisotopes. The major outlines of this pathway were completed by 1960.

THE EPIDEMIOLOGIC EVIDENCE

The epidemiologic side of the cholesterol-coronary connection unfolded in 1955 when John Gofman, a biophysicist at the University of California at Berkeley, used the newly developed ultracentrifuge to separate plasma lipoproteins by flotation. Gofman found not only that heart attacks correlated with elevated levels of cholesterol but also that the cholesterol was contained in one lipoprotein particle, LDL. Gofman also observed that heart attacks were less frequent when the blood contained elevated levels of another cholesterol-carrying lipoprotein, high-density lipoprotein (HDL).

The epidemiologic connection between blood cholesterol and coronary atherosclerosis was firmly established by a physiologist at the University of Minnesota, Ancel Keys, whose classic Seven Countries Study showed that the incidence of heart attacks in 15,000 middle-aged men followed for 10 years was linearly proportional to the blood level of cholesterol. Keys also found that the cholesterol level rose in proportion to the saturated-fat content of the diet. Men living in eastern Finland, where the mean cholesterol level was 260 mg/dl, had eight times more coronary deaths in a 10-year period than men living in a Japanese fishing village where the mean cholesterol level was 165 mg/dl. Men living in Italy, where the mean cholesterol level (200 mg/dl) was intermediate between that of Japan and eastern Finland, had three times fewer coronary deaths than in Finland and three times more than in Japan. Subsequent studies showed that this wide range of cholesterol levels resulted from a correspondingly wide variation of LDL levels in the blood.

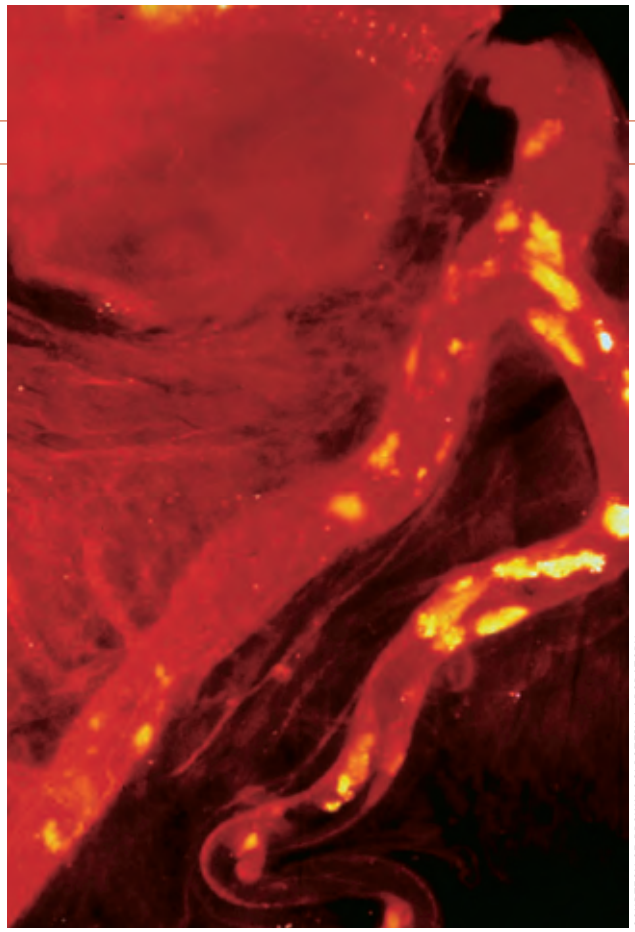
In 1974, the authors of this article discovered that the level of LDL in blood is controlled by the activity of a cell-surface protein we called the LDL receptor, which binds LDL and delivers it to cells where the lipoprotein is degraded; the cholesterol is then used there for metabolic and structural purposes. We also found that FH is caused by genetic defects in this receptor that ultimately block removal of LDL from the blood. These studies provided the first molecular link between LDL cholesterol and atherosclerosis.

THE THERAPEUTIC EVIDENCE

In 1976 Akira Endo, a Japanese scientist at the Sankyo Co. Ltd., discovered a fungal metabolite that could block cholesterol synthesis by inhibiting the enzyme HMG-CoA reductase. This discovery led to the first statin. In collaboration with Endo, we showed that the inhibition of cholesterol synthesis led to an up-regulation of LDL receptors, which explained how these drugs could selectively lower LDL, the bad cholesterol, without lowering HDL, the good cholesterol. We encouraged Merck & Co. Inc. to develop these drugs for therapeutic use, and in 1986 the FDA approved the first statin for human consumption. In 2003, more than 25 million people worldwide will take statins.

In 1994 the landmark "4S" (Scandinavian Simvastatin Survival Study) was completed. Sponsored by Merck and conducted by physicians in four Scandinavian countries, it showed for the first time that statins, by lowering LDL levels, could not only prevent myocardial infarctions but could generally prolong life. In several large multicenter trials, involving nearly 50,000 people followed for three to five years, treatment with statins lowered LDL levels by 25–35 percent and reduced the frequency of heart attacks by 25–30 percent—even in high-risk people who had "normal" LDL levels at entry. In these individuals, the high risk came from other predisposing conditions such as chronic smoking, hypertension, or diabetes. They benefited from statin therapy presumably because the predisposing conditions render the coronary arteries prone to inflammation at LDL levels considered "normal" in Western societies.

After nearly 100 years of exploration, we now have four lines of persuasive evidence—experimental, genetic, epidemiologic, and therapeutic—that implicate the cholesterol-carrying LDL particle as the primary cause of atherosclerosis. Very few, if any, chronic diseases of adults have ever been subjected to such intensive research, and in very few, if any, chronic diseases of adults has the cause been so convincingly demonstrated in so many ways. H



COURTESY OF BROWN-GOLDSTEIN LABORATORY

Clogged Arteries

Coronary arteries of a 50-year-old man who died of a myocardial infarction. They have been opened lengthwise to reveal the yellow deposits of cholesterol that constitute the hallmark of atherosclerotic plaques. Under ordinary conditions, the cholesterol-carrying particles (called low-density lipoproteins, or LDL) in the bloodstream infiltrate the tissue of the artery wall, where they are partially metabolized and some become oxidized. These oxidized lipids start an inflammatory reaction that leads to cytokine release, tissue damage, and scarring. The result over many decades is the buildup of atherosclerotic plaques that narrow the channel of the coronary artery. The process is accelerated by risk factors such as smoking, hypertension, and diabetes. For reasons apparently related to inflammation, some plaques become unstable. They ultimately rupture, leading to the formation of a blood clot—a thrombosis—that blocks the blood flow in the artery, producing myocardial infarction.

Y'S GUY

DAVID PAGE SAYS THE Y CHROMOSOME HAS A FEW SURPRISES UP ITS SLEEVE. BY RICHARD SALTUS

TWENTY-TWO YEARS AGO, David C. Page went fishing for DNA. Poking randomly at a collection of human DNA fragments in a laboratory gene-mapping experiment, the young medical student happened to pick up a Y-chromosome fragment. "When people ask me how I chose the Y to study," Page deadpans, "the correct answer is 'with a toothpick.' " ¶ Chance may have chosen the direction, but rampant curiosity and a measure of stubbornness have since propelled Page's quest to understand the Y—a strange, shrunken chromosome, once dismissed by many as a genetic wasteland too barren to be worth studying. ¶ Page, an HHMI investigator at the Massachusetts Intsitute of Technology's Whitehead Institute for Biomedical Research, has repeatedly proven the skeptics wrong. Now his odyssey has reached its most dramatic point yet: In the June 19, 2003, issue of *Nature*, a 40-person team led by Page reported on the decoding of the DNA sequence of the "male-specific," or MSY, region that makes up 95 percent of the Y chromosome. The MSY region

PHOTOGRAPH BY KATHLEEN DOOHER

David Page studies the Y—a strange, shrunken chromosome, once dismissed by many as a genetic wasteland too barren to merit much attention.

contains the gene that determines an embryo's sex—and was once thought good for little else.

DIVERSE LANDSCAPE

The MSY sequence was wrested from confusing arrays of 23 million letters of genetic code (nucleotides, or base pairs) in a marathon effort that one commentator, Huntington F. Willard, at Duke University's Institute for Genome Sciences and Policy, termed "heroic." A team of sequencing experts led by Richard K. Wilson at Washington University School of Medicine in St. Louis needed more than two years to sort out the maze of DNA sequence patterns in the MSY region. The effort was handsomely repaid: Page and colleagues have described a diverse landscape on the Y that contains 78 important protein-coding genes—hardly a genetic wasteland—and some surprising chromosomal features. The effort has "helped us rethink and throw off so many old, inaccurate ideas about the Y chromosome all at once," Page says. "We had glimpses of these things over the last six or eight years, but it took the complete sequence to show convincingly that these were not just anecdotes, but that they were the themes."

Each theme is a story in itself: how the Y chromosome and its female partner, the X, were once nearly identical but lost the ability to exchange most of their genes and went separate ways; how the Y dwindled to a fraction of its former size, gaining the reputation of a "rotting chromosome," yet harbors a critical set of genes for sperm production; the finding of an unexpected mechanism that enables the lonely Y to exchange DNA within itself (for the most part, it cannot exchange genes with the X chromosome); and the recognition that males and females differ genetically to a far greater degree than had previously been supposed—something that is certain to stir debate and further research.

Page gives much credit to two colleagues in his lab, computational biologist Helen Skaletsky and pediatric endocrinologist Tomoko Kuroda-Kawaguchi, for their zealous, almost obsessive, attention to detail in deciphering the meaning of the Y sequence. "Most people would either have given up or found shortcuts, and then we wouldn't know what we know," Page says. Another colleague, Steve Rozen, led the work that revealed the Y's odd gene-exchange method.

Most of the MSY sequence was in hand—and on the Internet—as much as three years before the *Nature* publication. Page and his colleagues spent that time reading and rereading the text "to see if we could extract some larger meaning from it." Page had never doubted it would be an amazing tale. "It was clear that the Y had a lot of surprises up its sleeve," he says.

HALL OF MIRRORS

The findings that Page's team described at a June press conference made headlines around the world. With his characteristic flair for metaphor, Page hailed the most spectacular Y-chromosomal feature as a "hall of mirrors" and a "crystal palace." He was referring to immensely long structures containing many repeated sequences and multiple copies of genes—scientifically termed "amplicons." In these regions, the researchers had identified eight complex and precise palindromes—segments along which the DNA code reads the same in one direction as the other.

Although palindromes are found on other chromosomes, Page says, they are not as large or as complex. The Y's palindromic sequences form pairs of symmetrical arms that extend in opposite directions from a central hub, spanning up to nearly 3 million nucleotides from tip to tip. Page calls this largest of the Y's eight palindromes "mind-boggling" because it accounts for a full one-thousandth of the entire genome. On six of the palindromes, the researchers identified eight distinct genes, expressed predominantly in the testes, that govern spermatogenesis, the making of sperm.

Some of the palindromes were first discovered several years ago, and they had given genome sequencers fits. DNA is decoded by breaking up the chromosome into small segments, then determining the order of nucleotides on those bits, and finally fitting them back together by matching up distinctive landmarks with the aid of powerful computer algorithms. But here, the long identical stretches offered few nucleotide differences for the scientists to use as landmarks; it was difficult to know even which arm of a palindrome a given piece was on.

"We had a big bin of things we couldn't assemble," says Wilson of Washington University. "It was analogous to a 10,000-piece jigsaw puzzle of ocean and blue sky and a little sailboat. All the pieces look the same. You're hoping you can find a small bird in the sky." For just this reason, the sequencers used DNA from the Y of a single man, an anonymous volunteer from the Buffalo, New York, area. Had the researchers combined DNA samples from several individuals for sequencing, as in the Human Genome Project, normal genetic variation would have washed out the tiny sequence differences the scientists sought in order to get a toehold on the Y. Success came slowly and iteratively, with the finding of differences among a few base pairs, the placement of those landmarks on a map, and then sequencing and mapping—over and over.

Francis S. Collins, director of the National Human Genome Research Institute at the National Institutes of Health, says, "More than any other center, the Washington University group perfected the map-based sequencing approach—and this was absolutely essential."

Once the hurdles were overcome, the hall-of-mirrors structure—with identical sequences on the palindrome's twin arms, capable of facing each other—could be seen as a bulwark against the relentless loss of genes that has been whittling away at the Y chromosome for eons. Such losses have resulted from the Y's inability to recombine, or exchange genes, with the X, as other chromosome pairs do to cull out harmful mutations and maintain their health. To retain at least some functionality, the Y can swap DNA internally: Evolution apparently provided a protective mechanism called "gene conversion," dependent on these extra gene copies and paired palindromes, for repairing mutated genes on the Y.

In the rest of the human genome, the 22 pairs of autosomes (non-sex chromosomes) line up with each other when cells are preparing to divide to create sperm and eggs for the next generation. At this time, the chromosome pairs often cross over each other and exchange chunks containing a few or many genes. In the gene swap, sequences having "typographical" errors involving only one or two genetic letters or large regions of missing or rearranged DNA that cause functional changes (mutations) can be rectified by substitution of normal sequences for the damaged ones.

The paired X chromosomes in female cells recombine this way. But the Y is the odd man out, so to speak, and lacking a trading partner,

Recombination Station

Unlike the two X chromosomes in females, the Y chromosome does not have a partner with which to swap genes in order to replace mutations. The Y appears to protect its genetic integrity by swapping multiple copies of the same gene within its own structure. These graphics suggest how a mutation on a palindrome in the Y (yellow band, figure 1) can be overwritten and thus repaired by a normal gene (2). Sometimes, however, the opposite happens—a mutation overwrites the correct sequence on the other arm and duplicates itself (3). To see these processes in animation, see the links at *www.hhmi.org/news/page5a.html*.

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untold numbers of genes have become irreparably mutated and lost over millions of years. (Because the genes that the Y does retain are handed down clonally, not sexually, from father to son to grandson and so on, the Y does not pass through the female line.)

OVERWRITING ERRORS

The process of gene conversion has been most thoroughly studied in yeast, but in these simple organisms, no palindromes are involved. On the Y chromosome, gene conversion occurs when the palindrome's twin arms swivel so that their identical sequences face each other, and if one arm contains a mutated gene, a normal copy on the opposite arm can overwrite the error. (Sometimes the opposite happens, however—the "bad" copy overwrites the correct sequence on the other arm.) Gene conversion occurs on a smaller scale elsewhere in the human genome, "but it's always been viewed as an event that's so occasional it's noteworthy when it happens," says Page. "What we're saying now is that in the case of the Y chromosome, it's standard operating procedure." In fact, when they traced back the gene configurations on the palindromes of the Y chromosome of both humans and chimpanzees, the researchers concluded that gene conversion was already present in our common primate ancestors.

As published in a second paper in the same issue of *Nature*, Page and his colleagues calculate that for every boy born in recent times, an average of 600 nucleotides have been swapped between palindrome arms. The effects of most of these exchanges would be unnoticeable. Evidence suggests that gene conversion repairs mutations on the Y as fast as they occur—good news for the chromosome's continued existence.

