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HHMI BULLETIN

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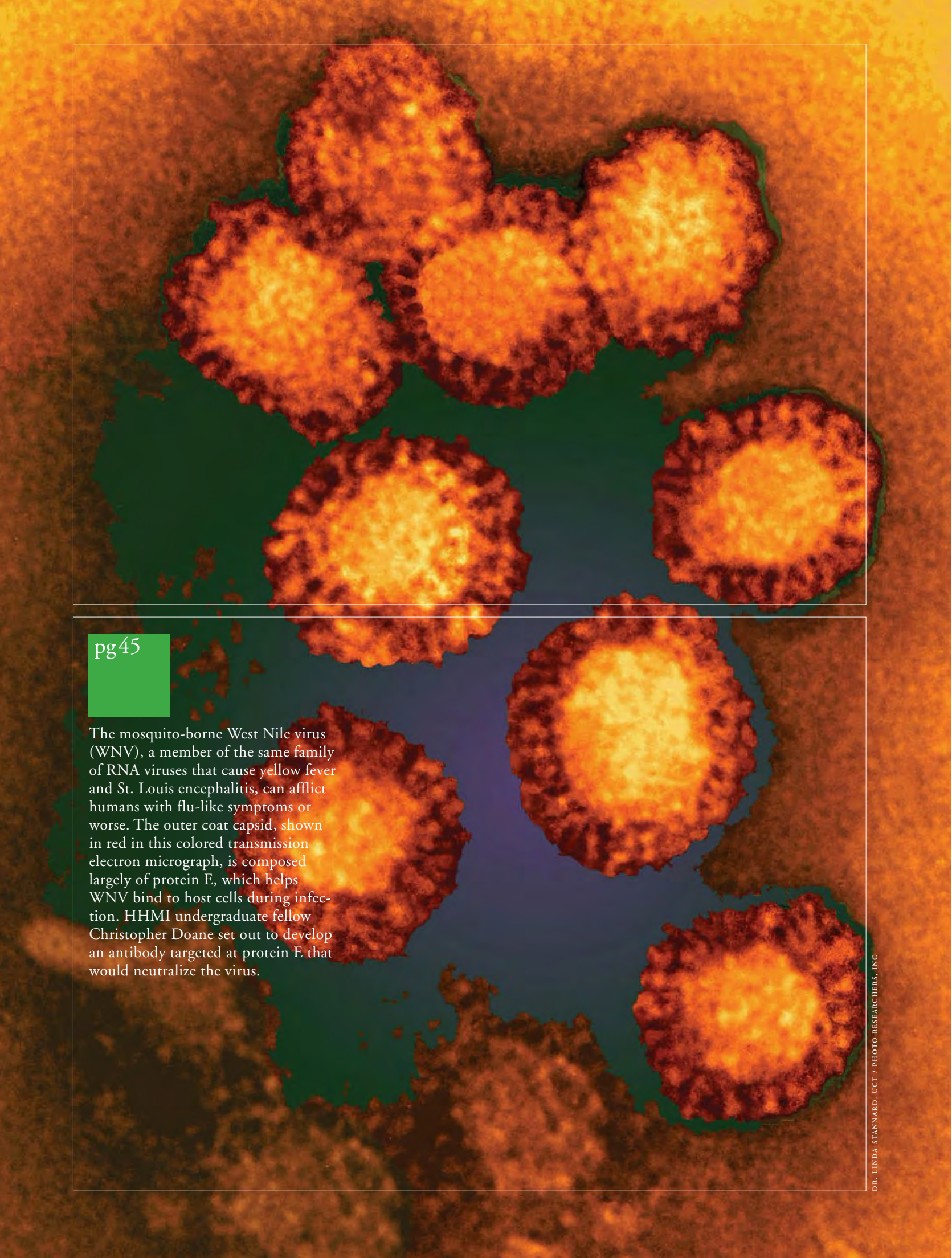
WINDOW TO INTERVENE

Sudden cardiac death kills as many as 300 young athletes each year. The root cause is often genetic. But now a new test offers answers and insight.



pg 45

The mosquito-borne West Nile virus (WNV), a member of the same family of RNA viruses that cause yellow fever and St. Louis encephalitis, can afflict humans with flu-like symptoms or worse. The outer coat capsid, shown in red in this colored transmission electron micrograph, is composed largely of protein E, which helps WNV bind to host cells during infection. HHMI undergraduate fellow Christopher Doane set out to develop an antibody targeted at protein E that would neutralize the virus.

DR. LINDA STANNARD, UCT / PHOTO RESEARCHERS, INC.

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DEPARTMENTS

FEATURES

Window To Intervene

[cover story]

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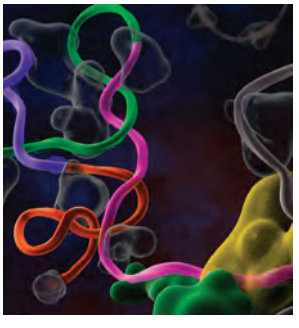
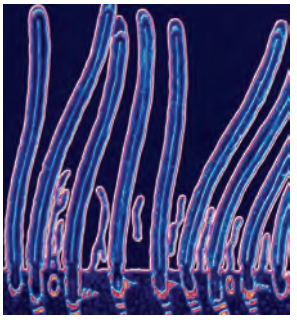


Alternative Splicing

Researchers have long assumed that one gene usually codes for one protein. But there's evidence that the rule instead is "one gene, many proteins." How does that happen?

Solving Big Questions

Poised to engage in some of science's largest riddles, seven researchers are appointed to be the first group leaders at HHMI's Janelia Farm Research Campus.



The Importance of Being Cilia

Once considered merely a vestige of evolution, cilia are in fact essential to many of the body's organs. As researchers learn more about cilia's role in disease, they're starting to pay this once-ignored organelle much more attention.

WWW.HHMI.ORG / BULLETIN

Visit the *Bulletin Online* for additional content and added features.

COVER IMAGE: JOHN HUET

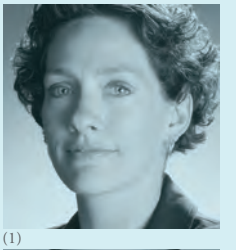
CONTRIBUTORS

Kathryn Brown is principal of EndPoint Creative, LLC, a communications company that works with science, medical, and technology organizations. Brown is a past contributing correspondent for Science, and her work has also appeared in *Scientific American*, *Technology Review*, *Discover*, *New Scientist*, and other national magazines. Among other honors, she won the National Association of Science Writers Clark/Payne Award for Science Journalism in 1999. (1)

After studying photojournalism at the University of Missouri, **Paul Fetters** spent five years in New York City shooting for *USA Today*, *Time*, *Newsweek*, and other publications. Now based in Falls Church, Virginia, he travels throughout the United States and the world completing photo assignments for corporate, nonprofit, magazine, and gallery clients. (2)

After earning a master's degree in medical and biological illustration from the Johns Hopkins School of Medicine in 1997, **Graham Johnson** moved to the Salk Institute to complete illustrations for the textbook *Cell Biology*, by Tom Pollard and Bill Earnshaw. He cofounded fivth.com and continued work in Boulder, Colorado as a freelance medical illustrator/animator. This fall, Johnson begins a Ph.D. program in molecular biology at the Scripps Research Institute, where he hopes to advance techniques for visualizing molecular interactions within cellular contexts. (3)

Paul Muhlrad did his doctoral dissertation on worm sperm (*A Genetic and Molecular Analysis of Spermiogenesis Initiation in* Caenorhabditis elegans). He writes from Tucson, Arizona, where he lives with his wife and two daughters. Between freelance assignments, he enjoys digital photography, riding his recumbent bike, and playing the banjo (but hardly ever at the same time). (4)



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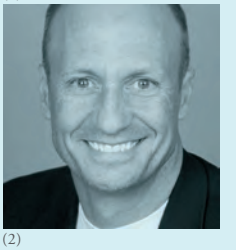

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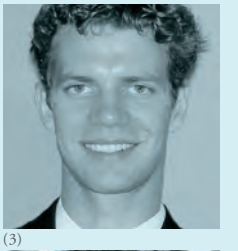

(3)

(4)

BROWN: PETER KROGH

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Thomas R. Cech
President
Howard Hughes Medical Institute

EXPANDING OPPORTUNITIES

More than 80 aspiring scientists converged on our headquarters earlier this year for an unusual 3-day meeting—and it wasn't simply the exuberant ice cream social that set it apart from the many other meetings HHMI hosts each year. Gathered in the Great Hall were more than 50 undergraduates, selected from among the thousands of students who participate in research projects that HHMI funds each year at liberal arts colleges and research universities. They were about to commence an experimental—and experiential—adventure, a summer of research in the laboratory of an HHMI investigator or professor. Last year's veterans also joined the throng to present their work and share their experiences.

So what made this meeting exceptional? The very presence of these students and what they represent for the future of science. Three years ago, HHMI quietly began a new initiative called EXROP—the Exceptional Research Opportunities Program—in an effort to encourage minority and disadvantaged students to consider careers in science. Through all the planning, two individuals offered inspiration and encouragement: James Gilliam, Jr., who served as a charter trustee of the Institute from 1984 until his untimely death in 2003, and Freeman Hrabowski, president of the University of Maryland, Baltimore County (UMBC).

I met Freeman more than a decade ago when Harold Varmus, then director of the National Institutes of Health, suggested that we might have common interests. He was right. Raised and educated in Birmingham, Alabama, at the height of the civil rights movement, Freeman is a mathematician who was initially recruited to UMBC to create a bold new program. The Meyerhoff Scholars Program began with a goal of developing a new generation of African American engineers and mathematicians. It has since expanded to encompass students interested in a wide variety of scientific disciplines.

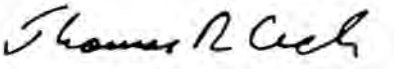
Like Michael Summers, an HHMI investigator at UMBC, I became a convert. Over the past decade, 16 Meyerhoff Scholars have spent summers in my lab in Boulder, Colorado, and each of them has gone on to medical or graduate school. The overall success rate of the program is equally impressive. Since 1993, when the first class graduated, roughly 80 percent of the more than 400 Meyerhoff Scholars have gone on to graduate or medical school. In biochemistry, UMBC is consistently among the national leaders in undergraduate degrees awarded to African Americans.

The success of the Meyerhoff program demonstrated to us that careful mentoring and high standards would be fundamental to expanding scientific opportunities to disadvantaged students. We quickly realized that it wouldn't work if we simply shipped our students cross country into high-powered labs: The scientists needed to have appropriate research projects and a commitment to mentoring, which typically involves both the investigator and a hands-on grad student or postdoc in the lab. A community of undergraduate students is essential, as is a good living environment. We began slowly. First, we asked those who administer our programs at the colleges and universities to nominate promising students from disadvantaged backgrounds or from groups traditionally underrepresented in the sciences. We believed they would know best who would benefit from the summer experience. Then we asked for volunteer mentors among HHMI investigators and the new HHMI professors.

That first year, we placed 32 students in the laboratories of 25 different scientists. As of this summer, a total of 143 students have participated in EXROP, along with 116 scientists. We're now looking at a variety of ways to foster community among this extraordinary group of young people by bringing them together in scientific symposia and through other activities.

That brings me back to Jim Gilliam, who long encouraged us to think creatively about how HHMI could increase diversity within the ranks of American science professors. The Gilliam Graduate Fellowships, created by HHMI in Jim's honor, will be awarded each year on a competitive basis to EXROP students pursuing a Ph.D. in the biomedical sciences. This issue of the HHMI Bulletin reports on the first six Gilliam Fellows (see page 49).

Jim Gilliam might seem like a remote figure to these young people. By the time they were born, he was long out of law school and well on his way to a distinguished career in government, business, and civic affairs. But the lesson of Jim's life carries a powerful message. As his 85-year-old father, known universally as "Senior," pointed out to the EXROP students, Jim Gilliam wasn't one to accept the status quo. That lesson holds as well in life as it does in science.



BART NAGEL

Night Watchmen

> If evolutionary biology looks at how life-forms arise and change, "astronomy pushes those questions even further.