The amplicon regions and their grand palindromes make up about one-quarter of the euchromatic (actively expressed) DNA in the MSY region. But in addition, the Page team described two other classes of genes, along with large amounts of heterochromatic (nonfunctional) DNA sequences. Twenty-nine genes on the Y are called "X-degenerate," which implies no moral weakness but instead refers to their origin. These genes are thought to be surviving relics of the ancient chromosomes from which the X and Y evolved, and they are identical to active genes on the X. The copies on the Y include 16 functional genes that are expressed throughout the body, where they perform housekeeping functions, while "many others are rotted-out hulks that no longer do any business," Page has commented.

A third category of DNA sequences in the MSY region is "X-transposed." These are bits of genetic code that jumped from the long arm of the X chromosome over to the Y, in a massive shift between 3 and 4 million years ago, after the ancestors of modern apes and humans split off from each other. Among these transposed sequences, the researchers could detect only two genes.

Page and his colleagues have constructed from the Y sequence data a history of human chromosome evolution. It begins some 300 million years ago, when our reptilian ancestors existed as males and females but sex determination was probably by some environmental factor, not sex chromosomes. At some point, one of an identical pair of ordinary chromosomes, or autosomes, acquired a mutation that determined maleness. Thus, the Y chromosome, with its sex-determining gene, was born. For a while it continued to recombine genes with its partner (now termed the X). But gradually the two stopped recombining along most of their length, apparently as a result of catastrophic events over millions of years in which large blocks of the Y became inverted, shutting down the DNA-swapping process one stretch at a time along the chromosome. (Two regions, one at each end of the Y, continue to recombine with counterparts on the X chromosome.)

At this point, the Y began to "rot." Absent recombination, genes mutated and became junk littering the MSY region, and the functional parts of the chromosome withered. "During the last 300 million years," Page says, "the Y chromosome has thrown overboard the vast majority of its genes, while today's X has largely retained the 1,000 or so genes from the ancestral autosome."

THE Y CHROMOSOME HAS THROWN OVERBOARD THE VAST MAJORITY OF ITS GENES.

Is there a chance that the Y could be headed toward an ultimate total eclipse? According to Page, one can imagine the Y shrinking down to nothing but the sex-determining gene (which, after all, is how many scientists viewed it in the "rotting Y" models of the 1960s). Fortunately for the species, the Y has managed to preserve its package of spermatogenesis genes—some of which migrated from other chromosomes—that are cocooned among the amplicons. "The Y is doing a pretty good job of holding its own," Page says.

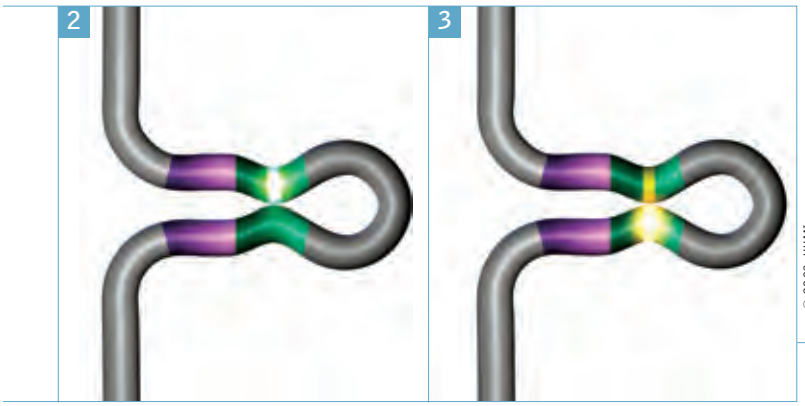
LIGHTNING ROD

While the newly discovered gene-conversion strategy appears to be stemming the tide of gene decay, Page says research on that question is only beginning, and much remains to be done on many fronts. One of the top priorities is to sequence Y chromosomes from other men, and from males of other species, to garner new details about the chromosome's evolution.

Mutations in spermatogenesis genes have already been implicated in forms of male infertility. Further analysis may lead to improved diagnosis and treatment.

Finally—and Page raises this point knowing it will be a scientific-political lightning rod—the finding of so much genetic information on the Y chromosome significantly widens the estimated genetic gap between men and women. "The difference between a male and a female comes down to trading the second X for a Y," he says. "This trade involves about 1 to 2 percent of the genome, and that completely dwarfs all the genetic polymorphisms [normal variation between individuals] in the human autosomes"—all the chromosomes excepting the X and Y. Put another way, the genetic difference between a man and a woman is about the same as that between a man and a male chimp, or a woman and a female chimp.

This genetic reality will be difficult for many people to embrace and may only stir up greater controversy about genetic determinism, Page acknowledges. But he suggests that the disparities may underlie differences in disease susceptibility between the sexes, for example, and they should not be ignored in further research. H



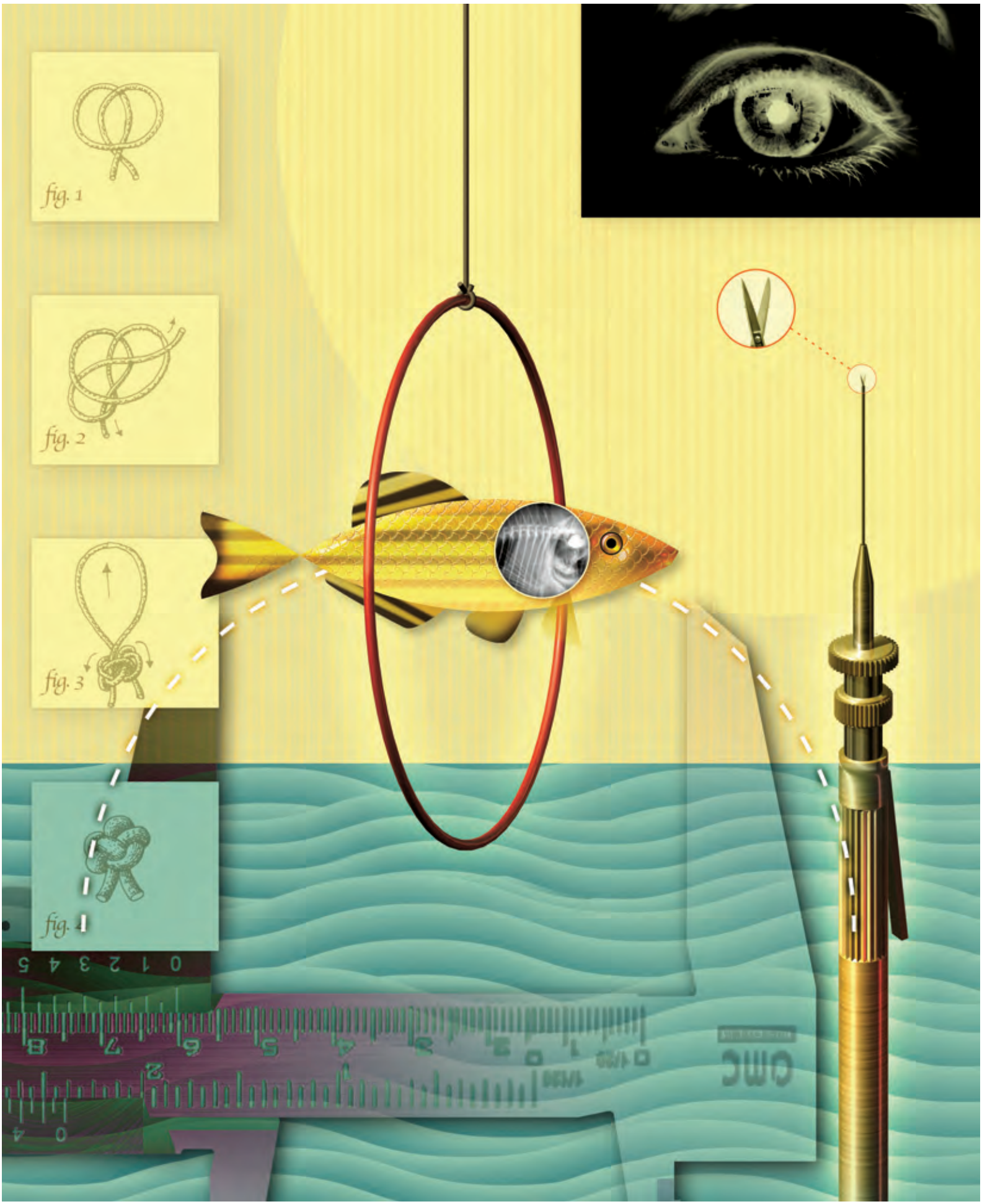


fig. 1
fig. 2
fig. 3
fig. 4

Neat Feats

To get where you want to go in science, sometimes you have to build your own road.

BY STEVE MIRSKY

ILLUSTRATION BY BRYAN LEISTER

Sometimes scientists know where they want their research to go, but find that there are no known roads to get there. Maybe the research technique doesn't exist. Or you need equipment that can't be ordered from a catalog. Or you need to work in spaces so small the challenge boggles the mind. What do you do?

Sometimes you just have to invent a way to get where you want to go. Here are several accounts of researchers who applied varying parts of inspiration and perspiration to create the ways and means of observing that which they wanted to see.

Cardiac Comeback

How do you do heart surgery on a fish whose entire body is less than two inches long? Very carefully, of course. And so, working very carefully, a team led by Mark T. Keating, an HHMI investigator at Children's Hospital in Boston and Harvard Medical School, performed surgery on hundreds of adult *Danio rerio*, better known as zebrafish. The researchers snipped 20 percent off the tip of the lower chamber (ventricle) of the fish's two-chambered heart to see whether the heart would regenerate. Keating already knew that zebrafish could regenerate fins, retinas, and spinal cords. Being a vertebrate like us made this animal a good candidate for researchers "trying to create a model system for heart regeneration," he says.

The bottom line was that after about two months, the hearts did regenerate, but the path to that finding, which was featured in the December 13, 2002, issue of *Science*, was noteworthy in its own right. The first problem was figuring out how to damage a vital organ about half the size of a pea and beating about 200 times each minute and have the fish survive. "We tried thermal injury, which is hard to control," says Keating. "And we tried radiofrequency ablation, which basically is also thermal injury and is also hard to control. And the injuries were not clean and reproducible." Cutting this Gordian knot, therefore, involved cutting. But what with? "A scalpel isn't a great instrument because you can't really hold on to the heart while you're cutting it," Keating explains. "We decided we needed scissors, and we wanted to get the sharpest, smallest scissors we could." Fine-gauge iridectomy scissors, ordinarily used by eye surgeons doing work on human irises, could do the job.

An incision on the fish's ventral side makes it easy to push the two-chambered heart out of the body while it's still connected to the rest of the cardiovascular system. Then the scissors are deployed to remove 20 percent of one ventricle of the heart—arduous trial-and-error efforts by Lindsay G. Wilson, a research associate in Keating's lab, revealed that about 90 percent of the fish could survive this procedure. The only problem is that a cut to the heart, not surprisingly, leads to profuse bleeding. A little pressure on the heart with gauze stops the bleeding and facilitates clotting. Then, Keating says, "we gently put the heart back in the chest" and, given that they are groggy coming out of anesthesia, "stimulate the fish a little bit to get them to swim, which passes oxygenated water through their gills." Two months later, the heart is full-size again.

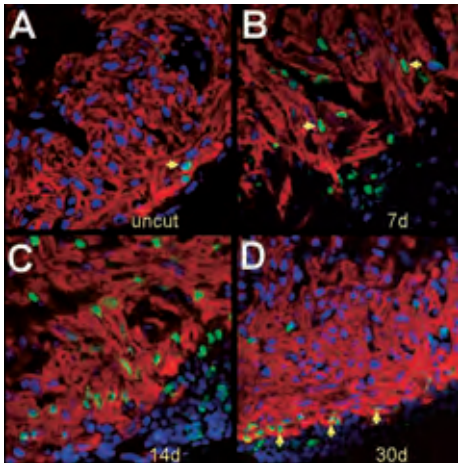
"There are really two aspects to this research," Keating notes. "First, we had to show that this beast can regenerate. And now we need to get to molecular mechanisms." The obvious hope is that understanding the genetics that govern regeneration in the zebrafish heart could lead to treatments that would induce damaged human hearts to regenerate. That fishing expedition, however, would be pointless had the zebrafish not cooperated.

If You Give a Mouse a Fez

Does a mouse live by smell alone? A mouse's brisk sniffing brings pheromones in its environment into contact with the vomeronasal organ (VNO), which sends signals to a distinct brain region known as the accessory olfactory bulb (AOB). Lawrence C. Katz, an HHMI investigator at Duke University Medical Center, and his colleagues were able to get deep inside the AOB of mice, in work that was featured on the cover of the February 21, 2003, issue of *Science*. They found that a mouse interprets another mouse as a distinct pheromonal pattern, similar to how the human brain recognizes another person via "face neurons" in the brain's visual-processing regions. But doing this research presented unique challenges, considering that a mouse AOB is the size of the head of a pin.

Most previous research focused on the responses, in vitro, of the peripheral sense organ—the VNO—to the application of pheromones or substances containing pheromones, such as urine. Technical obstacles have blocked the way to investigating how these peripheral responses are represented in the brain itself. Katz's team had something a little more true to life in mind. "The basic idea," he says, "is that we wanted to be able to monitor the activity of individual neurons in this unexplored area of the brain as an animal is engaged in natural social interactions with other mice."

But to record individual neurons in the AOB required the ability to target and manipulate incredibly tiny electrodes in behaving animals. "That's a tall order," says Katz, "because you need to advance and retract the electrode and have that electrode be stable enough so that you can actually record from a single nerve cell while the animal is moving."



These four images show the progression of cardiac regeneration in the zebrafish from prior to heart surgey (A) to 30 days postoperation (D). The arrows show where cardiomyocytes (heart muscle cells) proliferate during the process.

A mouse interprets another mouse as a distinct pheromonal pattern, similar to how the human brain recognizes another person via "face neurons" in the brain's visual-processing regions.

Fortunately, Minmin Luo, a postdoctoral fellow in Katz's laboratory, previously did bird-song research (as did Katz). And Luo was following the publications of a Bell Laboratories bird-song researcher, Michale S. Fee, who had created miniature instruments for studying the brains of zebra finches while they were singing. The three researchers adapted the bird apparatus for use in the similarly sized mice.

"Michale's developments really had two key components," Katz explains. "One is using advanced materials, like titanium, and micromachining techniques to sculpt a headpiece that is tiny and light enough to be mounted on the skull of a very small animal." The headpiece weighs about 2 grams, which even a mouse can tolerate, and looks like a tiny fez. On this fez, however, the traditional tassel is replaced by ultraminiature motors, which in turn control tungsten electrodes, which taper down to a width of only a few microns where their tips terminate in the AOB.

The other component is a feedback system that measures the strain on the cable. Should the mouse turn left, for example, it won't have to drag along the apparatus in that new direction. Instead, the cable's sensors send signals to a motor that tells the electronics residing in a support structure above the mouse to proceed in that direction as well. The unencumbered animal is thus free to act more normally.

Three micromotors allow Katz to advance and retract electrodes by remote control while listening to the activity of single cells as the animal behaves in various ways. "We can listen in on the activity of about half a dozen neurons at the same time if we wish, by recording from those different electrodes," he says. "We can really hear the staccato bursts of individual neurons, and we can continue to record from them while the animal is engaged in the full range of behaviors."

Katz believes this work to be the first that records nerve-cell activity in the brain while two animals are actively engaging in social interactions. ("We have one wonderful recording of the activity of a single cell in the brain while our mouse was actually mating," he says.) Being an HHMI investigator allowed Katz to venture into the uncharted waters of this research more comfortably. "I had no track record in this area," he acknowledges. "I can only imagine what would have transpired had I submitted a grant asking for funds to do recordings from awake behaving animals, never having done it before."

The Dendritic Dance

Karel Svoboda, an HHMI investigator at the Cold Spring Harbor Laboratory, also ponders the workings of brain cells through the window of the mouse—or, more accurately, through a window on the mouse.

Svoboda and colleagues recently discovered that some subtle and fascinating structural alterations take place in neurons as an animal learns. Dendrites and axons, on the input and output ends of a neuron, respectively, had been assumed to be constant in structure over long periods of time. But when Svoboda zoomed in on the dendrites, a more dynamic picture emerged, as detailed in the December 19, 2002, issue of *Nature*. Getting to that picture, however, required the combination of a designer mouse and some fancy microscopy.

Joshua R. Sanes, at the Washington University School of Medicine in St. Louis and a coauthor of the *Nature* paper, created a transgenic mouse strain that expresses the gene for green fluorescent protein (GFP) specifically in a small subset of neurons in the cortex—the part of the brain that interested Svoboda's group. GFP expression makes it possible to visualize the neurons, which glow green when hit with the proper wavelength of light. Sanes's original intent was for the protein to be produced at neuromuscular junctions. "But he found that the gene sometimes lands in a part

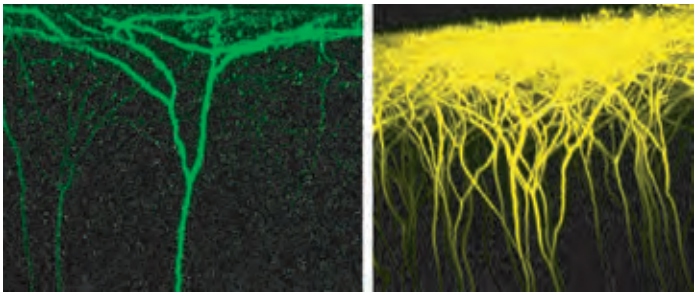
COURTESY OF KEATING LAB (ZEBRAFISH);
COURTESY OF MINMIN LUO AND LAWRENCE KATZ (MICE).

of the genome so that the protein is expressed in a subset of neurons in the cortex—the part of the brain that we're interested in," says Svoboda.

Magnetic resonance imaging (MRI) of the mouse brain would be a straightforward exercise, but using MRI to view a synapse would be like trying to see Pluto with opera glasses. "Synapses are only the size of the wavelength of light," says Svoboda. "The resolution limit of MRI is off by about 9 orders of magnitude." The device that can sneak into these tiny places is a two-photon laser scanning microscope, which not only persuades the GFP in the neurons to glow but captures images of the shimmering structures.

The idea is to throw a photon of a specific wavelength at the GFP, which will absorb that photon and then spit back a green one. But high-energy light scatters in living tissue, bouncing around before it can penetrate to the neurons. Lower energy light can penetrate deeper, but it doesn't pack the punch needed to excite the GFP—ordinarily. Should two low-energy photons arrive at a GFP molecule at the same time, however, they get absorbed together. As far as the GFP is concerned, the two low-energy photons are indistinguishable from a single high-energy photon. This two-photon absorption is normally a rare event. But modern pulsed lasers, which concentrate photons in brief pulses, allow two-photon absorption to be common enough to make deep brain imaging possible.

Fluorescent protein expression in cerebral neurons in two lines of transgenic mice, imaged in vivo. In one line, green fluorescent protein is expressed sparsely; in the other, yellow fluorescent protein is expressed abundantly.



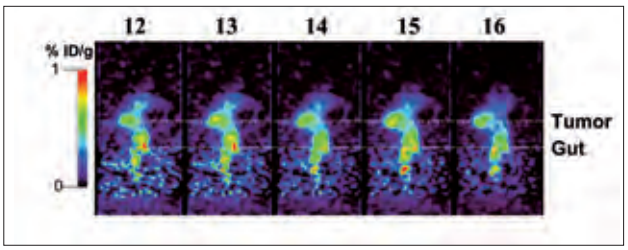
The microscope cannot, however, see through fur and the skull. "So you basically replace the skull with a small imaging window," Svoboda explains. "It goes right on top of the dura, the skin that covers the brain. The animals can live out their entire lives with this window in place."

By observing the same transgenic-mouse brains multiple times over weeks with the two-photon microscope, Svoboda and his colleagues could see tiny spines along the dendrites rising or receding. The rate of spine turnover increased as mice were exposed to novel experiences: As the organism learns, the brain clearly rewires itself. "This is the first demonstration," Svoboda says, "that there is synapse formation and elimination in the adult brain at a high rate."

Spying on T Cells

For the immune system to wipe out a tumor, it must succeed at multiple tasks. It has to generate T lymphocytes specific to the tumor, and those T cells must migrate to the tumor and then be able to destroy the tumor. While some treatments aim to enhance T cell migration and activity, the current ways of measuring their effectiveness mostly involve looking at the final outcome—via biopsy, for example. But Owen N. Witte, an HHMI investigator at the University of California, Los Angeles (UCLA), and colleagues have taken the first steps toward observing the T cell response in progress.

"We want to know what happens to populations of educated, active T lymphocytes"—T cells that have already eradicated a tumor in mice, thereby showing their "education"—"when they're infused into a recipient who has a tumor," Witte says. Witte transferred these educated cells to other immunodeficient mice—the only T cells available were thus the injected ones. Then the questions began. "Did the cells get to the site of the tumor?" he asked. "And if so, when did they get there, and how many of them got there? And did they multiply or expand upon their arrival there or elsewhere in the animal? In a sense, it was an analysis of the bookkeeping." Witte and his colleagues published their research in the February 4, 2003, issue of the *Proceedings of the National Academy of Sciences* (*PNAS*).



Taking a sequence of images of the mouse via microPET imaging technology, and using special "reporter" genes, researchers can track how cells that fight tumors locate their prey and act to destroy it. They hope that similar technologies will eventually help them study how well human beings react to clinical trials of therapies.

To transform the before and after snapshots into a moving picture of the process, Witte turned to a new technology developed by UCLA colleagues Simon R. Cherry, Sanjiv S. Gambhir, and Michael Phelps: small-scale positron-emission tomography, or microPET. Traditional PET constructs images by capturing the gamma rays produced when positrons emitted from a sample collide into their abundant, negatively charged counterparts, electrons. The resolution is "at best 0.1 cc [cubic centimeter], and more typically 0.5 to 2.0 cc," Cherry and Gambhir wrote in a 2001 paper in the *Institute for Laboratory Animal Research Journal*. The two researchers were able to tinker enough to get the resolution down to 0.006 cc, suitable for imaging small animals.

That microPET instrument needed something to measure, however, and UCLA biological chemist Harvey R. Herschman developed a process using a "reporter gene" to do the trick. The gene (herpes simplex type 1 virus thymidine kinase, which encodes the enzyme HSV1-TK) was incorporated into the T cells that Witte wanted to follow. When the enzyme was produced, it sequestered a fluorine isotope-labeled compound that generated positrons. The result was a concentration of positrons in the T cells, which made it possible to eavesdrop on their activities.

"It takes about a week for the T cells to figure out where to go, and to get to that site and begin to divide," says Witte. "It looks like they actually go to more traditional lymphoid sites, where antigen in the blood or some other set of signals begins to cause them to recirculate and eventually end up at the tumor."

The hope for the future, Witte says, "is that as we improve these technologies, they will find utility in human beings who are undergoing clinical trials to test therapies." H

"We had the genome, but now we needed a way for scientists to interactively explore it," David Haussler says.

Genes Seen

A computer scientist builds a picture window on the human genome.

SCIENTISTS AND THEOLOGIANS MAY DISAGREE about when and how human life began. But for HHMI investigator David Haussler, there can be no argument about when it *changed*: July 7, 2000. "Humanity walked through a portal that day," he realized then, "and we can't go back."

Haussler's epiphany occurred when his team posted the Human Genome Project's first draft on the Internet. "I turned on my computer and there it was, humanity's first nearly complete look at its own recipe, an almost boundless waterfall of As, Cs, Gs, and Ts cascading across my screen, chosen and ordered by the individual life struggles of our ancestors through billions of years of evolution. It was an indescribable moment."

Only weeks before, however, there seemed little possibility that the vast amounts of the project's data could soon be stitched together for ready display. Haussler, a computer scientist who directs the Center for Biomolecular Science and Engineering at the University of California, Santa Cruz (UCSC), had worried about this "genome assembly" problem since first being asked to join the effort by Eric S. Lander, director of the Whitehead Institute/MIT Center for Genome Research, one of the major laboratories contributing to the genome-draft data. Lander wanted Haussler to apply his computer algorithms to find the genes in the genome sequence, but given the fragmented nature of the data, this task proved exceedingly difficult. "Bits and pieces of information were coming to us from 20 different sequencing centers around the world," Haussler says. "It was a jumble."

Enter James Kent, then a UCSC graduate student, whom Haussler and growing legions of other admiring researchers consider a computer-programming genius. In a tour de force lasting only a matter of weeks, Kent crafted a large and sophisticated program, known as GigAssembler, which

By Jeff Miller | Photograph by Timothy Archibald

put together the first draft of the genome. "Jim saved the day and got the genome into the public domain, where it was available for free," Haussler says. And the public responded, downloading the draft in record numbers. Indeed, in the first 24 hours alone, Haussler's computers at UCSC churned out half a trillion bytes of data.

GOLDEN PATH

But that awesome day was only the beginning. "Jim and I had already started thinking about the next step," Haussler says. "We had the genome, but now we needed a way for scientists to interactively explore it." This was a challenge that Haussler's group was particularly well equipped to address, particularly on a campus where mathematicians and computer scientists were fast converging around two fundamental tools of the information age: Web-browsing technology and bioinformatics. Harnessing these new electronic technologies would ultimately produce the UCSC genome browser—dubbed the Golden Path—as well as its cousins at the European Bioinformatics Institute and the National Center for Biotechnology Information.

Over the past few years, researchers at laboratories around the world have become habitual users of the Haussler team's technology as they search for new clues about disease and potential therapies. Famed breast cancer researcher Mary-Claire King, at the University of Washington, is one of them. "The Golden Path is extraordinarily easy—and fun—to use," she says. "Our undergraduate students learn how to work with it in less than an hour and are hooked on genomics as a consequence." Moreover, it has already proven its worth as a premier investigative tool. "Using the UCSC browser over the past two years," says King, "we have identified a novel gene critical to breast cancer development. One of my postdocs identified a gene responsible for age-related hearing loss. And systemic lupus erythematosus, an extremely complex disease we have struggled with for 20 years, is now approachable because we can consider multiple genomic events simultaneously."

The popularity of the UCSC genome browser is no surprise to Haussler, simply because it helps scientists trek their way through areas that would otherwise be unnavigable. Regions of interest are not limited to the estimated 30,000–35,000 genes, which constitute only about 1.5 percent of the human genome. The other 98.5 or so percent, the non-protein-coding DNA that used to be dismissed as "junk," is known to possess gene-regulation and other cellular-control zones. Although areas devoted to these functions were once thought to constitute only a tiny fraction of the genome compared to protein-coding DNA, the opposite appears to be true. "We estimate that an additional 3–4 percent of the human genome, beyond the protein-coding DNA, may have an equally vital functional role," says Haussler.

His conclusion stems from analyses of the molecular evolution of the human genome that included comparisons to the genome sequences of the laboratory mouse and other mammals. By noting similarities, researchers ultimately determined the DNA regions that evolved from a common ancestor (called "orthologous" regions) and then measured the degree to which the DNA in those regions is conserved among the different mammalian species. Haussler refers to highly conserved areas of the human genome as "the living record of the difficult struggle of our ancestors to preserve the most critical parts of the message of life." Such regions constitute approximately 5 percent of the bases in the genome, according to his team. This result suggests that there are many non-protein-coding regions that perform vital functions, says Haussler, and it "defines a clear task for genomics: Find out what this selected 5 percent of the human genome does."

Mathematics—Haussler's first love—remains at the core of his genomic enterprise, which began 20 years ago at the University of Colorado. There, under the tutelage of his mentor Andrzej Ehrenfeucht, Haussler became interested in the mathematical analysis of DNA sequences. This was a time when there was considerable excitement about recombinant DNA methods and when the complete DNA sequences for selected bacteriophages were first being deciphered, along with parts of the *Escherichia coli* genome. The goal to find sites that most strongly drive protein expression motivated Haussler and two fellow graduate students, Gary D. Stormo and Eugene W. Myers (whom he counts among the "founding pioneers" of bioinformatics), to ponder computational methods for finding patterns in the sequences of nucleotides then being discovered. Few could have imagined the blossoming of technology since then that nowadays makes those early efforts seem clumsy and unsophisticated.

> The UCSC genome browser helps scientists trek their way through areas that would otherwise be unnavigable.

Nor could many people have envisioned the increasing numbers of students now happily poking their fingers into the very essence of life itself. "After all," Haussler asks, "isn't life basically just a complicated, self-perpetuating DNA program?" Such questions, tossed into the air with rhetorical flourish, have held a career-long fascination for this father of two, whose Hawaiian shirt and easy demeanor belie an almost flammable curiosity. "I think anyone [who trains to be a mathematician] strives for maximum simplicity. I'm a Platonist, looking for proof of the necessary structure inside the beauty and form of life."

Yet Haussler is the first to admit that simplicity is not always attainable. And he should know, having spent a decade trying to create a mathematical model of the ways by which synaptic junctions in a network of neurons are altered by experience. "I wanted to know how the brain adapts and learns at the most fundamental mathematical level." What he learned instead was that some complex natural systems do not break down cleanly into subparts. "When we subjected simulated neural networks to simple learning challenges, they naturally formed exceedingly complex, overlapping sets of interactions that successfully addressed these challenges," Haussler says. Their solutions were unanticipated and difficult to deconstruct. "I'm afraid that molecular evolution has addressed the challenge of creating self-perpetuating life in a similar manner, and that it will be difficult to fully unravel the solution it has found."

MATHEMATICIAN IN PARADISE

But on a more workaday level, Haussler believes that numerous discoveries of biomolecular processes with great medical importance are on the way. These discoveries will result from collaborations of math and computer-science experts with researchers such as microbiologists, cell biologists, and chemists—all of whose worlds once seemed parallel at best. "When I started in mathematics, I had no clue that I would be interacting with biology in this way," says Haussler, staring up at the towering redwoods outside his office window. "Who would have thought? I'm just a mathematician living in paradise."