DAVID KINGSLEY

RICHARD H. GOMER LEADS A DOUBLE LIFE. BY DAY, THE HHMI alumni investigator works as a developmental biologist, studying tissue-size regulation in slime molds at Rice University in Houston. At night, he often takes to the Texas countryside wearing another hat—that of astronomer. For Gomer, though, stargazing is more than a passing fancy. He approaches his nighttime avocation with the same robust dedication he applies in the lab. And he has significant results to show for it. He's part of a team that reports its findings in publications such as *The Astrophysical Journal*.

Gomer's split identity emerged during his undergraduate days. He had a knack for building electronic devices, so one summer he and a buddy, Keith Horne, took some junk parts and created a light detector for use with a telescope. It worked pretty well, he says, and even though Gomer changed his scientific focus from astronomy to biology soon afterward, his invention lived on. "I kept making the detector system fancier, adding more bells and whistles."

Thirty years later, he still designs new detectors and works as part of a team led by Horne, now an astronomer at the University of St. Andrews in Scotland. Gomer regularly hauls his gear to famous mountain-top telescopes, including the W. M. Keck Observatory in Hawaii, the Carnegie Institution's telescopes in Chile, and Caltech's Palomar Observatory.

Gomer likes the way astronomy enables him to combine observations from an x-ray satellite and from his homemade equipment with, say, theoretical physics. Not only is astronomy fun, he says, "it's nice to do something different and clean out your brain. When I get back to the biology lab, I'll be refreshed."

For another investigator-astronomer, amateur stargazing is one more way to understand where we come from.

Last January, biologist David M. Kingsley was busily gathering images of stickleback fish, which he studies for clues to the molecular basis of evolution, to accompany a paper he had submitted to *Science*. He finally settled on detailed anatomical drawings made by French naturalist Georges Cuvier in 1829. Craving a break from a grueling work week, the Stanford University School of Medicine-based HHMI investigator set up his backyard telescope and gazed at the moon. On that clear winter night, he spotted a peculiar cluster of three craters and started sketching them, wondering how they came to be. Kingsley next discovered a startling coincidence: One of the craters was named for Cuvier.

Speaking to analogous connections, Kingsley observes, "Our stickleback evolution project looks at how life-forms arise and change on Earth. Astronomy pushes those questions even further back: Where did the solar system and Earth come from? Or our galaxy? Or the universe?"

Kingsley's drive to observe heavenly sights often takes him to spectacularly beautiful places. In March, he headed to the remote Flinders Range of Australia to ponder the southern skies for 10 nights. "There we were in the middle of it all," recalls Kingsley. "Look down and you could see the very strata where life's complexity first evolved on our own planet. Look up and you could contemplate the evolution of entire galaxies, stars, clusters, and nebulae."

This past summer Kingsley escaped the city lights to spend time at one of his favorite places for stargazing: Lassen Volcanic National Park in northern California. And there's a practical bonus, he says. "These trips are a great chance to write papers. And I've written some of my best grants while holed up in the mountains with a telescope." ■

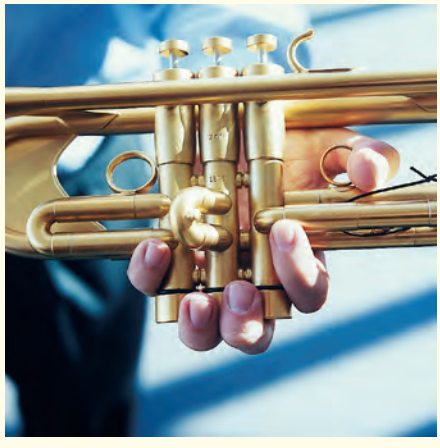
~Karen F. Schmidt~

RIGHT _ ASTRONOMY IS ONE FIELD IN WHICH A DEDICATED AMATEUR LIKE RICHARD GOMER CAN MAKE A MAJOR CONTRIBUTION.

GOMER: ROCKY KNETEN. KINGSLEY: KAY CHERNUSH

The Trumpet of the Zon

LEONARD ZON'S DREAM IS TO PLAY BOSTON'S SYMPHONY HALL—"EVEN IF I PLAYED 14TH TRUMPET IN THE JANACEK 'SINFONIETTA'"— A RARELY PERFORMED PIECE FOR ORCHESTRA AND 14 TRUMPETS.



WHAT SCIENTIST DOESN'T IMAGINE WALKing across the stage of the Stockholm Concert Hall to receive the Nobel Prize? Leonard I. Zon harbors a different fantasy. "I have this dream that someday I'll play with the Boston Symphony Orchestra," says HHMI investigator Zon, a Harvard physician, genetics researcher, and accomplished trumpet player.

Zon's dream is not the mere reverie of a dilettante. Zon is a serious musician who has performed everything from big-band swing to Bach and Mahler since he took up the instrument in fourth grade. He is currently the principal trumpet and senior member of the Longwood Symphony Orchestra, composed mainly of local doctors and health professionals, which performs four benefit concerts a year for medical charities. The orchestra is named for the Longwood Medical Area, a thicket of hospitals, research buildings, and pharmaceutical labs surrounding Harvard Medical School.

Squeezing in practice time requires ingenuity, especially when he's traveling. Zon has "blown" in stairwells and parked cars, for example—the latter coming as a surprise to students at Caltech, who once came upon him trumpeting in a rental car with the windows rolled up. Zon plays any of a wide array of instruments. His collection includes some custom-made trumpets and a "piccolo trumpet," a small horn that enables him to play certain standout high parts in Bach as well as the memorable solos in the Beatles' "Penny Lane."

Trumpet players are typified as fearless, outgoing, and confident, and true to form Zon is no shrinking violet. He recently cofounded, for example, an international organization to promote embryonic stem cell research. He's well known for innovative work on the genetics of blood development. And using the tiny, transparent zebrafish as a genetic workhorse, Zon has discovered a number of mutations that mimic human diseases; with his quirky sense of humor, he has named some of these mutations after teas (*earl grey, Darjeeling, jasmine*) or favorite wines (*merlot, shiraz, chianti*).

No one has yet discovered genes for musical talent, but Zon has no doubt that "a small number of them flow through families like mine." Both of his children are musical: Tyler, 11, plays the classical guitar, and Becky, 13, the flute.

Many doctors and scientists in fact have a musical bent, says Zon, as both involve discipline together with creativity. His own science and music are so deeply entwined, he says, that "when I'm writing a research paper, I think, 'How would I perform this on the trumpet?'" To make a convincing argument, that is, "I need to project everything I'm thinking at the moment, and the trumpet metaphor works very well for me." ■

-Richard Saltus-

> "When I'm writing a research paper, I think, 'How would I perform this on the trumpet?'
>
> LEONARD ZON

KATHLEEN DOOHER (3)

> "We later figured out that he had cloned *PAI-2* and I cloned *PAI-1*—two different genes that encode proteins with similar functions."
>
> DAVID GINSBURG

Separated at Birth?

RIGHT _ FEIGNING OUTRAGE, DAVID GINSBURG (LEFT) SAYS ABOUT HIS COLLEAGUE EVAN SADLER, "THIS GUY JUST KEEPS SHOWING UP EVERY TIME WE DO SOMETHING INTERESTING."

"SPOOKY" IS HOW HHMI INVESTIGATOR J. Evan Sadler describes the ways his career path has intersected that of fellow HHMI investigator David Ginsburg.

It all began some 30 years ago, when the two physician-scientists were 20-something medical students at Duke University. Being a year apart, "we didn't know each other at all," Ginsburg recounts, "but we discovered just a couple of years ago that we had dated the same girl!"

From Duke, the two headed to opposite ends of the country for training in hematology—Sadler to Seattle, Ginsburg to Boston. Still unaware of each other, they embarked on the same project: cloning the gene for von Willebrand factor (VWF), which when faulty can lead to von Willebrand disease, a blood-clotting disorder.

In 1984, Sadler—by that time a researcher at the Washington University School of Medicine in St. Louis—became an HHMI investigator. Ginsburg, with a lab at the University of Michigan Medical School, followed suit the next year. The two would finally meet—as presenters at an HHMI meeting.

"My lab had just cloned another blood-clotting gene, called plasminogen activator inhibitor (PAI), that we were very excited about," says Ginsburg. "And I was glad that I'd be breaking the news [at HHMI] about PAI.

"Evan gives his talk, about von Willebrand factor. And then at the end he says, 'I wanted to tell the group a little bit about this new gene we've just cloned—plasminogen activator inhibitor.' I couldn't believe it! We later figured out that he had cloned *PAI-2* and I cloned *PAI-1*—two different genes that encode proteins with similar functions."

In fact, Ginsburg and Sadler's meeting at HHMI sparked their collaboration. In the mid-1990s, when a postdoc in Ginsburg's lab identified a set of mutations in VWF that seemed to explain one form of von Willebrand disease, it turned out that Sadler's lab had gotten essentially the same results. "We decided, look, rather than trying to beat each other out, why don't we just submit our two papers together?" So they did, and both were published, back to back in the *Journal of Clinical Investigation*.

And in 2001, the two scientists—following contrary strategies and working with different collaborators—identified the gene *ADAMTS13* as the basis of thrombotic thrombocytopenic purpura, a blood-clotting disorder (see page 14). This time their papers came out within a month of each other.

Despite what might be viewed as stepping-on-of-toes, "we've remained great friends," says Sadler. And great colleagues, Ginsburg adds: "We share data, tell each other stuff, share reagents between our labs. Even though we've been competing over the years, it's in the way you'd like science to be but rarely is in practice. It's really been great." ■

-Paul Muhlrad-

SCOTT FERGUSON (2)

september '05

UPFRONT

A fall on poolside cement. A nick from a knife at a cookout. A tumble from a bicycle. We take for granted that the scrapes, cuts, and abrasions that result from such mishaps will scab and scar, and then fade. Yet behind that healing lies a series of highly orchestrated cellular responses within the body. HHMI scientists are beginning to uncover a richer understanding of which molecules trigger, guide, and halt the wound healing process.

The Logic of the Response

Working at the molecular level, HHMI researchers study how wounds heal—and probe genetic links between that process and cancer.

BELOW _ RESEARCHING WOUND RESPONSE, MARK KRASNOW (LEFT) AND MICHAEL GALKO CAN WATCH IT AT THE CELLULAR LEVEL, STUDY IT IN LIVE ANIMALS, AND ANALYZE IT RAPIDLY.

TIMOTHY ARCHIBALD

WHILE STUDYING A HOMELY "KISSING bug," British insect physiologist Sir Vincent Wigglesworth injured a spot on the creature's epidermis. He observed an inflammatory response and then watched the epidermis grow back under the outermost protective cuticle layer and reseal itself.

Wigglesworth documented his findings about how a wound heals in 1937. But even through today, basic understanding of wound healing hasn't progressed much beyond his work. Researchers, for example, still don't know much about the key genes involved and their specific roles.

Wanting to study wound healing at the molecular level, HHMI investigator Mark A. Krasnow was inspired to repeat Wigglesworth's experiment. Only this time, he and postdoctoral researcher Michael J. Galko were studying the tiny fruit fly (*Drosophila*)—and they were armed with the latest light and electron microscopes as well as techniques for creating mutants and analyzing gene expression. "Wigglesworth would have killed for these tools," says Krasnow.

Their cutting-edge approach allowed the Stanford University researchers to develop a powerful new model for the study of wound healing. "We've begun to dissect out the logic of the response, which has proved very difficult to sort out in vertebrates," says Krasnow. Because the ability to heal wounds exists in even the simplest animals and must have evolved early, he and Galko believe the core molecular controls in fruit flies will be similar to those in higher animals, including humans.

Galko figured out how to poke a hole in *Drosophila's* cuticle and epidermis without killing the insect. He identified a larval stage amenable to study—when the then-clear cuticle makes it easy to see fluorescent markers that signal gene expression. He also found a way to turn off a critical gene in the epidermis at this larval stage so that early development would remain normal and undisturbed. "We can now watch the wound response at the cellular level in transgenic larvae; study it in live animals, including mutants; and analyze it rapidly," Galko says.

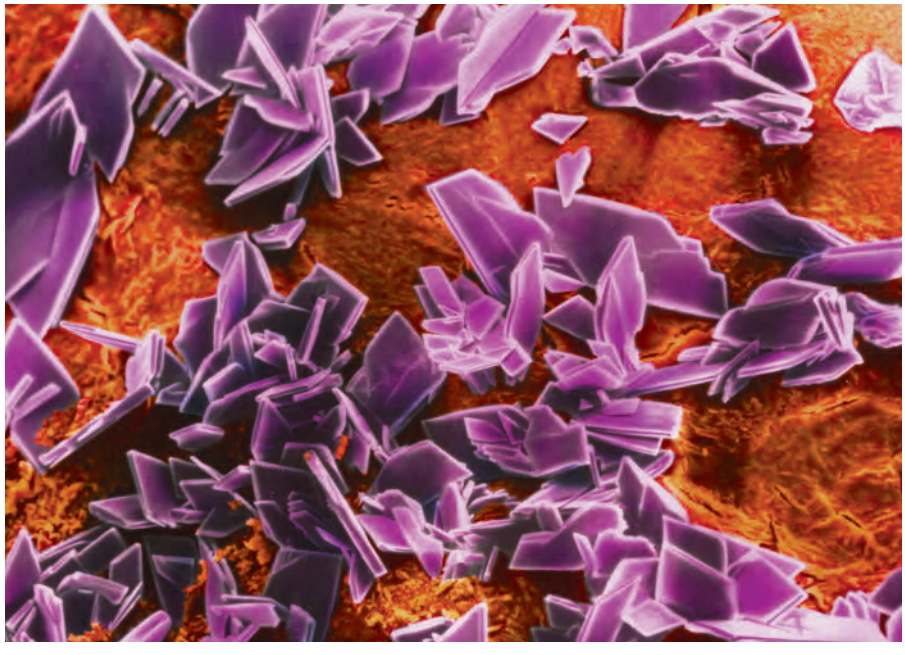
The Stanford researchers tested their model by knocking out two genes suspected of playing a role in wound healing: the transcription factor *lozenge* (which controls development of a specific kind of blood cell) and the gene that codes for Jun N-terminal kinase (an enzyme critical for programming development of epidermal sheets). Their experiments showed that the latter is required for epidermal closure, while the former is needed for scab formation. Although the two genes are involved in separate pathways, they clearly engage in some crosstalk. The researchers published their findings in the August 2004 issue of *Public Library of Science Biology*.

This work is a real breakthrough, says Paul Martin, a developmental cell biologist at the University of Bristol in England. "Now we have an immediate route to get at the genetics of this process; we can trawl through 50 to 100 genes and see what's important," he says. Indeed, Krasnow and Galko are now busily knocking out other genes and repeating their experiments on the mutants.

They're also getting help from a group down the hall at Stanford that is probing the link between wound healing and cancer. HHMI investigator Patrick O. Brown, Howard Y. Chang, and their colleagues took human fibroblasts—a kind of skin cell that plays a role both in normal tissues and in some tumors—and exposed them to serum derived from clotted blood. Then, using DNA microarray technology to see which genes were turned on, they identified a stereotypical pattern, or "wound-response signature," that involves about 500 genes. This information is helping Krasnow and Galko select candidate genes for testing in their *Drosophila* model.

Equally important, Brown's team confirmed that wound healing and cancer growth are truly related. For decades,

> "We found a striking and consistent tendency of tumors, compared with normal tissue, to express signature wound-healing genes."
>
> PATRICK BROWN



SPL / PHOTO RESEARCHERS, INC.

RIGHT _ WHEN THE SKIN IS CUT, BLOOD IS RELEASED ONTO THE EPIDERMIS (ORANGE). ALBUMIN PROTEINS IN THE BLOOD PLASMA HARDEN INTO CRYSTALS (PINK) OVER THE WOUND, JOINING WITH OTHER PROTEINS TO FORM A CLOT. ONE OF THE MANY MOLECULES INVOLVED IN HEALING, ALBUMIN HELPS MAINTAIN PROPER LEVELS OF HORMONES AND CALCIUM IN THE BLOOD, AND ALSO ASSISTS WATER FLOW BETWEEN TISSUES AND THE BLOODSTREAM.

researchers have theorized that "wound healing gone awry" makes tumor cells migrate and proliferate. One piece of evidence is that people suffering from chronic inflammation, which involves cycles of damage and repair, also have high rates of cancer. Last year, Brown's group finally found a link. "We found a striking and consistent tendency of tumors, compared with normal tissue, to express the signature wound-healing genes," he says.

"This is not at all surprising," says Martin, "but it's fantastic to have real molecular evidence." And, notes Brown, it raises the possibility that interventions targeting wound healing might also help treat cancer.

Already, the Brown lab's efforts suggest one potential medical benefit: a test to identify early those cancer patients who will likely need follow-up treatment such as chemotherapy. The researchers found that tumors of the breast, lung, and stomach were more likely to metastasize if they had a gene-expression signature suggestive of active wounds. They published the work in the March 8, 2005, issue of the *Proceedings of the National Academy of Sciences*. The team is now investigating ways to develop a simple "wound-response" test that would be useful in the cancer clinic. ■

-Karen F. Schmidt-

> "Wigglesworth would have killed for these tools."
>
> MARK KRASNOW

Frontiers of Science

At Columbia University, Darcy Kelley and colleagues have a new way to teach undergraduates science.

MARY HELEN BOWERS AND JENNIFER EVANS LEAN FORWARD, rapt, as their professors argue on the stage of Columbia University's Miller Theater. The debate: "Can we engineer consciousness?" The heated answers of a superstar faculty: "Yes," "no," and "maybe."

Bowers, 26, danced for a decade with the New York City Ballet before entering Columbia to earn a college degree. Evans, 18, is the daughter of scientists. Both are taking "Frontiers of Science," a novel course developed by Columbia neuroscientist Darcy Kelley and a cross-disciplinary assortment of colleagues to introduce undergraduates in all disciplines to research on the frontiers of science today and—more importantly—to the ways scientists think.

Bowers, Evans, and more than 300 other entering students attend weekly lectures by the cream of Columbia's scientists such as Horst L. Stormer, a Nobel Prize-winning physicist who used to head the physical research laboratory at the pioneering Bell Labs, and Kelley, one of 20 researchers nationwide named HHMI professors—each of whom received $1 million to help transform undergraduate science education.

For 85 years, Columbia's undergraduate education has centered around a core curriculum comprising literature, music, art, history, and philosophy. With the advent of "Frontiers in Science," for the first time science joins the fields that generate what this university calls "the great ideas of Western civilization." The course will be offered on a pilot basis for 5 years.

"Frontiers in Science" is designed to make science come alive for students who are not necessarily planning to become scientists. "Despite the necessity of a science-informed public," Kelley points out, "so-called nonscience students often are shunted into boring or irrelevant courses, if they take science at all." And the course is meant to be an inspiration for science majors as well. "Despite the need for interdisciplinary science," she notes, "the most able science students can be too narrowly educated."

The backbone of the course is a Web-based "book," *Scientific Habits of Mind*, developed by David J. Helfand, chairman of Columbia's Department of Astronomy and Astrophysics. It's far from your typical college text. Chapter headings read: "Expecting the Improbable," "Lies, Damned Lies, and Statistics," and "Discoveries on the Back of an Envelope." An anecdotal journey seen through a scientist's eyes, the book focuses on basic concepts in scientific reasoning and analytical thinking.

If the backbone is Helfand's book, and the heart and soul are the renowned scientists who lecture, the course's lifeblood comes from the Columbia Science Fellows, the postdocs who develop the curriculum and teach weekly undergraduate seminars.

While they are teaching, however, they're also learning. "The intellectual stimulation of working with people across disciplines has exceeded my wildest expectations," says Robin Herrnstein, who is doing a postdoctoral fellowship in astronomy. "I understand my own field better for having taught this course."

The interdisciplinary aspect of the course can be a challenge—and a plus. "I've really had to struggle," admits Jennifer Blanck, a postdoc in biology. "But some of my best seminars have been in other fields, because I'm learning too."

JENNIFER ALTMAN

ABOVE _ CONCERNED THAT "SO-CALLED NONSCIENCE STUDENTS OFTEN ARE SHUNTED INTO BORING OR IRRELEVANT COURSES, IF THEY TAKE SCIENCE AT ALL," DARCY KELLEY DECIDED TO DO SOMETHING ABOUT IT.

> "Despite the need for interdisciplinary science, the most able science students can be too narrowly educated."
>
> DARCY KELLEY

The debate that holds Bowers and Evans spellbound is the capstone of the course. In May 2005, the neuroscientists, physicists, geologists, and astronomers who shared cutting-edge ideas in their disciplines throughout the semester, gathered to ponder controversial questions and, as diplomats might say, to have a "frank exchange of views."

"Of course we will be able to engineer consciousness," states Stormer. "The brain is circuits. There is no reason we cannot mimic those circuits. There are no computers with consciousness because we're just not there yet."

Julio M. Fernandez disagrees. "We can't engineer something greater than ourselves," protests the biophysicist who studies the stability and folding dynamics of proteins.

"With all due respect, I think you've totally missed the point," replies psychologist Joy Hirsch, who defines consciousness as "the quality or state of being aware of something outside oneself."

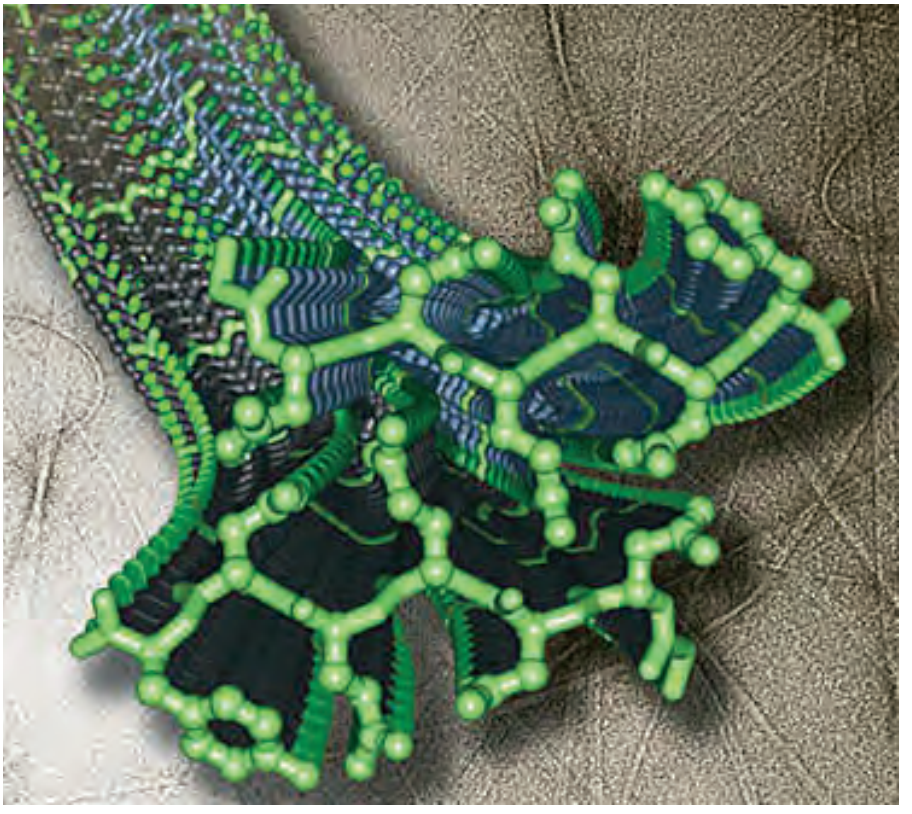
"With all due respect, your definition is trivial," Kelley retorts.

Full professors, fully engaged in intellectual battle—and the undergraduates love it. "It's refreshing," says Rachael Gargano, a political science/economics major. Wei-Jen Hsieh, a premedical student, calls it "fascinating." "They are so enthusiastic and passionate," Bowers remarks. Jennifer Evans has the last word: "They make science so much more human." ■

-Jennifer Boeth Donovan-

Hitting Pay Dirt on Amyloid Fibrils

An international collaboration helps solve the long-elusive structure of tiny proteins that figure in major diseases such as Alzheimer's.



DAVID EISENBERG, HHMI AT UCLA. IMAGE REPRINTED COURTESY OF NATURE, VOL. 435, PP. 773 TO 778.

"When you have to shoot a bunch of crystals rather than a single one, not only are the data degraded, there's too much data, which adds background noise.