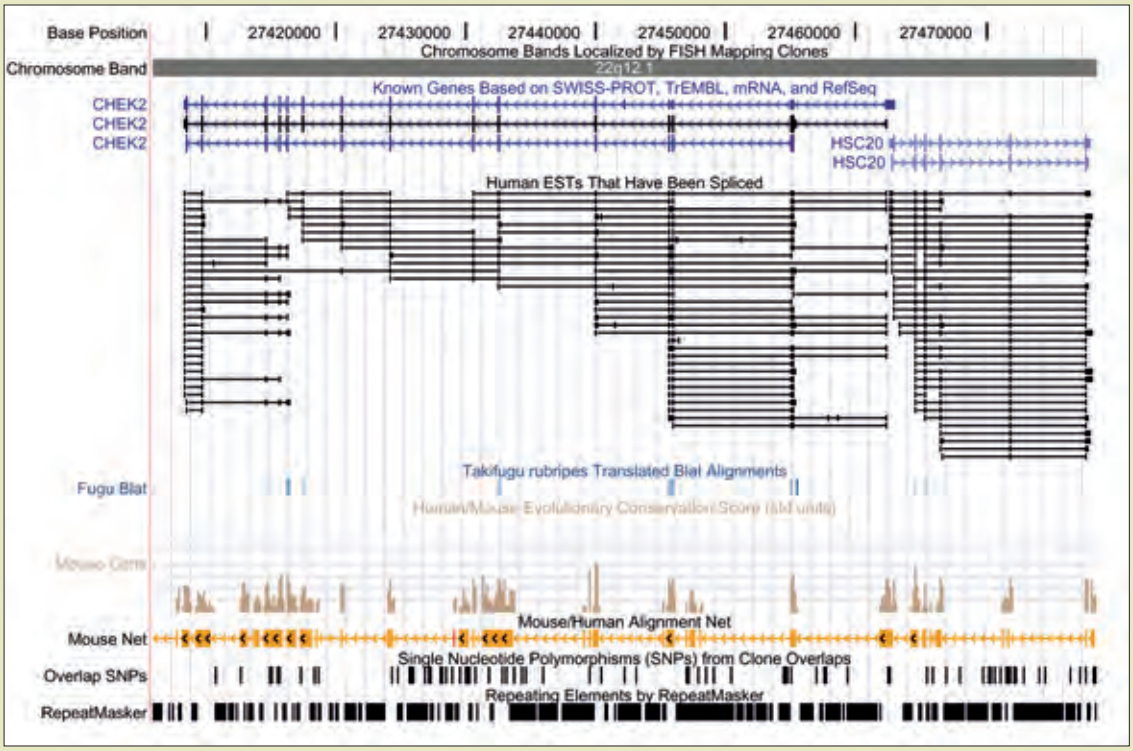
Feeding Hypotheses to the World

THE UNIVERSITY OF CALIFORNIA, SANTA CRUZ (UCSC) genome browsers (see *http://genome.ucsc.edu*) absorb mountains of data from genome sequencing and analysis projects around the world, including projects at UCSC itself, with the goal of creating coherent genome views. Or, as Haussler puts it, "We want an interactive, Web-based 'microscope' for each model animal genome."

With this versatile microscope, a user can zero in on a requested portion of the genome (and zoom out for a longer view) while simultaneously displaying information in annotation tracks (see chart). Users can also add their own tracks with proprietary data that will flash into place only on their own Web browsers or be shared only with close collaborators.

"Wherever you look in the genome," Haussler says, "you can bring up tracks of data that no one else has ever looked at before and also notice new correlations between different types of data previously studied only in isolation. The experience is like looking through a microscope. It is live discovery." Says Haussler: "We're happy to be feeding hypotheses to the world." **—JM**

A GENETIC SNAPSHOT



COURTESY OF HAUSSLER LABORATORY

This sample chart from the UCSC Human Genome Browser yields a rich lode of data about the evolution and operations of two specific genes on human chromosome 22,* CHEK2 *and* HSC20. CHEK2, *a "checkpoint" gene, senses when there has been DNA damage or a problem with DNA replication and sends a signal to halt the cell cycle while the damage is repaired. Mutations in* CHEK2 *can make one susceptible to many types of common cancers.

The lines in blue at the top of the chart show the location of three known splice variants of the* CHEK2 *gene. Splice variants are naturally occurring variations that give rise to different protein products from a single gene; some have little or no effect, but others are associated with human disease. Knowledge of specific splice variants can help those who are researching diseases and searching for therapies.

Most of our direct evidence for many human genes comes from the vast collection of copies of short segments of RNA transcripts known as expressed sequence tags, or ESTs (black lines in center of chart). By joining overlapping ESTs, one can assemble possible splice variants for a gene. The data here suggest that there may be more splice variants for* CHEK2 *beyond the three known variants displayed in blue above.

The next three lines are snapshots of evolution. The blue track marked "Fugu Blat" in the margin shows regions of the human genome where the DNA is similar to certain segments in the genome of the pufferfish* Takifugu rubripes, *despite the fact that these two species are separated by several hundred million years of evolution.

The next track (brown) shows similarities of the human genes to a region on the reverse strand of mouse chromosome 5 that evolved from the same part of the genome of our common mammalian ancestor.

The orange line that follows further analyzes our common ancestry with rodents. The hatched lines indicate parts that are unique to the human (primate) lineage.

The penultimate track shows the locations of single nucelotide polymorphisms (SNPs) in the human genome. SNPs are positions in the human genome where a single base shows a significant level of variation from person to person in the human population. Having a particular version, or "allele," of a SNP in a key functional region may be linked to susceptibility or resistance to disease.

The last track shows the locations of certain repetitive elements in the human genome. These are primarily transposons, a form of selfish DNA that repeatedly copies itself to new locations in the genome, eventually dying out and leaving the copies behind to accumulate mutations. H

PERSPECTIVE

How to Talk to the Public About Genes

Know where your audience is coming from.

By Celeste M. Condit

If you tell a woman she has a mutation associated with an increased risk of blood clots, or inform a community of its genetic predisposition to obesity and diabetes, will the disclosures actually help these people? Maybe. It depends in large part on how you talk to them.

Experts are often tempted to communicate with lay audiences as though they were grade-school teachers dispensing facts to children. But this aptly named "hypodermic needle" model of communication is based on a false assumption that experts can successfully "inject" a specific message into a given audience, and is widely discredited. People do not passively accept messages that they receive from experts or the mass media. For better or worse, they process information using their preexisting beliefs and knowledge.

PETER FREY, UNIVERSITY OF GEORGIA

That being the case, we shouldn't expect lay individuals to drop their perceptions of health or change their life practices merely because we tell them to. We have to be prepared for two-way conversations, which means learning someone else's language and gauging their knowledge and beliefs. This won't guarantee effective communication about medical genetics, but it is a necessary step.

Research in health communication is gradually clarifying the scope of lay knowledge, and it has already shown that the public knows a fair amount about the basics of heredity. For example, from high school biology classes, media coverage, and observation of others around them, most people have come to believe that some diseases seem to run in families and others don't. They know that even if a disease does run in the family, not every member will get it. They also know that traits are inherited from both parents, human beings share most of their DNA with each other, most genes have normal variations, and genes are the carriers of heredity.

In general, members of the public believe that health conditions are usually the result of both genes and personal behavior, and they may make surprisingly fine distinctions about this. For example, most understand that personal behavior plays a much larger role in the prevalence of lung cancer than many other cancers and that a predisposition to heart disease may be more difficult to overcome than a predisposition to diabetes.

Celeste M. Condit, *research professor in speech communication at the University of Georgia, works to enhance the translation of biomedical discoveries into improved public health by improving the communication process.*

On the other hand, members of the public know little or nothing about molecular genetics, which often results in a failure to understand that genes are working inside of us all the time. Most lay people associate genes with the making of a child, but they have absolutely no idea that genes are constantly being turned on and off in the adult, maintaining the routine operations of the body. This makes some crucial medical concepts difficult to convey. For example, despite the massive coverage of the dangers of cancer from exposure to the sun, most people don't realize that the sun causes *genetic mutations* that produce the cancers.

The word "mutation" is a powerful one in the public mind, and it provides an excellent illustration of the need to negotiate the gap between scientific jargon and lay understanding. To a geneticist, mutation is simply a technical term that describes a change in a DNA base. But although members of the public may understand the technical meaning of mutation, the term may also have other connotations based on general fears of technologically induced change. Participants in focus groups we have conducted, for example, described mutation as a "scary" word that brings to mind "a creature from the black lagoon."

Consequently, if you tell particular individuals that they have an allele that predisposes them to heart disease, diabetes, or cancer, you would probably not want to tell them that it's a mutation. Conveying the information in that way would bring up associations likely to decrease acceptance of the diagnosis—and for purely peripheral reasons. Far better to say that they have a "version of a gene" or a "genetic variation."

In part because in the process of translation technical precision can sometimes be lost, and summaries can be imprecise because they are incomplete, experts are often not comfortable translating their research into language for a lay audience. Nonetheless, we must make ourselves take this step. We have only begun to amass the technical knowledge about communication and public attitudes that we need in order to enter these conversations more effectively. But failing to take advantage of what we do know and not treating the lay public as a full conversational partner means not realizing the medical benefits of our growing knowledge of the molecular mechanisms of disease. H

Have Nobel, Will Mentor

Seventh graders learn science from a master.

Having recently retired to the Southwest, biochemist Stanley Cohen was getting restless. Cactus gardening, he soon came to learn, requires only so much attention, and he missed the kind of personal interactions he had in his lab back at Vanderbilt University.

So the e-mail announcement calling for volunteer scientists to assist middle school teachers seemed to be just what the doctor ordered. It sounded like fun, and Cohen thought he could do a pretty good job. But he had responded too late, and all the positions were filled. Perhaps he could have better spelled out his qualifications, Cohen thought. Hoping for another chance, he sent off a follow-up note with one more credential: "P.S. I was one of the recipients of the Nobel Prize in 1986 for discovering growth factors."

"I don't usually do that," Cohen confesses, "just when I want to get people's attention."

It worked. "This was too good to pass up," recalls Rachel Hughes, coordinator of the Scientist Teacher Alliance, an educational outreach program at the University of Arizona that is supported by a grant from HHMI. Hughes immediately phoned Trish Wheeler, one of her star teachers, who had also been late turning in her program application. An introductory meeting between Wheeler and Cohen sealed the partnership.

The Scientist Teacher Alliance aims to foster inquiry-based learning in elementary and middle schools by bringing together the talents and experiences of teachers and real scientists in practical, sleeves-rolled-up situations, explains Hughes, who modeled the program after a similar one at the University of California, San Francisco. "We wanted to move beyond the 'sage on a stage' approach typical of many scientist-in-the-classroom curricula."

Each Monday last spring, the 81-year-old Cohen, now an adjunct professor in the department of molecular and cellular biology at the University of Arizona, dropped in on Wheeler's two seventh-grade science classes at Tucson's Pistor Middle School. Rather than take over the teaching, however, Cohen served primarily as mentor and role model—and on occasion, surrogate grandfather.

PAUL MUHLRAD

Nobelist Stanley Cohen and a student discuss a specimen.

Early in the semester, the class investigated the effect of adding varying amounts of thyroxin—a developmental hormone—to a series of basins containing tadpoles. When the students returned to class after the weekend, they found that all the tadpoles exposed to the highest concentration of the chemical had died. Devastated, their first reaction was that they had failed as scientists. Cohen pointed out, however, that this is actually the way real science works, and he suggested that maybe they had actually discovered an important effect—the result of too much of a good thing.

"The kids got comfortable with the unexpected," Wheeler reports, and this led them to discover something else about the process of doing science. "Once the kids realized that they could *learn* from the unexpected, they saw that they really had to record things and be thorough about what the details meant."

Having an actual scientist on hand made the difference, she adds. Working with Dr. Cohen "was probably the most profound thing that the kids experienced in their seventh-grade year," she says. They carried their thoughts out further this school year than I had seen in previous years, and I attribute that to the fact that they had a genuine experience with a 'colleague.' "

At Cohen's final class session, two boys—Daniel Wilson, sporting punk-rocker hair spikes, black T-shirt, and a leather choker, and Gustavo Verdugo, with intense intelligent eyes and short black braids—gave testimony. Wilson recounted his explorations, with Cohen's guidance, of DNA fingerprinting, amphibian metamorphosis, and human blood types. Verdugo retold stories of Cohen's own renowned experiments with mouse tumors and chicken embryos and how they yielded medical tools for the fight against breast cancer. Cohen stood by beaming, gratified to see how much his students understood and retained. "That's remarkable—that was a long time ago," he said, patting Gustavo on the back. The boys cracked quick smiles, then turned back to their dissection specimen and continued their earlier conversation on mouse glands, blood types, and cancer inhibitors.

—PAUL MUHLRAD

Work Those Cells

Exercise can help cellular "power plants" stave off diabetes.

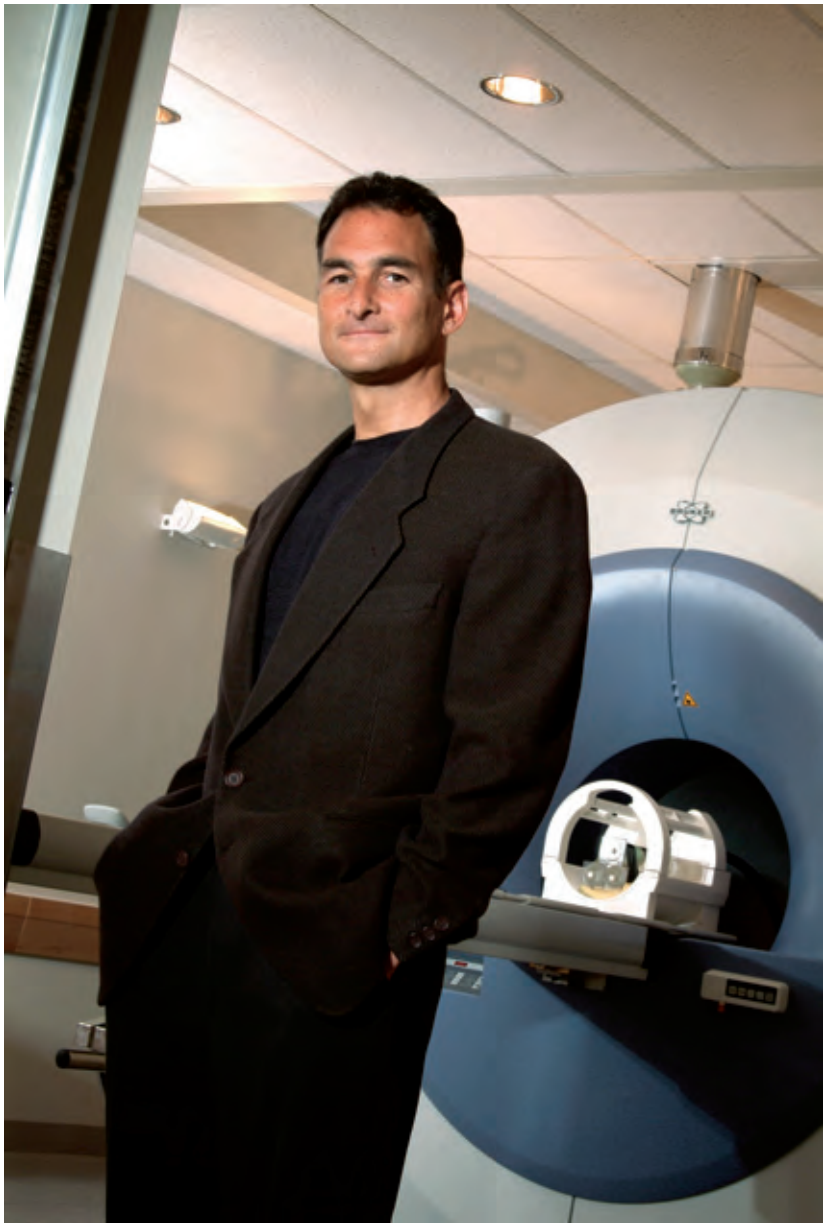
Back when Gerald I. Shulman was a medical resident and clinical fellow, he competed in triathlons and ran marathons in New York and Detroit. That was two decades ago, before he embarked on his career of studying the causes of type 2, or adult-onset, diabetes. What he didn't know at the time was that along with building a stronger heart and lungs, those workouts were helping him keep the mitochondria in his muscle cells fit—and thereby helping to reduce his own risk of diabetes.