COURTESY OF DAVID EISENBERG LAB

DAVID EISENBERG

LEFT _ NEW STRUCTURAL STUDIES SHOW THAT THE FILAMENTS THAT MAKE UP AMYLOID DEPOSITS LOOK LIKE NEARLY-CLOSED STUCK ZIPPERS. ONCE AMYLOID FIBRILS FORM IN TISSUES AND CELLS, THEY RESEMBLE A TOWERING STACK OF ZIPPERS, EACH TIGHTLY BONDED TO THE ONE BELOW.

A CHANCE MEETING WITH AN OLD FRIEND helped solve a problem that had stymied David Eisenberg's research team for years—and led to a breakthrough discovery. In 2000, the HHMI investigator's group at the University of California, Los Angeles (UCLA), identified a short peptide chain from the yeast protein Sup35, which, like a full-length protein, could form amyloid fibrils—thread-like abnormal protein deposits involved in a host of deadly disorders, including Alzheimer's disease, Parkinson's disease, type II diabetes, and the human counterpart of mad cow disease.

The next logical step, says Eisenberg, was to determine the peptide's atomic structure. This is a prerequisite to devising drugs that might prevent these lethal molecules from forming in the first place, says Jiri Safar, a scientist at the Institute for Neurodegenerative Diseases at the University of California, San Francisco (UCSF), "and developing diagnostic tools to detect their presence long before symptoms appear, to prevent irreparable damage."

To decipher the three-dimensional structure of this biologically important molecule, Eisenberg coaxed the proteins into forming crystals. That way, he could use a technique known as x-ray crystallography, which relies on the ability to get proteins into a crystal form. But the task proved daunting because the microcrystals formed by the peptide, which is composed of just seven amino acids, were impractically tiny—some 50,000 times smaller than the crystals researchers normally work with.

The UCLA scientists spent several frustrating years pursuing initially promising technologies that ultimately led nowhere. "We tried a whole bunch of tools using traditional x-ray methods as well as other methods," says Eisenberg, who is also director of the UCLA-DOE Institute for Genomics and Proteomics. "But when you have to shoot a bunch of crystals rather than a single one, not only are the data degraded, but there's too much data, which adds background noise. So we weren't getting a clear enough picture."

All that began to change in July 2003, when Eisenberg attended a conference in Crete. Over lunch with Swedish scientist Carl-Ivar Brändén, he spoke of his dilemma. "There's one person in the world who can help you," Brändén immediately responded, "Christian Riekel."

A crystallographer at the European Synchrotron Radiation Facility in Grenoble, France, Riekel had invented a highly focused x-ray camera capable of analyzing crystals as small as one micrometer in diameter—about 1/100th the width of a human hair—a technology 100 times more powerful than the best available in the United States. Another large plus was that a graduate student at the lab, Anders Madsen, had developed an effective way of manipulating microcrystals.

The UCLA team took their microcrystals to Riekel's lab, where they recorded accurate x-ray diffraction patterns from an individual microcrystal. With this diffraction pattern in hand, the researchers looked for the position of

the zinc atom in the structure, knowing that microcrystals grow only in the presence of zinc. "When I found the zinc atom, finding the others was easy," says Michael R. Sawaya, a research scientist at UCLA who contributed to the project. With the knowledge of the position of all the atoms, you can see the structure of the molecule. "Recognizing the features of the peptide was like seeing the familiar face of an old friend," adds Sawaya.

There was much dancing around the lab when, after 8 years of hard work, the three-dimensional structure was known. And the structure suggested to the researchers how the fibrils accumulate in brain tissue and why they are so infectious. At last they had all the puzzle pieces. The peptide molecules in the microcrystal assemble into a structure that resembles a tightly bound zipper that latches on to an identical structure, much in the manner of Velcro, which explains their great stability. This suggests how the amyloid fibrils keep growing: They latch on to either end of the structure, forming an almost indestructible spine-like chain. "This discovery is the pinnacle of years of hard work," says UCSF's Safar, "and is a significant contribution in our understanding at the atomic level of the way amyloids form."

The UCLA team hopes that unraveling the atomic structure will suggest ways to cap the growth of amyloids and pave the way toward formulating treatments that can inhibit this process. ■

-Linda Marsa-

Skin Deep

How skin forms layers.

HHMI RESEARCHERS HAVE NEW EVIDENCE THAT PUSHES ASIDE OLD THEORIES about how skin is able to create layers of different cell types while simultaneously forming a continuously self-renewing, protective barrier.

The discovery helps to explain how skin becomes "stratified" into different layers. The finding may yield new insights into the basic processes by which stem cells can both self-replicate to produce more stem cells and also mature and differentiate to form a tissue.

HHMI investigator Elaine Fuchs and colleague Terry Lechler at the Rockefeller University demonstrated that skin stratifies into layers in an unusual process involving asymmetric cell division—a fundamental developmental mechanism in which a mother cell gives rise to two distinctly different daughter cells. They published their findings on August 10, 2005, in an advance online edition of *Nature*.

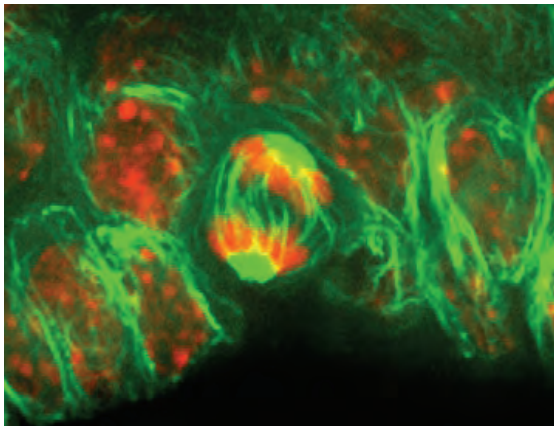
While their discovery of the basic process of skin stratification does not have immediate clinical implications, says Fuchs, insights from these studies may be applicable to asymmetric cell division in human stem cells.

"Understanding the processes of asymmetric and symmetric divisions in stem cells are of central importance to the stem cell field," she says. "It seems likely that the basic mechanistic process that we've documented in the embryonic skin stem cell will be utilized by other tissues and cell types, particularly stem cells." ■

-Dennis Meredith-

> "Understanding the processes of asymmetric and symmetric divisions in stem cells are of central importance to the stem cell field."
>
> ELAINE FUCHS

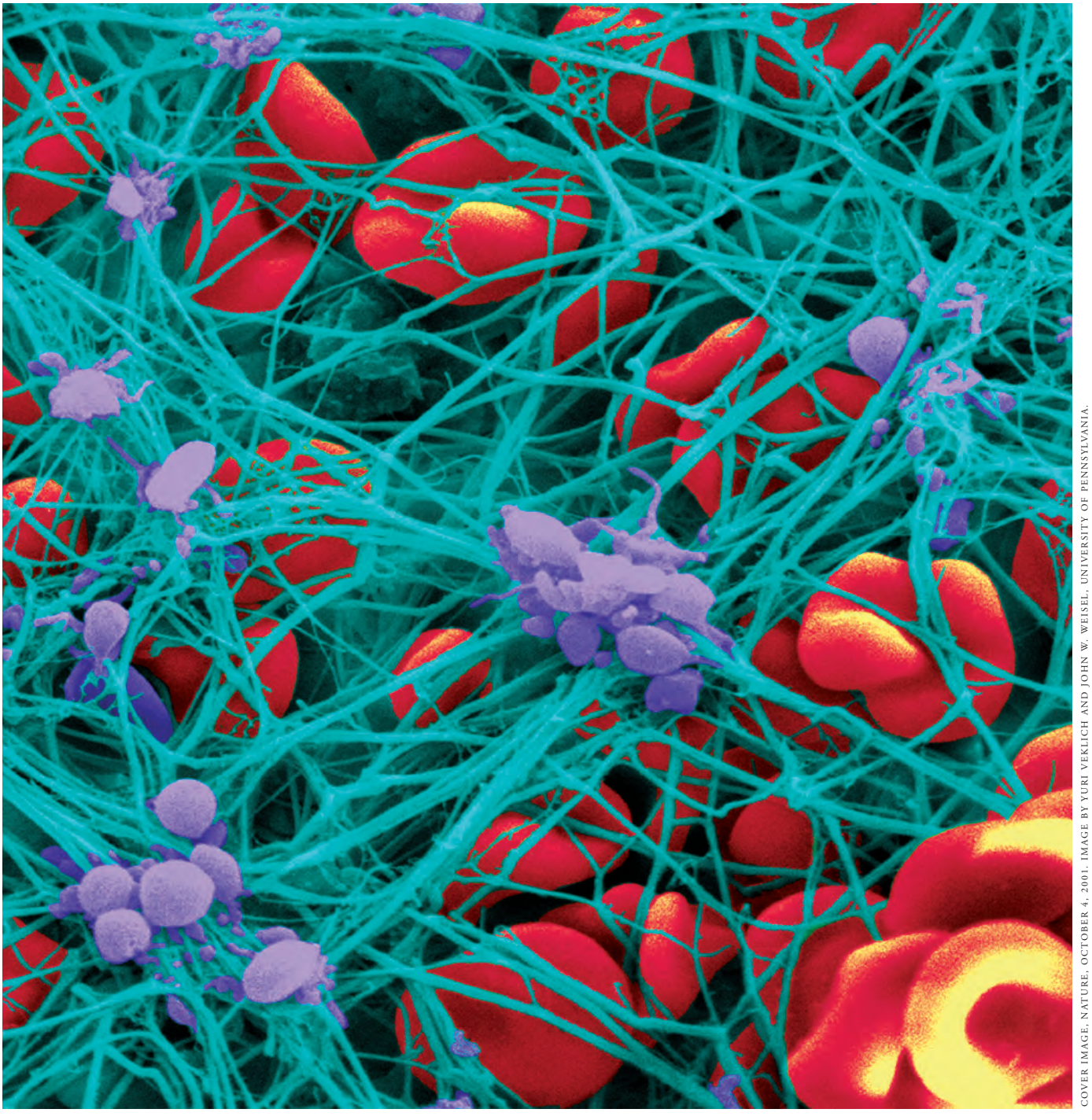
RIGHT _ SOME BASAL SKIN CELLS, AS SHOWN IN THIS CROSS-SECTION OF MOUSE SKIN, DIVIDE IN A PERPENDICULAR AND ASYMMETRIC FASHION, RESULTING IN ONE DAUGHTER CELL THAT REMAINS IN THE BASAL LAYER AND A SECOND, SUPRABASAL DAUGHTER CELL THAT MIGRATES TO HIGHER LAYERS OF THE EPIDERMIS. MICROTUBULES (GREEN) FORM THE MITOTIC SPINDLE ALONG WHICH DNA (RED) SEGREGATES DURING CELL DIVISION.



COURTESY OF TERRY LECHLER

FUCHS

COURTESY OF ROCKEFELLER UNIVERSITY



COVER IMAGE, NATURE, OCTOBER 4, 2001. IMAGE BY YURI VERKLICH AND JOHN W. WEISEL, UNIVERSITY OF PENNSYLVANIA.

Tracking a Perpetrator Gene

A single mutation can lead to a devastating disorder of the circulatory system.

ON MAY DAY 1999, JENNIFER CHAMBERLIN,* A 43-YEAR-OLD secretary in the Midwest, stayed home to recuperate from back surgery and spend time with her three daughters, on spring break from school. Walking into her kitchen, Jenn fainted and fell to the floor. She soon regained consciousness but never returned to her normal self. "It was like she was in a trance," says Jenn's husband, Tim. "She was always lying down and talked just when talked to." Doctors couldn't detect any physical ailments. "She might need to see a psychologist," a physician told Tim. Over the next 3 weeks, Jenn plunged deeper into mental darkness, crying out in delusional outbursts.

At the end of May, Jenn's platelet count had dropped precipitously to 16,000 per microliter—the minimum for a normal count is 150,000. Follow-up tests revealed that her red blood cells were breaking into shards. With those telltale symptoms, doctors finally diagnosed her with thrombot-

*The names of the patient and her husband have been changed.

OPPOSITE _ A BLOOD CLOT CONSISTS OF A PLUG OF PLATELETS ENMESHED IN A NETWORK OF INSOLUBLE FIBRIN MOLECULES. IN THIS COLORIZED SCANNING ELECTRON MICROGRAPH OF A BLOOD CLOT THAT FORMED IN VITRO, THE TEAL STRANDS ARE FIBRIN, THE PURPLE CLUSTERS ARE ADHERENT PLATELETS, AND THE RED OBJECTS ARE TRAPPED RED BLOOD CELLS.

ic thrombocytopenic purpura (TTP), a rare disorder of the blood-clotting system that was almost always fatal until the 1980s and 1990s, when doctors developed a crude but effective blood plasma transfusion treatment.

Scientists understood relatively little about the cause of Jenn's illness until recently. Researchers knew that platelets in TTP patients form spontaneous clots, or thrombi, within the narrowest blood vessels. As a result, circulating platelets become depleted, and red blood cells become shredded as they squeeze through the occluded vessels. The restricted circulation and anemia leave tissues starved for oxygen, leading to strokes, heart attacks, and failure of other critical organs. Jenn's May Day attack, her doctors suggest, was probably the first of several strokes resulting from TTP.

Doctors ordered plasma-exchange therapy, the only known treatment that might save her life. For three and a half hours every day Jenn lay in a hospital bed, hooked up to a machine via a large catheter in her arm vein. The machine filtered Jenn's blood, saving the cells and replacing the plasma portion with about one and a half volumes of donor plasma. The treatment worked. Jenn's platelet count rebounded, and her condition improved over 5 days. But on the sixth day her platelets dropped again, and she endured another plasma exchange. Then another. Ultimately, in just over 2 years, she underwent more than 145 exchanges.

One of Jennifer Chamberlin's hematologists is HHMI investigator J. Evan Sadler, from Washington University School of Medicine in St. Louis. Sadler and HHMI investigator David Ginsburg, at the University of Michigan, were both drawn to investigate TTP by their prior research on a key blood-clotting protein called von Willebrand factor (VWF). In the mid-1980s, the two physician-scientists independently cloned the gene for VWF and since then they have been studying the protein's roles in blood clotting. One of those roles, Sadler explains, is to make platelets adhere to the walls of injured blood vessels.

In 1996, two research groups, one led by Han-Mou Tsai at the Albert Einstein College of Medicine in New York and one by Miha Furlan in Bern, Switzerland, separately discovered that an unidentified enzyme in blood could cleave VWF but only if the protein was slightly unfolded. Such unfolding might occur when VWF, tethering platelets to a blood vessel, gets stretched in the current. Why VWF might get cleaved was anyone's guess, but an important clue soon followed.

The next year, Furlan's group made the crucial finding that children with a rare hereditary form of TTP lacked the VWF-cleaving activity in their blood, suggesting a link between VWF, the enzyme, and the disease. Then, in 1998, Tsai and Furlan, in separate studies, bolstered that notion by showing that most adults with the acquired form of TTP (including, as it turned out, Jennifer Chamberlin) produce antibodies against the still-unidentified enzyme.

The finding shifted researchers into high gear in their efforts to identify the VWF-cleaving activity. "We were then madly working to try to purify, to clone this protein," Sadler recounts. In collaboration with Dominic Chung at the University of Washington, who purified enough of the protein to determine some of its amino acid sequence, Xinglong Zheng, then a postdoctoral fellow in Sadler's lab, used a combination of bioinformatics and DNA sequencing to identify the gene.

Meanwhile, Ginsburg, Tsai, and colleagues were following a different strategy to identify the gene encoding the VWF-cleaving enzyme. Analyzing plasma samples from people with TTP in their families, the researchers found that, although plasma from children with the disease had no VWF-cleaving activity, plasma from their parents and some of their siblings (none of whom had TTP) showed about half the normal activity. This suggested that TTP is caused by a single recessive mutation in the gene responsible for the VWF-cleaving activity—that is, a mutation in one of an individual's two copies of the gene cuts down the enzyme activity, but for an individual to contract TTP, both gene copies must be mutated.

Gallia Levy, an M.D.-Ph.D. student in Ginsburg's lab, used this information in conjunction with genetic data from family members' DNA samples to determine the chromosomal location of the gene. Levy traveled to Cincinnati, to the lab of William Nichols, a former student of Ginsburg, who had the equipment and expertise for conducting the complex

continued on page 62

"It's one of these rare examples where we have an opportunity to take a basic research finding straight to the bedside. And it just isn't going to happen because of the financial realities of the pharmaceutical industry."

SCOTT FERGUSON

DAVID GINSBURG

HCM is a major cause of death in young athletes, but can be diagnosed in people of all ages. Because HCM is often asymptomatic, some patients may be unaware of their condition until it is detected during routine medical screening.

FOOTBALL PLAYER: CORBIS

WINDOW to intervene

SUDDEN CARDIAC DEATH KILLS AS MANY AS 300 YOUNG ATHLETES EACH YEAR. THE ROOT CAUSE IS OFTEN GENETIC. BUT NOW A NEW TEST OFFERS ANSWERS AND INSIGHT.

♥♥♥ GROWING UP, Wendy Borsari had reason to believe she had escaped the genetic heart condition that wreaked havoc on her family, killing three uncles and sending her mother into congestive heart failure. Borsari's regular childhood echocardiograms found no problems. When she was 18, doctors concluded Borsari was perfectly healthy. They were wrong.

In 1993, shortly after Borsari graduated from college, her doctor heard an irregular heartbeat during a routine exam. This time, an echocardiogram confirmed Borsari's fears: hypertrophic cardiomyopathy (HCM). A genetic abnormality that enlarges muscle cells in the heart's left ventricle, HCM can cause a sometimes-fatal irregular heartbeat, heart failure, exercise intolerance, and chest pain. With an HCM diagnosis in hand, Borsari began to actively manage her health, with frequent medical checkups and more careful health-related decisions. Today, as a

BY KATHRYN BROWN

39-year-old mother of two, she leads a pleasantly ordinary life in the Boston suburbs.

HCM is the primary cause of sudden death in people under 30. Young athletes in particular, researchers say, suffer sudden cardiac death at two to three times the rate of others of their generation. Although estimates vary, as many as 300 young athletes may die from sudden cardiac death every year in the United States alone—collapsing at soccer games or swim meets, for example, or simply during practice. One of Borsari's uncles died as a teenager, heading to the football field, roughly 50 years ago. (The other two died at ages 26 and 33.)

For patients and families, dealing with this uncertainty is costly, emotionally draining, and fraught with physical danger.

If HCM is detected in time, doctors can manage the condition with surveillance, lifestyle changes, and, sometimes, an implantable defibrillator. But young adults are not universally screened for heart conditions, and early HCM symptoms—such as shortness of breath—may easily be mistaken for more common conditions such as asthma. Although a family history of HCM suggests the need to test children, many—like Borsari—develop clinical symptoms later. For patients and families, dealing with this uncertainty is costly, emotionally draining, and fraught with physical danger.

Now, a new genetic test may allow early identification and diagnosis of those at greatest risk for developing HCM. The test can confirm an HCM diagnosis in patients who show clinical symptoms of the disease and can provide further information for individuals at risk for the condition. Administered by the Harvard Medical School–Partners Healthcare Center for Genetics and Genomics, the test detects mutations in eight genes that account for up to 70 percent of HCM in patients with clinical symptoms.

MOLECULAR MECHANICS

♥♥♥ **HHMI INVESTIGATOR** Christine E. Seidman and her husband, HHMI alumni investigator Jonathan G. Seidman, both of Harvard Medical School, developed the HCM test. The Seidmans have spent the past two decades identifying and explaining the molecular mechanics behind HCM. They tracked its incidence through families, analyzed the genomes of affected family members, mapped relevant disease genes, and, ultimately, pinpointed many of the telltale mutations that cause the condition. The couple currently is working to understand how these mutations trigger signaling molecules that cause heart muscle cells to grow abnormally.

Cardiovascular geneticist Jonathan Seidman brings a long-standing interest in the molecular causes of hypertrophy to the hunt for HCM genes. In particular, he has analyzed the mutations linked to the disease and currently heads efforts to determine how HCM genes may contribute to the risk of hypertrophy in other cardiac conditions.

Meanwhile, Christine Seidman sees HCM from the perspective of both researcher and physician. As director of the Cardiovascular Genetics Service at Brigham and Women's Hospital, she sees patients several days each month. Most days of any given week, however, she can be found at the lab bench. Her research efforts on HCM and other cardiac conditions, such as dilated cardiomyopathy, were recently recognized with her election to the National Academy of Sciences.

Christine Seidman's interest in heart disease emerged early in her career. After graduating from medical school in 1978, she spent roughly a decade in medical and research fellowships at Johns Hopkins and Harvard, specializing in internal medicine. She became intrigued by the research and clinical implications of the heart's unique biology. For example, she says, the cells of the mature heart don't divide, as cells in other tissues do. In another quirk, the heart can be caused to change shape by a diversity of disorders, from HCM to hypertension to coronary artery disease. But the change actually occurs in only two ways—hypertrophy, in which the heart walls thicken, or dilation, whereby the heart's volume increases. Either change limits the heart's blood-pumping ability, threatening to stop it completely. "Here you have a variety of pathogenic stimuli, and they all activate one of these two pathways to disease," Seidman says. If we could prevent these changes from occurring, she adds, "we could really help patients."

Christine Seidman established her lab at Harvard Medical School about 20 years ago, and began recruiting families with a history of cardiac hypertrophy or dilation for research studies. The lab homed in on HCM, painstakingly mapping disease genes to specific chromosomes and then fingering the genes themselves.

PROMISES AND PITFALLS

♥♥♥ **WORKING TOGETHER** and with colleagues, the Seidmans have linked HCM to mutations in structural proteins—such as the cardiac B myosin heavy chain and cardiac myosin binding protein C—found in heart muscle cells. These proteins help form the sarcomere, the pumping part of heart muscle cells. When mutated, the proteins apparently short-circuit the cells' normal flow of calcium that is needed to regulate cellular activity. As a result, the muscle cells grow unchecked, swelling into dramatically thickened heart walls.

Among individuals carrying an HCM mutation, however, it's impossible to know just when—or if—a heart actually will become hypertrophic. "You're born with an HCM mutation, but its clinical signs could take years to evolve," Christine Seidman says. Yet on the positive side, she notes, "we have a huge window to intervene. That's why I'm keen on gene-based testing."

The Seidmans developed a test, based on direct DNA sequencing, that screens a patient's blood sample for mutations of the genes most commonly implicated in HCM. Made avail-

Although the gene mutation responsible for causing HCM is inherited at the time of conception, it may take decades before there is clinical evidence of impaired heart function. The clinical spectrum of HCM ranges from asymptomatic individuals to those with exercise intolerance, chest pain, or disabling symptoms of heart failure.

RUNNER: GETTY IMAGES

ASIA KEPKA

HARVARD PHYSICIAN AND RESEARCHER CHRISTINE E. SEIDMAN DEVELOPED A DIAGNOSTIC TEST FOR HYPERTROPHIC CARDIOMYOPATHY (HCM), A GENETIC HEART ABNORMALITY THAT IS FREQUENTLY THE CAUSE OF SUDDEN DEATH IN YOUNG ATHLETES.

THE HCM TEST

The HCM test sequences DNA from a patient's blood sample, detecting mutations in the genes most commonly associated with HCM. The test is offered in two panels, HCM-A and HCM-B, for the following genes:

HCM-A

GENE	NAME
MYH7	myosin, heavy chain 7
MYBPC3	myosin-binding protein c, cardiac
TNNT2	troponin t2, cardiac
TNNI3	troponin i, cardiac
TPM1	tropomyosin 1

HCM-B

GENE	NAME
ACTC	actin, alpha, cardiac muscle
MYL2	myosin regulatory light chain
MYL3	myosin essential light chain, cardiac

Source: www.hpcgg.org/lmm

able to the public last year, the test is offered in two panels. The first, HCM-A, includes the five most common genes for HCM. Among patients with existing clinical symptoms, HCM-A offers a roughly 50 to 60 percent detection rate of a pathogenic mutation. The second panel, HCM-B, targets three other genes, adding another 5 to 10 percent to the detection rate of a pathogenic mutation. When both panels are analyzed, a disease-causing mutation is identified in 55 to 70 percent of individuals. The detection rate is highest among families in which clinical diagnosis is well established.