In a series of recent studies, part of Shulman's effort to explain the cellular mechanisms that cause type 2 diabetes and possibly provide important clues for improving treatment, the HHMI investigator and his colleagues at the Yale University School of Medicine showed that exercise increases the number of mitochondria in muscle cells and improves their functioning. Because they are the cell's "power plants," converting glucose and fatty acids from the blood into the energy needed for cellular activity, such enhanced mitochondria may help stave off diabetes as a person ages.

Although Shulman, a professor of medicine, is still lanky and fit, he no longer competes in athletic events. "I can't find the time anymore," he says, although he does bike to work whenever he can and hikes on weekends with his wife and scientific collaborator, Kitt Falk Petersen, an assistant professor of medicine at Yale.

Shulman, Petersen, and their colleagues use nuclear magnetic resonance (NMR) spectroscopy, an imaging technique that employs magnetic fields to trace radio signals emanating from nonradioactive tracer isotopes, to measure the metabolic processes within muscle and other cells of living subjects. This method allows the investigators to assess biochemical differences between the metabolisms of diabetic and normal individuals—or, in the case of a recent study, changes in their mitochondrial function that underlie the development of insulin resistance.

Insulin is a hormone, produced in the pancreas, that controls glucose metabolism in cells. Inside cells, glucose is either stored (as glycogen) or used up for energy. Insulin resistance—which interferes with a cell's ability to metabolize glucose and fatty acids—is believed to be the underlying cause of type 2 diabetes, the most common chronic metabolic disease among the elderly. "Approximately one in four individuals over the age of 60 has type 2 diabetes," says Shulman. "If you add impaired glucose toler-



GALE ZUCKER

Gerald Shulman says exercise training is likely to be effective in preventing or even reversing type 2 diabetes.

ance, you're talking about 40 percent of the population. It's staggering."

For its study of mitochondrial function, published in the May 16, 2003, issue of *Science*, Shulman's team compared healthy individuals aged 61 to 84 years to 18- to 39-year-olds who shared similar body types and levels of activity. An initial study showed that the elderly participants were markedly more insulin-resistant compared with their younger counterparts, with muscle tissue responsible for most of that resistance.

What happens to us as we age that leads to increased insulin resistance in muscles? Shulman wonders. Although obesity is a major risk factor for developing type 2 diabetes, in this case the elderly volunteers were lean and had amounts of body fat similar to the younger control subjects.

Sitting at a computer in his office, Shulman brings up an image of a dissected mouse that had been genetically altered to eliminate body fat. Despite that, the mouse still had severe insulin resistance. Although it was almost totally devoid of fat, it did have large amounts of fatty acids that had accumulated within muscle and liver tissue. Shulman and others have found that the higher the amount of fatty acid metabolites within a person's cells, the more resistant that person will be to insulin. "It's not how much fat that matters in insulin resistance," he concludes. "It is how it gets distributed within cells."

Further NMR measurements for the study indeed revealed that the elderly participants had much higher levels of fat accumulation in their muscle and liver cells. In addition, with the aid of a technique Shulman developed to measure mitochondrial function, an NMR comparison showed that the older people had 40 percent lower levels of mitochondrial activity in their leg-muscle tissue compared with younger subjects. This finding suggests that insulin resistance and the relatively high prevalence of diabetes in the elderly arises from an increase in fatty acids within cells that may result from an age-associated decline in mitochondrial activity.

Age, however, need not result in increased insulin resistance. In a 1996 study, Shulman and colleagues found that exercise can dramatically reduce insulin resistance in skeletal muscle. Using NMR, his team found that three sessions of 15 minutes on a stair-climbing machine four times a week increases insulin sensitivity in both normal and insulin-resistant offspring of diabetic parents because of a twofold increase in glycogen synthesis in muscle. "It's clear that exercise training," he says, "is likely to be an effective means of preventing or even reversing type 2 diabetes."

C. Ronald Kahn, president of the Joslin Diabetes Center and professor of medicine at Harvard Medical School, agrees that such findings show "it is possible through exercise to change mitochondrial function in muscle. There are many reasons for exercise in the elderly. This adds one more." These findings could also provide a target for the development of medications to improve mitochondrial function, he notes.

Meanwhile, Shulman is studying young insulin-resistant offspring of parents with type 2 diabetes to see whether they show abnormalities in mitochondrial function similar to those of the elderly participants in his *Science* study. "These individuals have a high likelihood of developing type 2 diabetes," he says. "If they have alterations in their mitochondrial activity, as I think they will, then these data would suggest that genes responsible for mitochondrial biogenesis and/or function might be responsible for type 2 diabetes." From there, he plans to search out which genes may be responsible for the mitochondrial dysfunction in muscle. "I love the way that this is coming together," he says. **—MARC WORTMAN**

New Grants Support International Collaboration

As a global enterprise, science often relies on collaborations between scientists who live far from each other. To encourage such partnerships, the Institute has awarded 14 minigrants totaling $194,600 to HHMI international research scholars and an EMBO/HHMI young investigator for work on collaborative projects (EMBO is the European Molecular Biology Organization).

The minigrants were created as an alternative to the 2003 scientific meeting of international research scholars, which was postponed this year because of political uncertainties in many countries. One of the primary purposes of the annual meeting is to encourage collaborative research among these scholars, who at present hail from 29 nations.

Awards will support collaborations between laboratories as distant from each other as Argentina and Israel, the Czech Republic and Brazil, Australia and Mexico, and Chile and Russia. Several of the projects focus on infectious diseases and vaccine development. Grantees and their countries are:

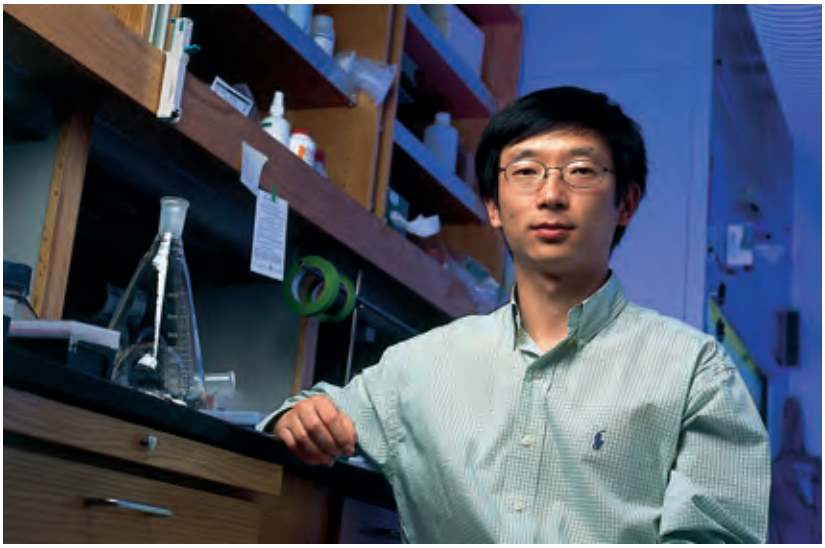
- Thomas Egwang, *Uganda*, and Chetan Chitnis, *India*
- Saulius Klimašauskas, *Lithuania*, and EMBO/HHMI Young Investigator Janusz Bujnicki, *Poland*
- Robert G. Korneluk, *Canada*, and R. Chris Bleackley, *Canada*
- Mariano Jorge Levin, *Argentina*, and Shulamit Michaeli, *Israel*
- Marie Lipoldova, *Czech Republic*, and George Alexandre DosReis, *Brazil*
- Roberto Mayor, *Chile*, and Andrey Zaraisky, *Russia*
- László Nagy, *Hungary*, and Patricia Torres Bozza, *Brazil*
- Jacek Otlewski, *Poland*, and Janusz Bujnicki, *Poland*
- Magdalena Plebanski, *Australia,* and Ross Coppel, *Australia*
- Magdalena Plebanski, *Australia;* Susana López, *Mexico*; and Carlos F. Arias, *Mexico*
- Vladimir I. Polshakov, *Russia,* and Olga Dontsova, *Russia*
- Edda Sciutto, *Mexico*, and Fernando Alberto Goldbaum, *Argentina*
- Virginijus Siksnys, *Lithuania,* and Jacek Otlewski, *Poland*
- Julio Urbina, *Venezuela,* and Miguel Angel Basombrío, *Argentina*

A Key in Search of a Lock

Prodding stem cells to be neurons.

One of Sheng Ding's favorite activities outside the lab is scrambling up the twisted granite boulders of Southern California's Joshua Tree National Park. By finding just the right combinations of grips, foot placements, and body English, Ding can mold his body to the cracks and outcroppings, surmounting virtually any obstacle he encounters. It's a similar power of combinations that excites him about chemistry.

Earlier this year, Ding, then an HHMI predoctoral fellow at The Scripps Research Institute, combined a set of chemical building blocks to assemble a molecule that can spur embryonic stem cells to become neurons. Now an assistant professor of chemistry at Scripps after just having completed his Ph.D., the 28-year-old chemist hopes his research will eventually lead to treatments for regenerating patients' damaged or diseased organs. Ding and his colleagues at Scripps and the Genomics Institute of the Novartis Research Foundation reported their findings in the June 24, 2003, issue of the *Proceedings of the National Academy of Sciences* (*PNAS*).



ROBERT BURROUGHS

Sheng Ding uses the power of chemical combinations in research he hopes will lead to treatments for regenerating damaged or diseased organs.

Embryonic stem cells are open to pursuing almost any destiny, but the paths toward specific cell fates must pass through carefully secured gateways. And the keys to those doors are the signaling molecules that traverse between and within stem cells, instructing them to differentiate into muscle, intestine, bone, or any other cell type. In an attempt to unlock the gateway toward a nerve-cell fate, Ding and his colleagues designed more than 50,000 small-molecule "keys" and tried them all, one by one.

Starting with 10 different synthetic "core scaffold" molecules, the researchers added various combinations of atoms and chemical groups to create a wider and more diverse set of compounds. They divided those molecules and then attached still other side groups, repeating the cycle once more until they had created thousands of distinctly shaped chemicals, each separated in its own bar-coded reaction vessel.

Using a robotic workstation, team members added a sample of each compound to mouse embryonic stem cells growing in 384-well culture plates. Initial tests identified several promising molecules that seemed to initiate the process of turning the cells into neurons. Ding and colleagues then made 50 new compounds by further tweaking the structure of the best candidate. One of those fine-tuned compounds, dubbed TWS119, proved particularly potent for stimulating mouse embryonic stem cells to become neurons. Cells bathed in the chemical developed the characteristic wiry network of axons and dendrites that neurons use to transmit their electric impulses. The cells also tested positive for proteins and other molecules found only in neurons or cells on their way to becoming neurons.

How did TWS119 perform its magic? The molecule's shape and chemical properties enabled it to bind to a molecule that controls stem cell differentiation, and that binding cleared the path to the neuronal cell fate. Ding and his colleagues had identified a key in search of a lock, exploiting the binding power of TWS119 to find the molecule with which it interacts—that is, the "lock" itself. In a technique called affinity chromatography, they attached molecules of TWS119 to tiny beads and then mixed the beads with stem cell extracts. Whatever molecules in the extract that could bind to TWS119 should then have become tethered to the beads. After the researchers gently washed the beads to remove all nonbound molecules, one prominent protein stayed attached.

That protein, called glycogen synthase kinase-3β (GSK-3β), turned out to be a familiar one to biologists. Protein kinases, enzymes that attach phosphate groups to other proteins, are a major class of cellular signaling molecules. Biologists had recognized GSK-3β's role in many other aspects of cellular regulation, but its involvement in nerve cell differentiation had only been hinted at. TWS119 inhibits GSK-3β's enzymatic activity, but Ding says that more work is needed to figure out exactly why this leads stem cells to become nerve cells. "TWS119 is just our first step to demonstrate that we can rationally screen small molecules to identify interesting leads," he explains.

Developing TWS119 is only the latest of Sheng Ding's many accomplishments since emigrating from China in 1996. He graduated from the California Institute of Technology in 1999 with a host of honors and is author or coauthor of 15 scientific papers, with several more on the way.

—PAUL MUHLRAD

Getting an Early Read on Dyslexia

To get baseline data, first you have to get the kids to sit still.

When Elise Temple decided to go to college after living on a farm and raising two kids, she had no idea that watching and helping her kids develop would eventually help focus her career in science. Daughter Chloe and son Dylan, she says, "were in some ways my first test subjects." Today, based in large part on her early fascination with the way her children gained and processed knowledge, Temple studies childhood dyslexia.

As an HHMI predoctoral fellow in the laboratory of John D. E. Gabrieli at Stanford University, Temple sought to determine whether the neurological basis for dyslexia could be identified at the critical stages when children are learning to read. To study that question, Temple wanted to use functional magnetic resonance imaging (fMRI), an invaluable noninvasive test that measures oxygen uptake and, by proxy, brain activity. But there was a problem—the test requires that the patient lie absolutely still. Most research groups have shied away from studying children because their abundant energy and short attention spans make them unlikely subjects for an fMRI. As a result, Temple says, "we know very little about what's going on in the brains of children as they learn things like speech and reading, even though huge changes are taking place."

So, braving a group of 32 kids aged 8 to 12 years (20 with dyslexia and 12 controls), Temple applied her own abundant energy—along with her experience as a mom, playgroup leader, and PTA member—to the task. She reassured parents, put kids at ease in the fMRI machine, and rewarded them with Pokémon trading cards. The successful results gave her baseline data to use in the next phases of the study.

Temple and her colleagues knew that adult dyslexics lack activity in the left temporoparietal cortex, an area of the brain involved in the processing of language sounds. Hypothesizing that breaking down sounds is critical to reading, they indeed saw in the preliminary study that dyslexic children also lack the sound-processing activity of normal readers when trying to do simple reading tasks such as rhyming and sounding out words.

Next, the researchers wanted to know whether a training program that built sound-processing and oral language skills could affect brain function in the dyslexic children. The 20 children with dyslexia were trained on a computer program designed to improve how youngsters distinguish spoken language. They spent nearly two hours a day, five days a week, for eight weeks on a series of word and sound games. Then the children returned to the lab for another fMRI scan.

Given that this study was the first of its kind in children, getting clean results was an achievement in itself. Beyond that, however, the significant changes in brain activity after training gave the team some very useful data. The dyslexic children showed increased activity in the left temporoparietal cortex, bringing them closer in brain function to children with normal reading abilities. They also showed new activity in the parts of the right-brain hemisphere that mirror language areas in the left and are not normally involved in language sound processing. The researchers concluded that these changes probably helped compensate for the children's still-incomplete sound processing. On top of the evidence seen in brain images, the children's scores on reading tests taken after the training program showed significant improvement over those taken before.

After her work was published in the March 4, 2003, issue of the *Proceedings of the National Academy of Sciences*, Temple received 200 e-mails from parents wanting to know how to help their dyslexic children. But while she cautioned that it's too early to provide a prescription, she was at least able to respond with a hopeful message. "This study and others," says Temple, "suggest that the key problem dyslexics have is understanding the sounds of the words they are trying to read—that's where energy needs to be directed."

Now an assistant professor at Cornell University, Temple plans to chart the brain activity of normal children learning to read. She wants to get down to age 5, admitting that it will take a "certain kind of magic" to be able to test children that young, but believing she can draw on her experience so far with kids and fMRI. Being a student, researcher, and mother all at once kept her grounded, Temple says, and "constantly aware of these bizarre connections children make." Temple and her family—husband Knox, and Chloe and Dylan, now 11 and 16 years old—have come a long way since tending chickens and goats and harvesting hay on a communal farm 55 miles northwest of Eugene, Oregon.

Eventually, Temple hopes to apply her research to education strategies that serve normal and learning-challenged children.

Meanwhile, Gabrieli credits her with showing that fMRI studies combined with training programs can be successfully done in dyslexic children when critical brain development is taking place. "This is a sensitive, good tool to look at the disorder and to approach treatment in children," he says.

—KENDALL POWELL

Elise Temple's research is inspired in part by her daughter and son, who "were in some ways my first test subjects."

MARC BRYAN-BROWN

STEVE MUREZ

Philippe Sansonetti researches bacterial dysentery, which infects 160 million people each year, and claims 1 million lives.

Bacteria Hijacks Pathway

This lethal pathogen is an ingenious invader.

Every year in the developing world, 160 million people contract shigellosis, or bacterial dysentery. One million of them—mostly young children—die. This heavy toll has moved the World Health Organization to make the development of a vaccine against the disease-causing bacterium *Shigella* a high priority.

Shigellosis is currently treated with antibiotics, which tend to be ineffective for two reasons. In regions where the disease is endemic, antibiotic resistance is alarmingly high and growing with each passing year. Moreover, the drugs don't work unless they are taken while the infection is in its earliest stages.

Philippe Sansonetti, an HHMI international research scholar at the Pasteur Institute in Paris, believes he has discovered the reason for *Shigella*'s early window of vulnerability to antibiotic treatment. In the process, he may have stumbled onto a previously unknown mechanism of bacterial spread: Microbes can hijack signaling pathways between host cells, effectively forcing the host cells to support their invasion. His team's findings were published in *Nature Cell Biology* in August 2003.

The first thing *Shigella* does on arrival in the colon—perhaps having entered the body in a morsel of contaminated food or by contact with an infected person—is to penetrate the epithelial cells that line the colon. After this initial invasion, says Sansonetti, the pathogen spreads through the cells that make up the lining—moving like a passenger walking through the cars of a train without entering the space on either side of the cells. By adopting this strategy, the invading bug avoids competition with the myriad bacteria that already reside in the gut. Just as important, it protects itself from attack by cells of the body's immune system, which typically kill foreign invaders by physically engulfing and digesting them. Because *Shigella* passes directly from one colon cell to the next, immune cells never come into contact with it. This helps explain why antibiotics are not effective against *Shigella* once it invades the colon lining.

"A lot of the deaths observed in endemic areas are due to late antibiotic delivery to babies, thus leaving the bug ample opportunity to spread in the intestinal tissue," says Sansonetti. The destruction of that tissue and resulting bloody diarrhea can kill malnourished infants.

In their search for a way to halt *Shigella*'s advance, Sansonetti and Guy Tran Van Nhieu have been working with a tantalizing clue: In 2001, they found that *Shigella* cannot spread in cultured HeLa cells, epithelial-like cells commonly used as a research model. This curious finding led them to focus on gap junctions—tiny channels through which neighboring cells exchange signaling molecules such as calcium and cAMP. Unlike epithelial cells of the colon, HeLa cells lack gap junctions. Perhaps, reasoned Sansonetti and Tran Van Nhieu, that absence correlates with the bacterium's inability to spread in HeLa cells.

One way of testing this hypothesis is to artificially induce HeLa cells to "develop" gap junctions. Using genetic engineering techniques, the two researchers created HeLa cells that expressed connexins, the biochemical building blocks of gap junctions. When they exposed these transfected cells to the *Shigella* pathogen, the infection's spread was explosive. Within six hours, all the cells forming an epithelial layer in a lab dish were infected, and no amount of washing or antibiotic treatment could halt *Shigella*'s progress.

It was already known that when *Shigella* invades an epithelial cell, it does so by forming a protrusion into the cell, and that once inside, the bacterium exists temporarily in a tiny cavity called a vacuole. It quickly escapes this bubble and sprouts a tail of actin filaments, which enables it to "swim" freely in the cell's soupy cytoplasm. "It looks as if the rationale for this bacterium moving intracellularly is for the bacterium to pass from one cell to another," says Sansonetti. In other words, the invader doesn't penetrate the cell simply to take shelter from the immune system; its aim seems to be to move methodically through the entire epithelial cell layer.

From the safety of a cell it has successfully invaded, *Shigella* forms another protrusion into an adjacent cell. Sansonetti and Tran Van Nhieu wondered how it does this. Does it penetrate the second cell by brute force or by some other mechanism? The researchers' discovery that gap junctions are involved in *Shigella*'s spread suggests something subtle: The bacterium is in fact modulating the signals passing between cells.

To test this concept, they loaded natural and engineered HeLa cells with a fluorescent calcium probe and traced it using sensitive imaging equipment. They found that calcium signaling was severely perturbed in engineered cells that were exposed to the bacteria. To their surprise, that perturbation appeared to be ordered, with huge fluxes of calcium arising from different cells, appearing on the monitoring screen like regular heartbeats. These "heartbeats" were amplified as new cells became infected and their own connexin-dependent signaling systems were recruited to the bacterium's cause.

By blocking calcium receptors in the cells, the researchers found they could halt the ordered flux, but, disappointingly, not the spread of infection. They had much better success, however, when they blocked receptors for ATP: This halted the bacterium's spread. Sansonetti surmises that the observed calcium pulses are secondary effects of a more fundamental disruption involving ATP.

It is not yet clear how this finding can be exploited therapeutically, Sansonetti notes, because blocking the host's signaling pathways to prevent bacterial spread would disrupt the cell's own healthy functioning. But it does shed light on some interesting mechanisms set in motion by *Shigella* as it works its way through the lining of the colon.

—LAURA SPINNEY

Free Thinking

Lithium might slow the progress of Alzheimer's disease.

For decades, millions of people with manic depression have found relief in lithium. It now appears that the drug might help sufferers of Alzheimer's disease as well. Researchers led by HHMI investigator Peter S. Klein have found that in mice the drug may block formation of the brain-clogging protein deposits that are hallmarks of Alzheimer's.

Klein's team demonstrated that lithium shows an ability to block an enzyme, glycogen synthase kinase-3α (GSK-3α), that's key to producing amyloid plaque, the material that accumulates around nerve cells of Alzheimer's sufferers. This plaque is triggered to form when amyloid precursor proteins (APPs) break apart to produce amyloid-β (Aβ) peptides, which congregate outside nerve cells in the brain.

GSK-3α also catalyzes formation of protein snarls, known as neurofibrillary tangles, that are found inside the neurons of people with Alzheimer's. Klein and his colleagues at the University of Pennsylvania School of Medicine published their findings in the May 22, 2003, issue of *Nature*.

In their initial experiments on cultured hamster ovary cells engineered to produce APP, the researchers showed that lithium markedly reduced production of amyloid peptides. "It was especially gratifying to find that lithium worked effectively in the known therapeutic range of the drug," says Klein. Importantly, the team's studies of lithium's effects found that the drug inhibited APP processing late in the production pathway and did not inhibit processing of another protein, called Notch, that's important in a wide range of cellular functions.

In other cell culture studies, the researchers knocked out the activity of two forms of GSK-3, showing that GSK-3α is specifically required for producing Aβ peptide. And when they engineered cells to overexpress GSK-3α, they saw an increase in Aβ peptide production.

"There is a concern that inhibiting both forms of GSK-3 could produce side effects such as cancer," says Klein. "But the fact that GSK-3α is specifically required means that we might be able to design drugs to inhibit only that form and bypass side effects that might occur by inhibiting both forms."

Finally, the researchers treated a mouse strain engineered to have Alzheimer's-like pathology with lithium levels analogous to those in patients taking the drug. The strain showed reduced Aβ production and reduced formation of amyloid plaques (see figure).

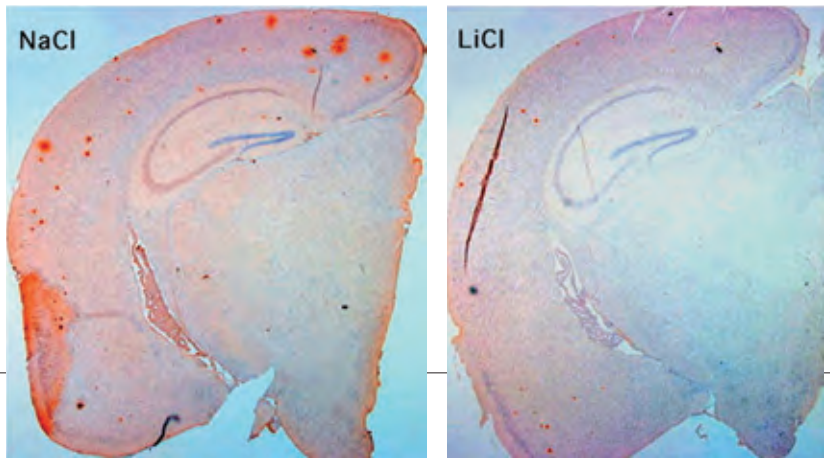
"There are several levels of significance for this work," says James R. Woodgett, an HHMI international research scholar at the Ontario Cancer Institute in Toronto, Canada. "It reveals a new means by which Aβ peptide production can be reduced, through inhibition of GSK-3α. It also opens up a new area of research for understanding the mechanism by which GSK-3 selectively modulates amyloid precursor processing."

Klein emphasized that more basic research needs to be done to pinpoint the mechanism of lithium's action before clinical testing should take place.

"Lithium has been used for over 50 years, and it's been a godsend for the control of manic depression," he says. "There are potential side effects that can't be ignored, but the benefits outweigh the risks in these patients. While the side effects of lithium appear to be more common in older patients, the possibility that lithium might slow or prevent Alzheimer's disease justifies further, albeit careful, study."

—DENNIS MEREDITH

Sections of brains from mice with Alzheimer's disease mutations, stained for amyloid plaques. The sodium chloride–treated controls (NaCl) have many plaques (orange). In brains from a parallel group treated with lithium chloride (LiCl), there is a marked reduction in the number of visible plaques.



CHRISTINA WILSON AND VIRGINIA LEE

HHMI LAB BOOK

RESEARCH NEWS FROM HHMI SCIENTISTS

IN BRIEF

New Bugs at the Zoo Ten newly identified mycobacteriophages—viruses that infect a range of bacteria, including those that cause tuberculosis and leprosy—have been found in the monkey pit at the Bronx Zoo and other locations. The studies also uncovered evidence that mycobacteriophages undergo constant random genetic mixing in the wild, producing a mélange of recombinant viruses. Researchers: **Graham F. Hatfull** and **William R. Jacobs Jr.**
www.hhmi.org/news/hatfulljacobs.html

Quick-Change Artist New findings indicate that one strategy used by the human immunodeficiency virus-1 (HIV-1), a common strain of the virus that causes AIDS, is to evade the body's immune system by continuously changing the arrangement of large sugar molecules that stipple its protein coat, thereby blocking antibody-docking stations. Researcher: **George M. Shaw**
www.hhmi.org/news/shaw2.html

Muscle Breach A protein that is defective in two types of muscular dystrophy also appears to be important in repairing the damaged membrane of a muscle fiber. This discovery reveals the first known component of the muscle-repair machinery. Further studies of the protein and its relatives could lead to a better understanding of disorders that affect cardiac and skeletal muscles. Researcher: **Kevin P. Campbell**
www.hhmi.org/news/campbell5.html

Coaxing Hair Growth Research has shown that the delicate interplay of two chemical signals coaxes stem cells into becoming hair follicles—a finding that may lead to better understanding of hair growth and hair follicle development. Researcher: **Elaine Fuchs**
www.hhmi.org/news/fuchs2.html

"Kinome" Yields Colon Cancer Clues

VOGELSTEIN

KAY CHERNUSH

MARKOWITZ

GEOFFREY PANKHURST

In the first such effort to exploit sequences provided by the Human Genome Project, scientists have performed a systematic analysis of mutations in a family of genes implicated in a variety of cancers—colon cancer in particular. As a result, the researchers identified mutations in genes for enzymes—tyrosine kinases—that occur in perhaps a third of colon cancers. These genes might now serve as a new target for drugs.

HHMI investigators Bert Vogelstein, at the Sidney Kimmel Comprehensive Cancer Center at The Johns Hopkins University School of Medicine, and Sanford Markowitz, at Case Western Reserve University and University Hospitals, together with colleagues, screened a database of genes that produce protein kinases—collectively referred to as the "kinome." When altered, as in circumstances when genetic mutations turn on a tyrosine kinase in the absence of a normal activation signal, the enzyme signals cells to divide continually, a hallmark of cancer. This process, called "constitutive activation," provides ideal drug targets, says Vogelstein. There is ample precedent: The drug Gleevec, used to treat chronic myeloid leukemia, acts on such a target.

The researchers identified the regions of the proteins most responsible for normal enzyme activity in all 138 known tyrosine kinase and similar genes. They then compared them with the corresponding regions in 35 colorectal cancer cell lines, finding mutations in the kinase domains of 14 genes. The team subsequently analyzed another 147 colorectal cancer cell lines in the same way for kinase-domain mutations, discovering 46 of them.