After developing the test, the Seidmans transferred the technology to the nonprofit Harvard Medical School–Partners Healthcare Center for Genetics and Genomics. The center's laboratory of molecular medicine actually performs the test, as one of many genetic tests—including a different test for unexplained cardiac hypertrophy—available to the public. (The Seidmans do not receive any profit from these tests.) But the center has a broader mission: to incorporate genetics and genomics into clinical medicine. To that end, the center maintains a computer database with the medical records, including HCM test results, of patients in the Partners Healthcare system, which includes Massachusetts General and Brigham and Women's hospitals.

Tapping this database, participating cardiologists can easily weave a patient's HCM diagnosis into his or her clinical care. Ultimately, the center's goal is to learn whether genetic test results improve that care. "Many labs provide genetic testing, but we're different because we're also interested in incorporating the knowledge of genetics and genomics into the practice of clinical medicine," explains Raju Kucherlapati, scientific director of the center and a geneticist at Harvard. "This same attitude is what makes Christine's work special. She not only discovers genes, but also understands patients and clinical outcomes. So she's in a good position to assess how genetic tests for cardiovascular conditions can change the practice of medicine."

Already, the HCM test illustrates the promises and pitfalls of applied genetics. Among its promising points, the test can provide critical information. Families with an HCM history, for instance, often want to know whether a child carries a mutation linked to the condition. If so, parents may steer him or her toward sports and hobbies that are not overly strenuous. In addition, patients previously diagnosed with general cardiac hypertrophy can take the test to identify, or possibly downplay, HCM as the cause. Finally, patients with suspected HCM can learn which mutation they carry—useful knowledge,

as several of the mutations can result in more severe forms of the condition.

However, like any other test for a complex genetic condition, the HCM test has gaps. "It's good at finding mutations in eight key genes, and that probably accounts for most cases of unexplained cardiac hypertrophy," says Allison Cirino, a genetic counselor at the Cardiovascular Genetics Center of Brigham and Women's Hospital. "But there are still other HCM genes out there, and a negative test result cannot rule them out."

Cost is another drawback. Because the comprehensive HCM test screens for more than 250 possible mutations across 14,188 base pairs of nucleotides, it is highly technical and thus expensive. Panel A alone costs $3,000 and Panel B costs $1,150. Alternatively, checking family members for a known mutation costs $250. Major insurance companies have covered the test for patients who received preapproval, Cirino notes.

Finally, some people may fear that the HCM test—or any genetic test—could publicly label them as vulnerable or diseased, leading to genetic discrimination from health- and life-insurance companies, or even employers. That's one reason, Christine Seidman suspects, why some who've taken the HCM test since last year have done so by mail—downloading test forms from the Internet and then quietly sending in blood samples with payments—as opposed to working with a cardiologist, who would keep permanent medical records. Still, Kucherlapati notes that most test takers are referred by physicians. So far, he adds, at least several hundred people have taken the HCM test.

Despite the challenges, many other medical scientists consider the HCM test—the only such test currently available—to be a step forward. "This test is an important advance, and the Seidmans deserve credit for creating something needed and new," says Barry J. Maron, director of the Hypertrophic Cardiomyopathy Center at the Minneapolis Heart Institute Foundation. "As is the case with any test, there are limits—particularly cost and false negatives—that must be overcome. But now we can at least aspire to having an HCM diagnosis in a timely fashion."

TO USE A NEW TOOL WISELY

♥♥♥ **BORSARI, WHOSE FAMILY** has long participated in the Seidmans' research, agrees. "Any scientific progress in HCM testing is a move in the right direction," she says. "We now have something else available to help us make wiser decisions in the future." Borsari adds that when her own son and daughter were born—in 2000 and 2003, respectively—she immediately had researchers test their blood for HCM mutations. (She prefers to keep the results confidential.)

As the Seidmans' research evolves, their findings could improve future versions of the HCM test—or, possibly, lead to therapies that treat the condition. For example, in studying the genetically engineered mice that the Seidman lab uses to unravel HCM's molecular mechanisms, the researchers could discover molecular signals that kickstart abnormal growth in heart muscle cells, leading to hypertrophy. The ability to block such signaling could form a major therapeutic advance.

Looking ahead, Christine Seidman says that society—from doctors and patients to

The center's laboratory of molecular medicine performs the test as one of many genetic tests available to the public.

HOW DO PATIENTS GET AN HCM TEST?

There are two basic routes to the HCM test: The first, and most widely recommended, is through a cardiologist. The patient gives a blood sample at the doctor's office, which is then sent to the molecular medicine lab at Harvard Medical School–Partners Healthcare Center for Genetics and Genomics. About 6 weeks later, the cardiologist receives the results and reviews them with the patient. ♥ Alternatively, individuals can download test forms directly from the center's Web site (*www.hpcgg.org*), submit a 7-milliliter blood sample in a test tube by overnight mail, prepay for their test, and receive results in 6 weeks. ♥ Although formal genetic counseling is not part of the HCM test—it is available but not required—the center does have a staff counselor to answer specific questions by telephone. Meanwhile, independent genetic counselors work in all major urban areas nationwide (to find one, see the Web site of the National Society of Genetic Counselors *www.nsgc.org*). Many patients benefit from speaking with a qualified counselor, who can explain how a test works and what results mean, as well as answer questions.

In May 2005, HHMI researchers published research in **Genes & Development** showing that they had induced adult heart muscle cells to proliferate in adult animals. Researchers said the studies provide a framework for exploring the molecular mechanisms that might one day make possible clinical regeneration of damaged heart muscle. According to Mark Keating, an HHMI alumni investigator at Harvard Medical School and Children's Hospital Boston and senior author of the paper, "These findings represent the first step toward showing that drugs that eliminate p38 activity could reduce scar tissue formation and enhance cardiac regeneration after cardiac injury." Keating said the formation of scar tissue in damaged hearts is the major reason myocardial infarctions lead to subsequent abnormalities and compromised heart function.

MORE INFORMATION AT HHMI NEWS ONLINE

www.hhmi.org/news/keating8.html

TETRARCHS 4:04 AND 4:16PM 4TH NOVEMBER 2001
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ALTERNATIVE SPLICING

GENETIC SPLICE AND DICE

RESEARCHERS HAVE LONG ASSUMED THAT ONE GENE USUALLY CODES FOR ONE PROTEIN. BUT THERE'S EVIDENCE THAT THE RULE INSTEAD IS "ONE GENE, MANY PROTEINS." HOW DOES THAT HAPPEN?

IN FEBRUARY OF 2000, HHMI INVESTIGATOR S. Lawrence Zipursky was trying to sort out what he had originally expected would be a relatively simple problem. He and his colleagues at the University of California, Los Angeles (UCLA), had discovered a fruit fly gene that encoded a protein on the surface of nerve cells that helped them migrate and connect to the correct cells. The team was analyzing the makeup of that protein, which they called Dscam—for Down syndrome cell adhesion molecule—because of its similarity to a human protein of the same name. They were surprised to find a segment of the protein that did not match the human version and suspected the fruit fly gene might produce a few subtly different proteins. • The situation turned out to be far more complex. The team found that the *Dscam* gene in fact produced numerous variants. And when they pored over the newly released sequence of the fruit fly genome, they realized that *Dscam* had the potential to generate a mind-boggling 38,016 distinct forms of proteins. • It's now known that *Dscam* is an extreme example of "alternative splicing"—a variable but carefully regulated adaptation of a routine RNA modification process that allows a single gene to give rise to multiple versions of a protein. While most cases are more modest than *Dscam*, the reality of alternative splicing has still managed to turn on its head the "one gene, one protein" principle that has guided genetics for more than half a century. • The ability of alternative splicing to exploit limited genetic information to generate a multitude of proteins is important because each cell in an organism depends on a highly specialized set of proteins to carry out its unique function. Even within a single cell, the set of required proteins varies as a function of the stage of development and changing environmental conditions. With the latest estimates putting the content of the human genome at only 20,000 to 25,000 genes, most scientists believe a one-to-one ratio of genes to proteins just cannot be enough.

BY JENNIFER MICHALOWSKI

Photograph by Christopher Bucklow

Variants Per Gene

DOUGLAS BLACK, another HHMI investigator at UCLA, points out that alternative splicing allows for much more complexity than the size of the genome suggests. It's too soon to know just how big the human *proteome* is, but "it's certainly much larger than the number of genes," he says. "The analyses that have been done seem to say that most genes have two or three splice variants per gene." And while not all of these transcripts (RNA segments) produce functional proteins, there are other genes that generate far greater diversity—hundreds or even thousands of forms.

The first example of alternative splicing in cells—there were earlier examples in viruses—was found in a gene called *IgM* in 1980. It was considered an anomaly. "But if you were paying attention, you started to see more and more examples of genes that were alternatively spliced," says Black. Computational biologists are now trying to assess the frequency of alternative splicing more accurately. "If you now ask how many of the known human genes are producing more than one splice variant, almost everyone says at least 50 percent," Black says. "Some people argue that 70 percent have alternative splicing. And while nothing as complicated as *Dscam* has been discovered in mammalian cells, there are many transcripts that can produce hundreds of proteins."

Cells use alternative splicing to increase protein diversity toward a host of biological ends. Some of the best-studied examples derive from fruit fly development, where the splicing of several related genes dictates whether an embryo will develop into a male or a female. Similarly, alternative splicing can allow one gene to generate different proteins in different tissues—many of the highly specialized proteins in the brain, for example, come from differential splicing of genes that are also expressed in other tissues. Cells can even modify splicing in response to changing conditions: An ion channel transcript studied by Black's lab produces a protein whose sensitivity to calcium depends on which exons (segments of DNA that encode a protein's amino acid sequence) are included.

CELLS USE ALTERNATIVE SPLICING TO INCREASE PROTEIN DIVERSITY TOWARD A HOST OF BIOLOGICAL ENDS.

Recent work in the lab of HHMI investigator Robert B. Darnell at the Rockefeller University shows that not only can alternative splicing tweak the structure of a single protein, but it may also be a means of regulating entire pathways. In the first genome-wide screen for the targets of a tissue-specific splicing factor, Darnell showed that Nova, a protein found only in the brain, controls the splicing of 49 mRNAs to produce proteins not found in other tissues. Almost all of these proteins help nerve cells transmit their signals across the synapse.

"We don't know this yet, but one can guess that different exons may change the way these proteins interact or send messages," Darnell says. "If that is so, by changing these exons, you can modulate the quality of the synapse in a very powerful way—'powerful' meaning very regulated." Darnell expects that as similar studies are done with other splicing factors, they too will be found to regulate similarly coherent groups of proteins.

Mutations that alter splice sites often cause entire exons to be excluded, severely damaging the encoded protein; these types of mutations are often associated with human disease. In the case of alternative splicing, however, a cell usually splices a single transcript in multiple ways to generate an assortment of proteins. So some mutations that alter a splice site or a nearby regulatory sequence have subtle effects—shifting the ratio of the resulting proteins without entirely eliminating any form. Several human diseases illustrate that these sorts of mutations still have the potential to be devastating. Alternative splicing errors are known to contribute to some growth deficiencies; a urogenital disorder known as Frasier syndrome; a type of cystic fibrosis; and a condition known as frontotemporal dementia and Parkinsonism. In the latter case, mutations disrupt the normal splicing pattern of a transcript called *tau*, which produces a protein that helps give a nerve cell its shape. Normally, the cell uses the transcript to produce six different forms of tau protein. Interfering with splicing, however, can result in an excess of some of those forms, causing tau to clump together in the brain and bring about progressive dementia.

Rules of Regulation

AS IT BECOMES clear that alternative splicing is more the rule than the exception, scientists are realizing that, to make effective use of the enormous amount of data being generated by genome-sequencing projects, they must understand how the cell's splicing machinery processes that information. "Proteins are the major workhorse of the organism. If we want to have a really big-picture view of how organisms develop and function, we need to know what all the proteins are," says Brenton R. Graveley, an associate professor at the

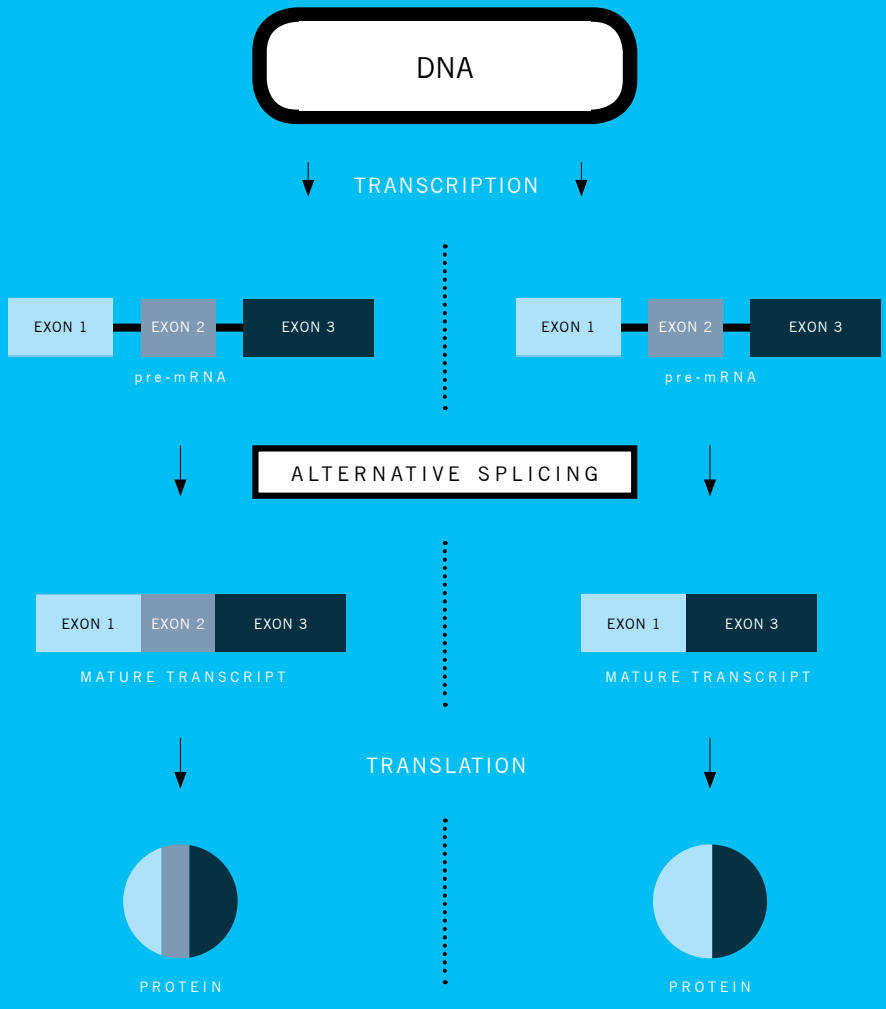
THREE OF THE HHMI INVESTIGATORS INVOLVED IN SPLICING RESEARCH

1. Robert Darnell
Robert Darnell studies degenerative brain disorders that are provoked by an immune response to certain cancers.

2. Douglas Black
Douglas Black researches the regulation of pre-mRNA splicing in differentiated cells, particularly neurons.

3. Lawrence Zipursky
Lawrence Zipursky is interested in uncovering the mechanisms by which neurons make highly specific patterns of connections during development.

DIRK WESTPHAL, KIM SIMMONDS, MARK HARMEL



FUNDAMENTALS

Two Types of Splicing

Fundamentally, alternative splicing is little different from routine, or constitutive, splicing.

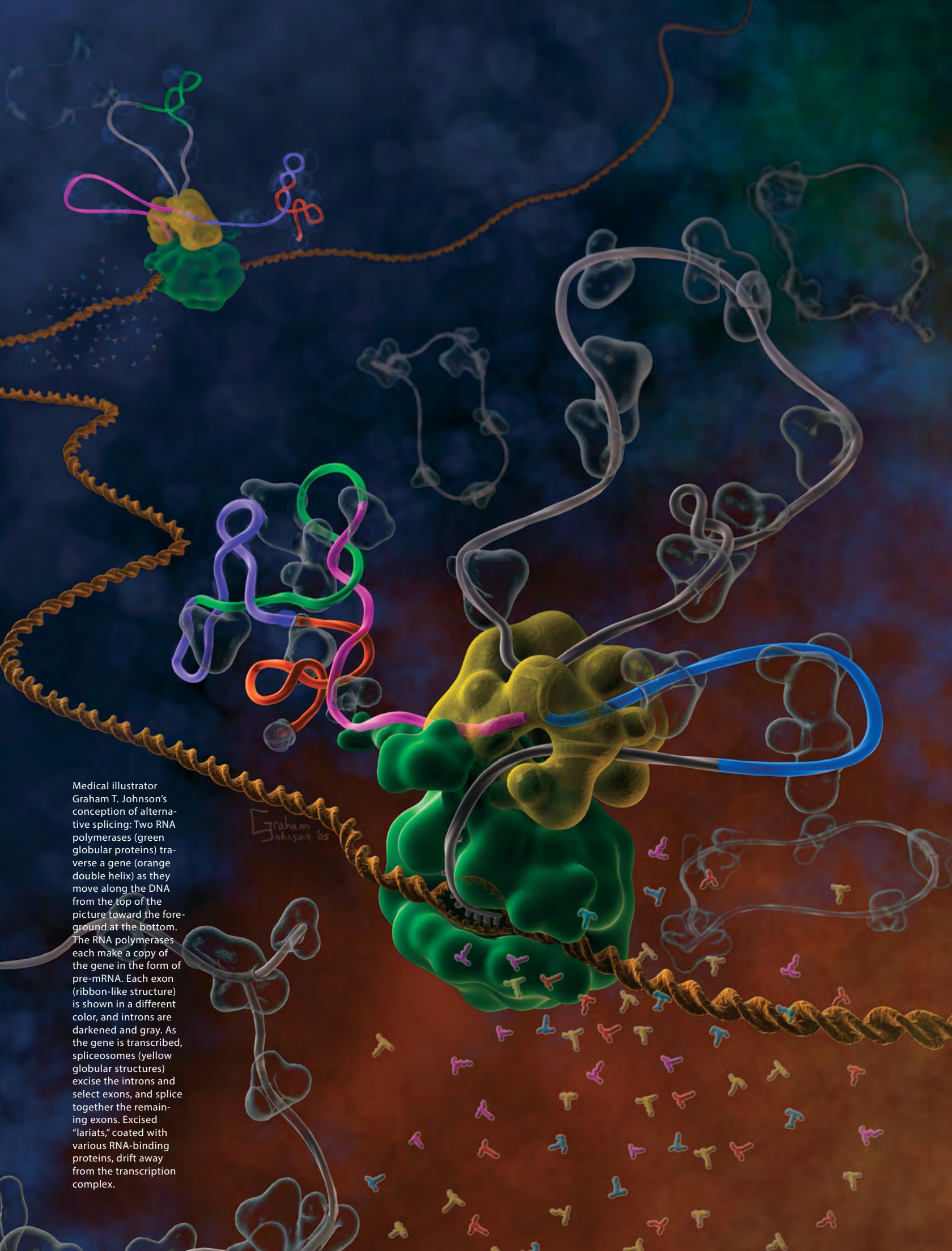
Scientists have known since the 1970s that, in eukaryotic organisms, gene sequences that encode proteins are interrupted by segments of noncoding DNA known as introns. These regions, which can make up more than 90 percent of a gene, are transcribed into RNA along with the interspersed coding segments, or exons. Because they cannot be used during the translation of RNA to protein, they must be removed.

A complex of proteins and RNA known as the spliceosome is responsible for identifying splice sites on an RNA molecule, snipping out the appropriate segments, and reconnecting the severed transcript.

For some transcripts, this process is always the same: Introns are removed and the remaining exons are pieced back together. But for alternatively spliced transcripts, things are more complicated. A subset of exons is often removed along with the introns (the most prevalent form of alternative splicing in mammals); in other cases (most commonly in plants and lower animals), introns are retained in the final transcript. The inclusion or exclusion of each segment determines the structure of the resulting protein, and whether to remove an alternatively spliced exon is a decision the spliceosome must make each time a gene is transcribed, with the assistance of a sizable collection of regulatory molecules.

SPLICING AND TRANSCRIPTION

To begin the conversion of genetic information into proteins, cells produce a precise RNA copy of a gene using the DNA sequence as a template. In all but the simplest organisms, these transcripts are not functional mRNAs until they undergo a molecular editing process, when the cell snips out unintelligible or unwanted bits of genetic sequence. This splicing process often occurs while an RNA molecule is still being assembled, and for alternatively spliced transcripts, how quickly the cell's transcription machinery pieces together the RNA can influence which bits are discarded. • The spliceosome (the mass of proteins and RNA that controls genetic splicing) recognizes some splice sites more readily than others, ordinarily ignoring weaker sites in favor of stronger ones located nearby. But according to work by Alberto R. Kornblihtt, an HHMI international research scholar at the University of Buenos Aires, slowing down the transcription of certain genes can alter this selection process. • The effect is seen in genes where a weak splice site is transcribed before a stronger one. Ordinarily, the spliceosome would opt for the stronger site, but when transcription slows, there is a delay during which the weak site has been transcribed, but the stronger one does not yet exist. A spliceosome working on an incomplete transcript has no choice but to use the weaker splice site and consequently produces a different protein than is generated when transcription proceeds more rapidly. • Cells alter transcription rates to modulate the amount of proteins they produce. For genes whose splicing is coupled with transcription, this strategy may alter not just the quantity of those proteins but also some important aspect of their structure, Kornblihtt says. He also thinks this effect can explain why splicing is often coordinated between distant regions of the same gene. It's common for the inclusion of one exon to relate directly to the inclusion of another—which may mean that the splicing of each region depends on how quickly transcription proceeds. One transcription rate may mean that both exons are included, for example, while at a faster rate, both will be excluded.



Medical illustrator Graham T. Johnson's conception of alternative splicing: Two RNA polymerases (green globular proteins) traverse a gene (orange double helix) as they move along the DNA from the top of the picture toward the foreground at the bottom. The RNA polymerases each make a copy of the gene in the form of pre-mRNA. Each exon (ribbon-like structure) is shown in a different color, and introns are darkened and gray. As the gene is transcribed, spliceosomes (yellow globular structures) excise the introns and select exons, and splice together the remaining exons. Excised "lariats," coated with various RNA-binding proteins, drift away from the transcription complex.

University of Connecticut Health Center. "In cases like *Dscam*, where you have 38,000 different proteins that are made from that gene, knowing just one of them is not terribly useful."

THE GOAL OF BEING ABLE TO PREDICT SPLICING FROM A GENE SEQUENCE IS STILL TANTALIZINGLY OUT OF REACH.

Christopher Burge, an associate professor at the Massachusetts Institute of Technology, likens the state of the splicing field to an earlier phase in the ongoing quest to decipher the genetic code. "Back in the early sixties," he says, "they didn't know whether it was a triplet code, a doublet code, a tetramer code, or not of fixed length. And I think that's where we are with splicing. We don't yet know what the code looks like."

To delve into that code, Burge has scoured human and mouse transcript databases—enormous collections of gene sequences generated from mRNAs—for features that distinguish alternatively spliced exons. The presence of a splice site, Burge says, is not sufficient to know that splicing will occur; nearby enhancer and repressor sequences—short segments of RNA that serve as landing pads for regulatory proteins—are equally crucial. The splicing of a single exon, he estimates, is likely promoted by at least three to seven enhancer sequences.

By analyzing the complete sequence information of genes known to be alternatively spliced in both mice and humans, Gene Yeo, a graduate student in Burge's lab (now a fellow at the Salk Institute for Biological Studies), developed a profile of a "typical" alternatively spliced exon and later identified another 2,000 exons that fit the description. Of these, at least 70 percent appear to undergo alternative splicing in both humans and mice.

Burge has focused his analyses on those alternative splicing events that are conserved between species, because he believes they are most likely to be functionally important. "It's a diverse collection of genes," he says, "but there are significant biases in what kinds of genes undergo conserved alternative splicing." These exons are more likely to be in genes that are involved in development, he says. "They're more likely to be transcription factors. They're more likely to be expressed in different brain regions."