The most difficult part, says Vogelstein, was discerning which mutations would trigger cancer from the large number of harmless variations in kinase genes. To do so, research team member Alberto Bardelli, an HHMI research associate at Johns Hopkins, painstakingly compared hundreds of changes in cancer-cell genes—including dozens of alterations that hadn't been seen before—with the corresponding genes in normal tissues of the same patient. The results indicated that about 30 percent of colorectal cancers have at least one mutation in the tyrosine kinome, meaning that these cancers will theoretically be vulnerable to drugs that can block the enzyme's action.

"We envision that in the future, each patient with colon cancer could have a diagnostic analysis to determine which kinases are activated by mutation—an easy task once you know which ones to look for," Vogelstein says. "Then, that patient could be treated with a drug that specifically targets that kinase." The scientists reported their findings in the May 9, 2003, issue of *Science*.

More broadly, Vogelstein says, "I think it's a perfectly reasonable speculation to suggest that ultimately a great majority of cancers will be found to have at least one drug-targetable mutation, and this could lead to new avenues for individualized therapy." H

This Is Your Brain on Noise

Continuous background noise—so-called "white noise"—delays development of the auditory cortex, researchers report, potentially affecting the ability to both hear and learn spoken language. But white noise may not be all bad. It may actually just delay the period during which the brain adapts and rewires itself as part of normal early development. Such "plasticity" could have major implications for treating developmental problems.

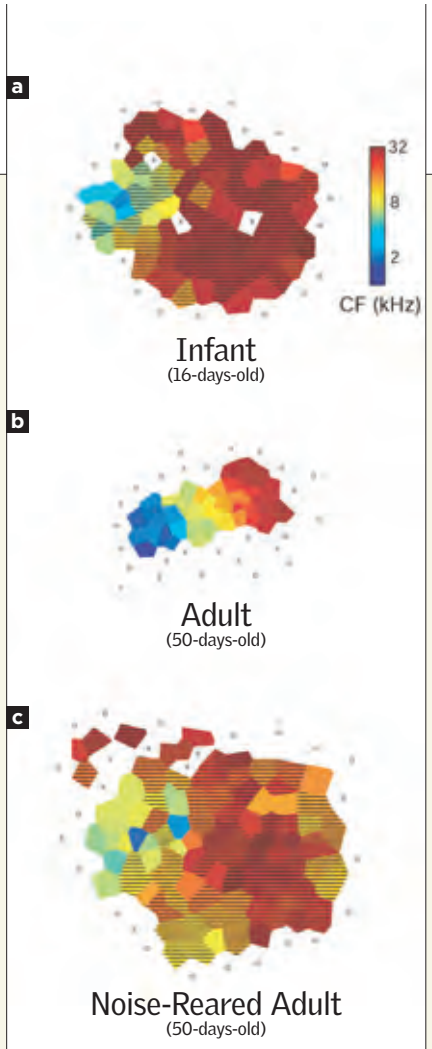
HHMI medical student fellow Edward F. Chang and otolaryngology professor Michael M. Merzenich at the University of California, San Francisco, already knew that in newborn rats the brain's auditory portion is still under construction and that during the first month of life the auditory cortex neurons begin to develop dramatically and organize. During this phase, says Chang, the brain is especially sensitive to sound. Chang and Merzenich wanted to use this knowledge to explore how auditory cortex organization is regulated during early development.

The researchers exposed rat pups to moderate levels of white noise, just loud enough to mask normal environmental sounds, and they examined the effects of that noise on brain development. They found that animals exposed to white noise continuously for up to three months from birth had auditory neuron organization virtually identical to that of normal 16-day-old pups.

"That suggests that the developing brains in such animals are highly sensitive to experience—in particular, the experience of patterned sensory input," says Chang. He and Merzenich reported their findings in the April 18, 2003, issue of *Science*.

Chang and Merzenich also found that normal sounds were more important to the timing of the "critical period" (the stage early in life when the visual and auditory cortexes develop) than physical age alone. Subsequent experiments in adult rats showed that the brain could still reorganize its circuitry despite a relatively advanced age—that is, it could initiate and maintain the critical period—even though the brains of normal rats ordinarily cease rewiring at that age.

"These findings suggest that noise can have devastating effects on the rate of development of the brain," Chang says. But they also suggest that "once you improve the condition of the sensory environment, the brain can catch up and reorganize." This plasticity offers hope for children at risk of developmental delays. H



FROM CHANG, E. F. AND MERZENICH, M. M. 2003. *SCIENCE* 300:498-502. © 2003 AAAS.

Developmental organization of the auditory cortex is delayed by noise, shown by "maps" representing sound frequency in the auditory cortex. The infant auditory cortex (a) is large, poorly organized, and overdominated by high frequencies. By the end of the first month of life, the map has become smaller and more refined, and has balanced sound frequency distribution (b). When infants are exposed to moderate-level white noise that masks normal environmental sounds, the cortex retains an immature organization into adulthood (c).

HHMI Lab Book written by Steven I. Benowitz

IN BRIEF

Tactical Striker The first clinical trials of a new type of cancer treatment—based on blocking the action of cytotoxic T lymphocyte-associated antigen 4, which inhibits activated immune system T cells—indicate that this approach enhances attacks on tumors while sparing the body's own tissue.
Researcher: **James P. Allison**
www.hhmi.org/news/allison2.html

Resistance Is Futile Working with mice genetically engineered to develop resistance to the antileukemia drug Gleevec, researchers have shown that using a second drug, PKC412, overcomes such resistance. PKC412 provides complementary activity against Gleevec's main target, a tyrosine kinase enzyme. Patients with cancers that can be blocked by such kinase inhibitors might one day be treated with drug cocktails custom-made to their specific case.
Researcher: **D. Gary Gilliland**
www.hhmi.org/news/gilliland4.html

They Wnt Thataway A purified version of a protein called Wnt, a powerful signaling molecule involved in triggering development and cell proliferation, can cause blood-forming stem cells to proliferate as well. This discovery suggests novel ways to restore the blood-forming systems of cancer patients whose stem cells have been destroyed by chemotherapy.
Researcher: **Roel Nusse**
www.hhmi.org/news/nusse.html

Lupus Look-Alike Researchers have discovered that a type of genetic malfunction—abnormalities in a protein called Ro 60-kDa—causes an autoimmune disease in mice that resembles systemic lupus erythematosus in humans. The discovery suggests that the protein might normally play a protective role in the body by "hiding" defective ribonucleoproteins from attack by the immune system.
Researchers: **Sandra L. Wolin** and **Richard A. Flavell**
www.hhmi.org/news/wolin.html

■ Seven HHMI investigators were elected fellows of the American Academy of Arts and Sciences. They are **Thomas D. Albright**, The Salk Institute for Biological Studies; **Philip A. Beachy**, The Johns Hopkins University School of Medicine; **Carolyn R. Bertozzi**, University of California, Berkeley; **Jennifer A. Doudna**, University of California, Berkeley; **Stephen J. Elledge**, Baylor College of Medicine; **Dan R. Littman**, New York University School of Medicine; and **Arthur Weiss**, University of California, San Francisco.

■ **Kristi S. Anseth**, an HHMI investigator at the University of Colorado at Boulder, won the 2003 Allan P. Colburn Award for Excellence in Publications by a Young Member of the Institute from the American Institute of Chemical Engineers.

■ Two HHMI investigators were elected to membership in the American Philosophical Society: **Richard Axel**, Columbia University College of Physicians and Surgeons, and **David Eisenberg**, University of California, Los Angeles.

■ Two HHMI investigators, **Gregory S. Barsh** at Stanford University School of Medicine and **Val C. Sheffield** at the University of Iowa College of Medicine, each won the 2003 E. Mead Johnson Award For Research in Pediatrics from the Society for Pediatric Research.

■ **Peter E. Baumann**, who completed an HHMI postdoctoral fellowship before joining the Stowers Institute for Medical Research, is one of 20 Pew Scholars in the Biomedical Sciences for 2003.

■ **Denis Baylor**, professor emeritus of neurobiology at Stanford University School of Medicine and a member of the HHMI Scientific Review Board, and **John D. Scott**, an HHMI investigator at Oregon Health & Science University, were elected to the Royal Society of London, the United Kingdom's national academy of science.

■ Six HHMI investigators were among 56 physician-scientists elected to membership in the Association of American Physicians. They are **Morris J. Birnbaum**, University of Pennsylvania School of Medicine; **James M. Cunningham**, Brigham and Women's Hospital and Harvard Medical School; **Jeffrey M. Friedman**, The Rockefeller University; **D. Gary Gilliland**, Brigham and Women's Hospital and Harvard Medical School; **Ben Margolis**, University of Michigan Medical School; and **Leonard I. Zon**, Harvard Medical School.

■ Worm Work Wins Prize

MARK YOUNG

Cornelia I. Bargmann, an HHMI investigator at the University of California, San Francisco, has been named the winner of the 2004 Dargut and Milena Kemali Foundation International Prize for Basic and Clinical Neurosciences. Awarded to a scientist under the age of 45, the prize will be presented in July 2004 at the Congress of the Federation of European Neuroscience Societies in Lisbon, Portugal.

Bargmann studies the genes that affect neuronal development and behavior, using the nematode *Caenorhabditis elegans* as a model organism. The award recognizes her pioneering work in the cellular, molecular, and developmental basis of chemosensory transduction in the *C. elegans* nervous system and the implications of her results for an understanding of the biological formulation of behavior.

■ **Patrick O. Brown**, an HHMI investigator at Stanford University School of Medicine, won the 2003 BioTech Helsinki Prize and the 2003 Promega Biotechnology Research Award. Brown was the first winner of the Helsinki Prize, which is presented to a life scientist whose research is of global significance for society. The Promega Award is given by the American Society for Microbiology to recognize contributions to the application of biotechnology.

■ **Carlos Bustamante**, an HHMI investigator at the University of California, Berkeley, has been named winner of the Biophysical Society's 2004 Founders Award for his pioneering role in single-molecule physics.

■ **Edward M. F. De Robertis**, an HHMI investigator at the University of California, Los Angeles, was elected to corresponding membership in the Latin American Academy of Sciences.

■ **Brian J. Druker**, an HHMI investigator at the Oregon Health & Science University, received the David A. Karnofsky Memorial Award from the American Society of Clinical Oncology, recognizing innovative research that has changed the practice of oncology. He also received the 2003 Braunschweig Prize, at 50,000 euros the largest research prize awarded by a German city, for research in molecular cancer therapy.

■ **Ronald M. Evans**, an HHMI investigator at The Salk Institute for Biological Studies, received the 2003 Alfred P. Sloan Jr. Prize from the General Motors Cancer Research Foundation. Evans shared the prize with Pierre Chambon, director emeritus of the Institute for Genetics and Cellular and Molecular Biology in Strasbourg, France. Worth $250,000, the award is given for contributions to basic research in cancer.

■ **Stanley Fields**, an HHMI investigator at the University of Washington School of Medicine, shared the 2003 Jacob Heskel Gabbay Award in Biotechnology and Medicine with Roger Brent of the Molecular Sciences Institute. Given by the Jacob and Louise Gabbay Foundation, the award recognizes scientists for outstanding biomedical research early in their careers.

■ Four HHMI investigators, two HHMI professors, and an international research scholar were elected to fellowship in the American Academy of Microbiology. Investigators are **Eduardo A. Groisman**, Washington University School of Medicine; **William**

R. Jacobs Jr., Albert Einstein College of Medicine; **Paul Modrich**, Duke University Medical Center; and **Thomas A. Steitz**, Yale University. Also elected were HHMI professors **Jo Handelsman**, University of Wisconsin–Madison, and **Graham F. Hatfull**, University of Pittsburgh, and international research scholar **B. Brett Finlay**, University of British Columbia, Canada.

Richard L. Huganir, HHMI investigator at The Johns Hopkins University School of Medicine, received the 2004 Santiago Grisolia Chair Prize given by the Fundación Valenciana de Investigaciones Biomédicas in Spain. The prize recognizes contributions to neuroscience and biomedicine.

Nancy Hutchison, HHMI precollege grant program director at the Fred Hutchinson Cancer Research Center in Seattle, won the 2003 Bruce Alberts Award for Excellence in Science Education. The award is given by the American Society for Cell Biology.

William R. Jacobs Jr., an HHMI investigator at Albert Einstein College of Medicine, won the 2003 Gardner Middlebrook Award from BD Diagnostic Systems. The prize recognizes lifetime achievement in the field of mycobacteriology and tuberculosis.

Simon W. M. John, an HHMI investigator at The Jackson Laboratory, received the 2004 Cogan Award from the Association for Research in Vision and Ophthalmology in recognition of research that relates directly to disorders of the human eye or visual system.

Eric R. Kandel, an HHMI investigator at Columbia University College of Physicians and Surgeons, won the 2003 Paul Hoch Award from the American Psychopathological Association. Kandel was also named an honorary fellow of the American College of Psychiatrists for distinguished service in psychiatry.

Randal J. Kaufman, an HHMI investigator at the University of Michigan Medical School, received the 2003 Van Wezel Prize from the European Society for Animal Cell Technology.

Rebecca McClaine, an HHMI medical student fellow at Duke University Medical Center, won the 2003 Gertie Marx Resident Research Award from the Society for Obstetric Anesthesia and Perinatology, a prize recognizing accomplishments of a junior investigator. This is the first time that a medical student has won the award.

Ruslan Medzhitov, an HHMI investigator at Yale University School of Medicine, received the Cancer Research Institute's 2003 William B. Coley Award for Distinguished Research in Basic and Tumor Immunology.

Craig C. Mello, an HHMI investigator at the University of Massachusetts Medical School, was one of four winners of the 2003 Wiley Prize in the Biomedical Sciences, recognizing work that has opened new fields of research or furthered novel concepts in a biomedical discipline.

Tamar Schlick, an HHMI investigator at New York University, received the 2003 Agnes Fay Morgan Research Award for achievement in chemistry. The award is given by Iota Sigma Pi, a national honor society for women in chemistry.

Allan C. Spradling, an HHMI investigator at the Carnegie Institution of Washington, won the 2003 Edwin Grant Conklin Medal from the Society for Developmental Biology.

Bruce D. Walker, an HHMI investigator at Massachusetts General Hospital, won the 2003 Commitment to Children Award from the Elizabeth Glaser Pediatric AIDS Foundation. The award recognizes contributions to children's health and AIDS research.

Xiaodong Wang, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas, received the 2003 Norman Hackerman Award in Chemical Research from the Welch Foundation. The award recognizes the work of young chemical scientists.

Masashi Yanagisawa, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas, won the 2003 Bristol-Myers Squibb Foundation's Award for Distinguished Achievement in Cardiovascular Research.

Sara L. Young, director of the American Indian Research Opportunities program at Montana State University, was one of 10 individuals to receive the 2002 Presidential Awards for Excellence in Science, Mathematics, and Engineering Mentoring. Young runs the Montana Apprenticeship Program, funded by a grant from HHMI.

Honors for Duke Researcher

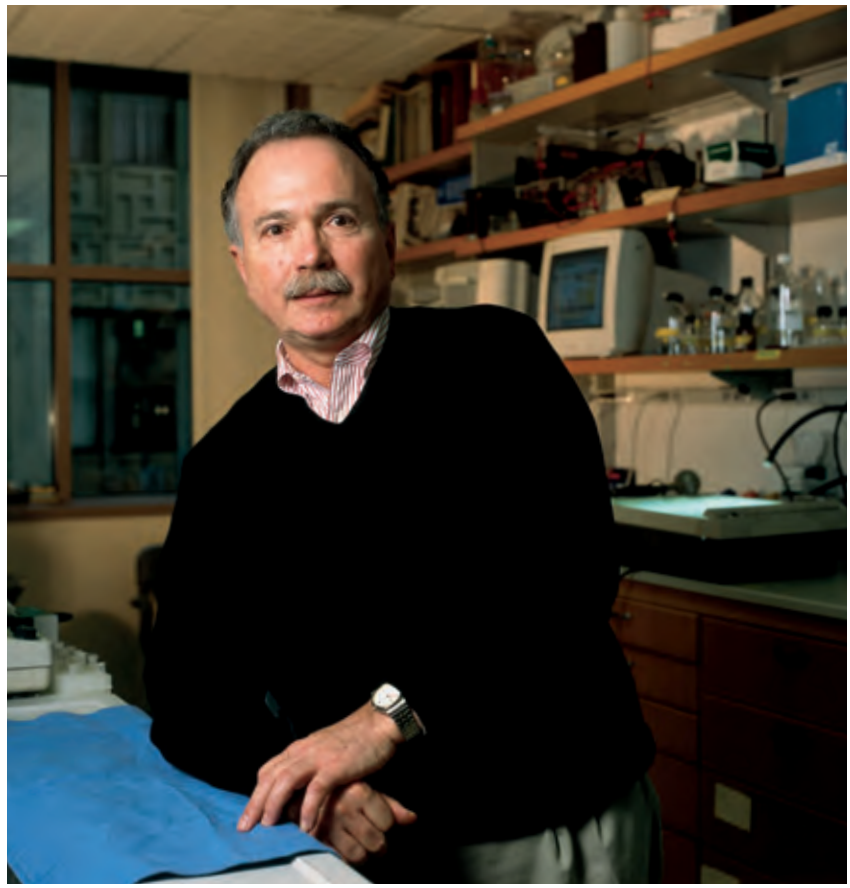
SCOTT DINGMAN

Robert J. Lefkowitz, an HHMI investigator at Duke University Medical Center, has been named the winner of the 2003 Lefoulon-Delalande Foundation Grand Prize for Science from the Institut de France. Recognizing Lefkowitz's discoveries in the field of helical transmembrane receptors, the prize will be presented in January 2004 at a meeting of the Société Française de Cardiologie.

Lefkowitz also won the 2003 Endocrinology Prize from the Fondation IPSEN, a French foundation that supports biomedical research, mainly in the neurosciences.

A cardiologist and biochemist, Lefkowitz's research on the adrenergic receptors and G protein–coupled receptors formed many of the foundations of modern receptor biology. Moreover, he has taken his research from bench to bedside in the development of a molecule that inhibits β-adrenergic receptor kinase, a protein that prevents the heart from responding to hormone stimulation. Lefkowitz's work has broader clinical implications as well—for the development of treatments for epilepsy, heart disease, and pain.

Betting on the Individual



BARBARA RIES

A country doctor inspired David Clayton to pursue a career in science.

More than 50 years ago, an unintentional encounter with the sharp end of a farm hoe sent young David A. Clayton to see the family physician, Lauren Wunsch, in rural Plattville, Illinois. Today, Clayton can still picture the doctor's kindly face as he stitched the cut in the boy's forehead.

Dr. Wunsch impressed him with his wisdom and powers of analysis—and he provided a glimpse into the world of science. Clayton, now HHMI's vice president and chief scientific officer, also recalls Dr. Wunsch's office, with its hefty medical textbooks and mysterious bottles of "chemicals." It was a revelation to Clayton that a job could be so fascinating; the only ones he knew as a teenager were detasseling corn for 65 cents an hour and working all night at a gas station. Dr. Wunsch's learned corner of Plattville inspired the young man to pursue a career in science.

Clayton did his undergraduate work at Northern Illinois University, where he excelled, so impressing his freshman-chemistry professor that he was offered a lab job—a rarity even today for an undergraduate. Starting out washing flasks and beakers, Clayton soon was participating in the lab's research, and during his junior year, he published a paper in *The Journal of Organic Chemistry*. This unusual feat, he believes, helped him get into the California Institute of Technology, where he attended graduate school.

After doing postdoctoral work at the City of Hope National Medical Center near Los Angeles, Clayton joined the faculty of Stanford University Medical School in 1970. He served for 26 years there, first as a professor of pathology and later of developmental biology.

Clayton came to HHMI in 1996 as a senior scientific officer and was named vice president for science development in 2000. Today, as the Institute's chief scientific officer, he is in charge of its core programs in science, including HHMI investigators, and he was a driving force behind the decision to create Janelia Farm, the planned state-of-the-art scientific laboratory complex in Virginia.

Despite his growing administrative responsibilities, Clayton has managed to keep up his own research on mitochondrial genes and related diseases. He recently moved his lab from Stanford to a building on the National Institutes of Health campus, where he tries to spend at least a small part of each week. "It's critical to maintain an active level of scientific investigation," he says. "At the end of the day, the most stimulating thing that any real scientist can do is to keep thinking creatively. That will spill out into everything else. And there's no greater satisfaction than the feeling of completing a piece of work that leads to a new understanding of some scientific puzzle."

Still, Clayton enjoys the current balance in his career between the scientific and the administrative. He especially admires HHMI's unique approach to the support of scientific research—identifying the most talented people and supporting them, rather than funding the research itself.

"We bet on the individual," he says. "We put our investment in someone willing to take risks and hope that in five years we will know something important from that person that we don't know today."

Clayton thinks the most exciting biomedical research areas in the coming years will include regenerative biology, biochemistry at the single-cell level, and the fundamental aspects of memory and thought. "When all is said and done, I think those are among the most interesting areas for scientists," he says. "We have much to learn."

For relaxation, Clayton likes nothing better than to visit the 200-acre family farm in Plattville. He and his wife Lauretta—who have three children, all in college now—travel there whenever they can, usually several times a year. "It's very peaceful, a real stress-free environment," he says.

At the farm, Clayton checks in with his two lovingly restored vintage cars, a 1967 dark blue Pontiac GTO and a 1977 red Corvette.

He still drives the GTO. "It's not a museum piece," he says. "I drive it whenever I go there." But he's not sure how well it would fare in the Washington area. "It has a lot of 'go' but very little 'stop' because the brakes are undersized. So I think taking it on the Beltway might be dangerous."

—MARLENE CIMONS

IN MEMORIAM

Nestor Villarosa Santiago

Nestor V. Santiago, vice president and chief investment officer of HHMI, died on June 12, 2003, of a heart attack. He was 54 years old. Santiago, who joined HHMI in 2000, was responsible for managing the Institute's investment department and endowment, which stood at $11 billion at the end of June.

"Nestor's death is a double loss for HHMI—a loss at the professional and personal levels," said Institute President Thomas R. Cech.

Santiago was "a brilliant strategist, and a successful one," Cech said. "He achieved investment returns for the Institute that have enabled us to continue funding our research and education programs during trying economic times. He loved being associated with HHMI and had the highest regard for its success in supporting the very best biomedical research, both in the United States and internationally. As part of the leadership group of the Institute, he had valuable insights and a unique angle on many of the problems we face."

ROY KARTEN

At a funeral mass held June 18 at Holy Trinity Catholic Church in Georgetown, HHMI Trustee Garnett L. Keith, chairman of SeaBridge Investment Advisors, L.L.C., spoke of the personal loss in Santiago's death: "It would be easy to dwell on Nestor's analytical abilities and the way his facile mind could balance the risks and potential rewards in an opportunity. But those who knew him well place those skills second in the list of why we loved to work with Nestor. At the head of the list were Nestor's personal qualities: warmth, caring, fairness, and a modesty which was all the more remarkable in light of his exceptional ability and repeated accomplishments."

Santiago steered the HHMI endowment successfully through turbulent financial markets during the past three years, providing investment returns that outperformed the markets. At HHMI, Santiago and his colleagues in the investment department restructured the endowment portfolio with the goal of maximizing returns while ensuring a steady stream of income for the Institute's programs.

Throughout his career as an investment manager, Santiago used his skills and knowledge to serve organizations with a broad mission to benefit society. Before joining HHMI, Santiago was head of the investment office at the International Monetary Fund, where he was responsible for a $3.8 billion globally diversified portfolio. Previously, Santiago worked for two decades at the World Bank, serving as a financial analyst and planner in positions throughout Asia, Africa, and Latin America before becoming director of the Bank's pension department.

Santiago did graduate work in economics and undergraduate work in chemical engineering at the University of the Philippines, where he graduated magna cum laude. He went on to earn an M.B.A. degree with high distinction from Harvard Business School. A chartered financial analyst, he served on the boards of the Emerging Market Growth Funds, Inc., and the Bank Fund Credit Union. He was a CREF Trustee of TIAA-CREF and was a financial adviser to the Margaret McNamara Memorial Fund.

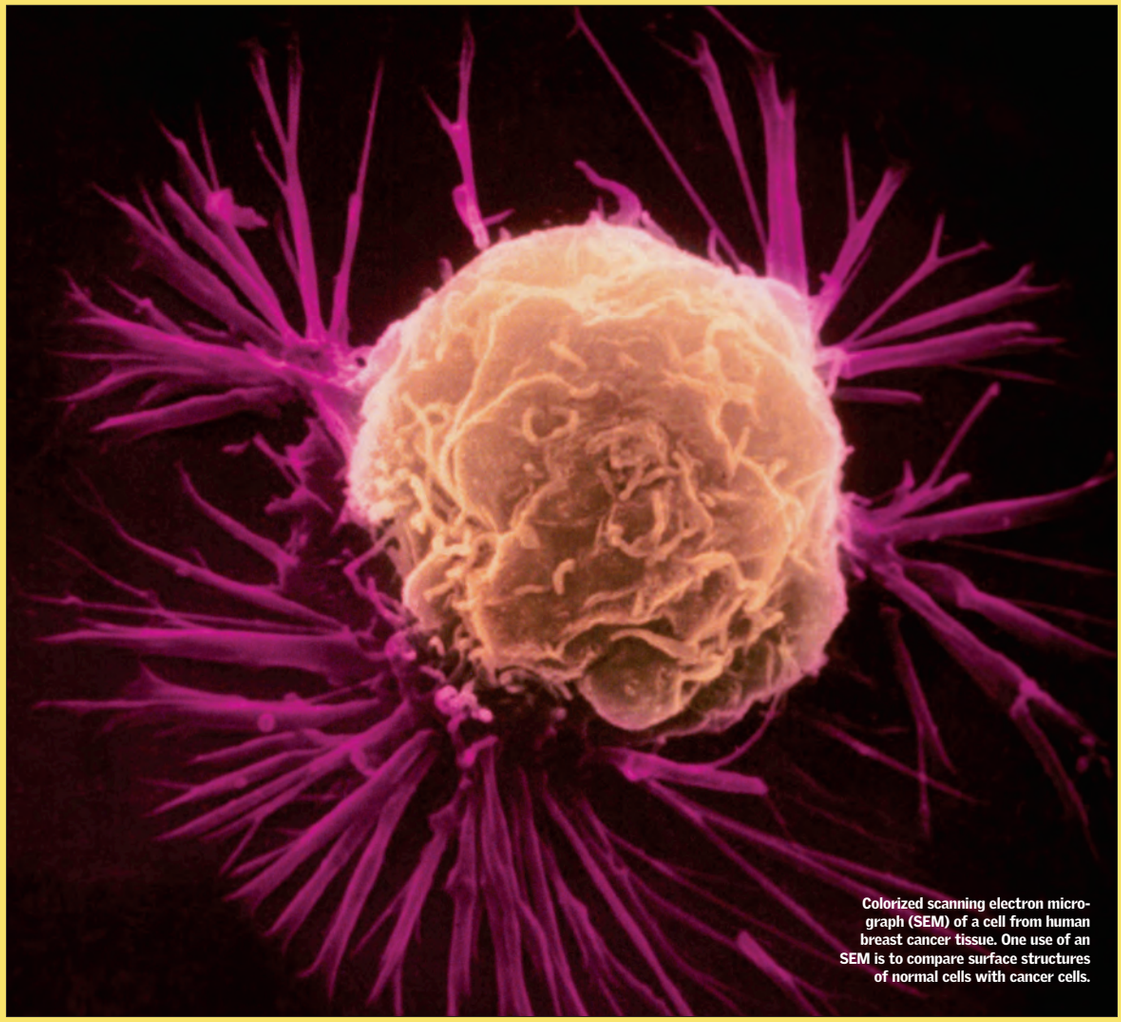
Santiago also was a trustee of the Washington Opera, a member of the investment committee of Arena Stage, and a past chairman of the finance council and an active member of Holy Trinity Catholic Church.

Santiago is survived by his wife, Aurora, and daughter, Nina, both of Washington, D.C. He is also survived by three siblings: Cielo Santiago Myers, Mercedita Santiago Nolledo, and Teodoro Villarosa Santiago, Jr., as well as numerous nieces, nephews, aunts, uncles, and cousins.

In 2000, Santiago and his siblings established the Santiago Fund to benefit the Nueva Ecija National High School, which is located in the Nueva Ecija province about 70 miles north of Manila. Crowded and underfunded, the school serves many needy but talented students with limited educational opportunities, particularly in science and mathematics. To memorialize Nestor's personal commitment to the school and promote the Institute's science education mission, HHMI has created an endowment to contribute to the Santiago Fund in support of a highly qualified science teacher. Contributions are being matched dollar-for-dollar by HHMI up to an aggregate maximum of $50,000.

" 'Nestor the Investor' has left the Institute strong, positioned to continue supporting the very best," Cech told those gathered at Santiago's funeral. "He has left his department strong, well able to carry out the mission that they began together with Nestor and will now carry on by themselves. And Nestor, the soft-spoken, humane man, has left part of himself in each one of us—we cannot go through a day without rejoicing at what we have learned from him." H

IN THE NEXT ISSUE



Colorized scanning electron micrograph (SEM) of a cell from human breast cancer tissue. One use of an SEM is to compare surface structures of normal cells with cancer cells.

NIH

Breast Cancer Metastasis

Researchers have identified a set of "rogue" genes that accelerate the spread of breast cancer from its primary site to a secondary location in bone marrow. The finding suggests that different cancers might bear unique "gene expression signatures" that govern and guide metastasis. Are there implications for the treatment of other cancers?

The Mysterious Centromere

Centromeres, peculiar pieces of DNA, have a vital job—they direct the shuffling of chromosomes during cell division. And while centromeres may hold keys to cancer and birth defects, their exact workings are a mystery, leading one researcher to label them "the ultimate black box of our genome."

The Rocky Road of Rare Diseases

Dedicated to the treatment of rare eye diseases, two investigators developed an unprecedented genetic testing program that tells patients exactly what has gone wrong in their genes. In the process, they found themselves embroiled in debates involving insurance coverage, genetic counseling, and intellectual property protection.

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