Burge's group has also observed that alternatively spliced exons tend to fall between the segments of a gene that encode the functional units, or domains, of a protein. For most alternatively spliced transcripts, he says, "presumably both forms would produce stable, folded proteins that would have the same enzymatic activity. They would just differ in these regions that might affect other properties of the protein—maybe its localization or its regulation." By altering proteins' locations inside a cell or their sensitivity to activators or inhibitors, most conserved alternative splicing events, Burge expects, produce "subtle, but perhaps very important differences."

While Burge explores the sequence clues to how transcripts are spliced, others are trying to unravel the convoluted system of regulatory proteins that contribute to splicing decisions. It's an extensive network of positive and negative regulators, some ubiquitous and some expressed only in specific cells, and Black thinks that easily hundreds of proteins could be involved.

Connecticut's Graveley, for example, focuses on the fruit fly *Dscam* gene, which he considers the perfect model for studying the intricate systems that regulate alternative splicing. Graveley saw Zipursky's first paper about *Dscam* the day it was published in the journal *Cell* (June 9, 2000); thinking "this is too good to be true," he started designing experiments that very day.

Eliminate the Proteins

SINCE THEN, Graveley and his colleagues have found 47 proteins that alter *Dscam* splicing, and they expect to turn up more. Their strategy has been to systematically eliminate each of the 250 or so proteins in the fruit fly that bind to RNA—good candidates for splicing regulators—and examine the effects of such knockouts. Some of the molecules they've identified in their screen are generic splicing factors, thought to be required for the splicing of many genes in a variety of cell types; others are novel, perhaps controlling a more defined set of spliced exons. Most of the factors regulate the inclusion of a single exon within *Dscam*.

CONTINUED ON PAGE 64

SECRETS OF THE SPLICEOSOME

Cells invest significant resources in carrying out and regulating splicing—both constitutive and alternative. In mammals particularly, the machinery for identifying splice sites, snipping out introns, and reconnecting the severed transcript has long been known to be a large complex of regulatory and catalytic components, both protein and RNA. • Melissa J. Moore, an HHMI investigator at Brandeis University, says that not long ago, she estimated that 50 to 70 proteins participated in the splicing process. So it came as a surprise when, in 2002, her lab and others purified the spliceosome in various stages of the process and found about 100 proteins at each phase. The proteins known to come and go during splicing total about 300. • These studies, Moore says, focused on constitutively spliced exons, which are more efficiently recognized and removed than those that are alternatively spliced; many of the molecules regulating the latter process have probably been left out of recent structural models. "My guess is that the list of proteins affecting splicing is not complete yet. We haven't even begun to scratch the surface of the proteins that affect alternative splicing," she says. • Scientists are still debating how the components of the massive complex arrive at a splice site, but according to HHMI investigator Michael Rosbash, also at Brandeis, "It's rather difficult to imagine how alternative splicing would take place if the spliceosome was preassembled." While some researchers argue for a model in which the spliceosome is at least partially preassembled, recent studies from Rosbash's lab and another have demonstrated that, at least in yeast, loading of the spliceosome occurs in a stepwise fashion. • Rosbash has found that the five small nuclear ribonuclear particles (snRNPs) at the core of the splicing complex load sequentially onto the transcript, presenting an opportunity for regulation. "A lot of alternative splicing regulation could take place at the level of which snRNPs jump on where, and in what order," he notes. "It just gives you many more degrees of freedom." Although there's no evidence yet of this stepwise assembly in other organisms, "the core mechanisms are so similar that it would be shocking if something as fundamental as this were not conserved between humans and yeast," Rosbash says.

Q

Solving Big Questions

BY KATHRYN BROWN

POISED TO ENGAGE IN SOME OF SCIENCE'S LARGEST RIDDLES, SEVEN RESEARCHERS ARE APPOINTED TO BE THE FIRST GROUP LEADERS AT HHMI'S

JANELIA FARM RESEARCH CAMPUS.

Some scientific problems get solved at 3:00 a.m., with a jolt of insight in the dark. Some need 5 years of solid, steady work. And then there are the Big Ones—looming, bewitching scientific riddles that could take 100 years to crack. *How does the human brain work? What is consciousness?* It takes a rare researcher to pursue big questions with creativity and focus. But HHMI recently found seven such scientists. In June, HHMI announced the selection of seven group leaders for its Janelia Farm Research Campus. Slated to open next year in Ashburn, Virginia, Janelia Farm is HHMI's first freestanding research community. The $500 million campus will bring together small research groups in a highly collaborative environment to tackle fundamental biomedical problems that linger unsolved in traditional research settings. "HHMI always has sought to support the most original, creative science," says Janelia Farm Director Gerald M. Rubin, also a vice president at HHMI. "Janelia Farm extends this effort, opening a new frontier for the Institute." Rubin calls the campus a "once-in-a-generation opportunity" for science to build something new. Janelia Farm's first set of group leaders have diverse backgrounds, from mathematics and physics to computational biology and genetics. Their wide-ranging

interests include mathematical theories of brain design, gene and genome function and structure, the neural circuits behind specific behavior, and image analysis.

TOGETHER, THESE GROUP LEADERS WILL LAUNCH AN AMBITIOUS AGENDA. Janelia Farm will pursue two basic, and intertwined, goals: identifying the general principles that allow neural circuits to process information and developing imaging technologies and computational methods for image analysis. › Put simply, Janelia Farm scientists will develop new ways to peer inside a working brain, revealing brain anatomy and function in unprecedented detail.

To do that, each group leader will head a small team of up to six scientists. These interdisciplinary teams will work side by side, breaking down big scientific questions into smaller steps. When new tools—from specialized microscopes to specific computer programs—are needed, they (and support staff) will simply build them.

GREAT ADVENTURE: › Group leader Nikolaus Grigorieff, currently an HHMI investigator at Brandeis University, calls Janelia Farm "a great adventure." Grigorieff develops cellular image-processing techniques based on electron microscopy. "To win grant funding in academics, you generally have to propose research that's practically guaranteed to be successful, and that can be boring," says Grigorieff. › "But at Janelia, we're going to be given resources and time, with the trust that we'll have good ideas. That's very different." While at Janelia Farm, Grigorieff plans to collaborate with neurobiologists, perfecting imaging techniques to spotlight activity at brain synapses.

Group leaders are roughly equivalent to academic professors in that they're intellectually independent and direct a research team of postdoctoral fellows, graduate students, and technicians. But that's where the similarities end. Unlike their academic peers, Janelia Farm group leaders will not teach, write grant proposals, or do administrative tasks. › Instead, they will devote their full attention to research, with minimal distractions.

In return, Janelia Farm group leaders agree to spend 75 percent of their professional time focused on research at the campus. In their remaining time, leaders can attend conferences, review for journals, give outside seminars, and pursue other scientific activities. Group leaders also may use part of this time to consult for industry, in accordance with HHMI policies.

That schedule suits group leader Sean Eddy, currently an HHMI investigator at Washington University in St. Louis. Eddy knew he wanted to join Janelia Farm 5 years ago, when he first heard Rubin describe the campus. "I'm the kind of guy who prefers to work with my own hands," says Eddy, a computational biologist who builds mathematical tools to probe genomes. "I enjoy being a professor, but I'm constantly training, training, training. At Janelia Farm, *I* will get to do the science." While at the campus, Eddy plans to adapt his computational techniques to the study of neural circuits.

› RUBIN ENVISIONS JANELIA FARM AS "A COMBINATION THINK TANK AND RESEARCH LAB," with an unstructured, open environment that invites brainstorming and long, meaningful debate. The plan for the campus emerged as HHMI leadership realized that some biomedical research problems cannot be adequately addressed in a university environment. Developing new tools for biological discovery, for instance, requires diverse expertise. But university scientists often work in distant departments, with little opportunity to collaborate extensively.

HHMI modeled Janelia Farm after the best features of two widely respected institutions: the Medical Research Council Laboratory of Molecular Biology (MRC LMB) in Cambridge, United Kingdom, and AT&T's Bell Laboratories in the United States. Although these organizations have had different missions, both incorporated small research groups, generous funding, and top support services.

In addition to the incoming group leaders, HHMI has appointed two senior fellows to Janelia Farm: Nobel laureate Sydney Brenner of the Salk Institute for Biological Studies in La Jolla, California, who is the former director of MRC LMB, and Charles V. Shank, former director of Lawrence Berkeley National Laboratory, who also worked at Bell Labs. The senior fellows will advise Rubin, spend several weeks a year in residence at Janelia Farm, and help shape its research program.

The incoming group leaders and their current affiliations

DMITRI B. CHKLOVSKII
COLD SPRING HARBOR LABORATORY, NEW YORK

SEAN R. EDDY
HHMI INVESTIGATOR AT WASHINGTON UNIVERSITY SCHOOL OF MEDICINE IN ST. LOUIS

NIKOLAUS GRIGORIEFF
HHMI INVESTIGATOR AT BRANDEIS UNIVERSITY, MASSACHUSETTS

"YES, THERE'S SOME RISK THE EXPERIMENT WILL FAIL, AND WE'LL HAVE TO READJUST. BUT IF WE'RE SUCCESSFUL, WE'LL CREATE A DIFFERENT WAY OF DOING BIOMEDICAL RESEARCH."

› "There are three critical ingredients for a leading lab: stable funding, top scientific leadership, and scientific focus," says Shank. "Janelia Farm has all three. This HHMI effort is poised to make fundamental contributions to the understanding of neural networks, laying the foundation for a new understanding of the human brain."

The research programs at Janelia Farm naturally extend HHMI's commitment to offering creative scientists freedom from research constraints. Janelia Farm's campus and scientific program will complement HHMI's long-standing investigator program, which currently consists of more than 300 researchers at 64 universities throughout the United States who have the freedom and flexibility to push the bounds of biomedical research. › HHMI recently announced the selection of 43 of the nation's most promising biomedical scientists as new HHMI investigators.

LIKE HHMI'S INVESTIGATOR POSITIONS, JANELIA FARM APPOINTMENTS ARE TEMPORARY. Group leaders will be appointed for an initial 6-year term. At that point, outside experts and HHMI leadership will review each group leader's contribution, including research progress, collaboration, and mentoring. Successful group leaders will be offered a 5-year renewal, with an invitation to stay at Janelia Farm. (The review process will be repeated every 5 years.) Those leaders also can choose to move on, as HHMI investigators, to another U.S. research institution.

If successful group leaders develop interests that stray from Janelia Farm's mission, they may be offered renewal, without an invitation to stay. Those leaders, too, will be offered HHMI investigator appointments at other research institutions. Finally, group leaders who are not renewed will be given 2 years of research support.

PURE SCIENCE: › As a postdoctoral fellow at the University of Wisconsin–Madison, incoming group leader Julie Simpson says this is the perfect time in her career to experience Janelia Farm. "This is a unique opportunity, and I'm fortunate that the timing works so well," Simpson says. "I'm delighted to be going because Janelia is a chance to do pure science." Simpson investigates the neural basis of particular behaviors, such as motor control, in the fruit fly *Drosophila*.

To recruit the first wave of Janelia Farm group leaders, HHMI looked for scientific researchers in the fields of biology, chemistry, computer science, engineering, mathematics, and physics who were interested in neuronal circuits and imaging. "We also considered applications from exceptionally talented individuals working outside these defined areas," adds Rubin. › HHMI used both targeted recruitment and an open international competition, which yielded more than 300 applications. Those applications were reviewed by groups of HHMI researchers, supplemented as needed with physicists, engineers, and computer scientists.

This fall, HHMI plans to announce a second open application process. This round will include a December application deadline, resulting in offers to perhaps five additional group leaders by the spring of 2006. **LATER NEXT YEAR, WHEN JANELIA FARM OPENS ITS DOORS, HHMI WILL BEGIN CONTINUALLY RECRUITING FOR ADDITIONAL STAFF.** By 2009, when the campus is fully operational, it will include 24 group leaders and a permanent research staff of about 300 scientists.

Known for his entrepreneurial verve, Rubin acknowledges that Janelia Farm is a risky endeavor. › "We're doing an experiment here," he says. "Yes, there's some risk the experiment will fail, and we'll have to readjust. But if we're successful, we'll create a different way of doing biomedical research." ■

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UNIVERSITY OF WÜRZBURG, GERMANY

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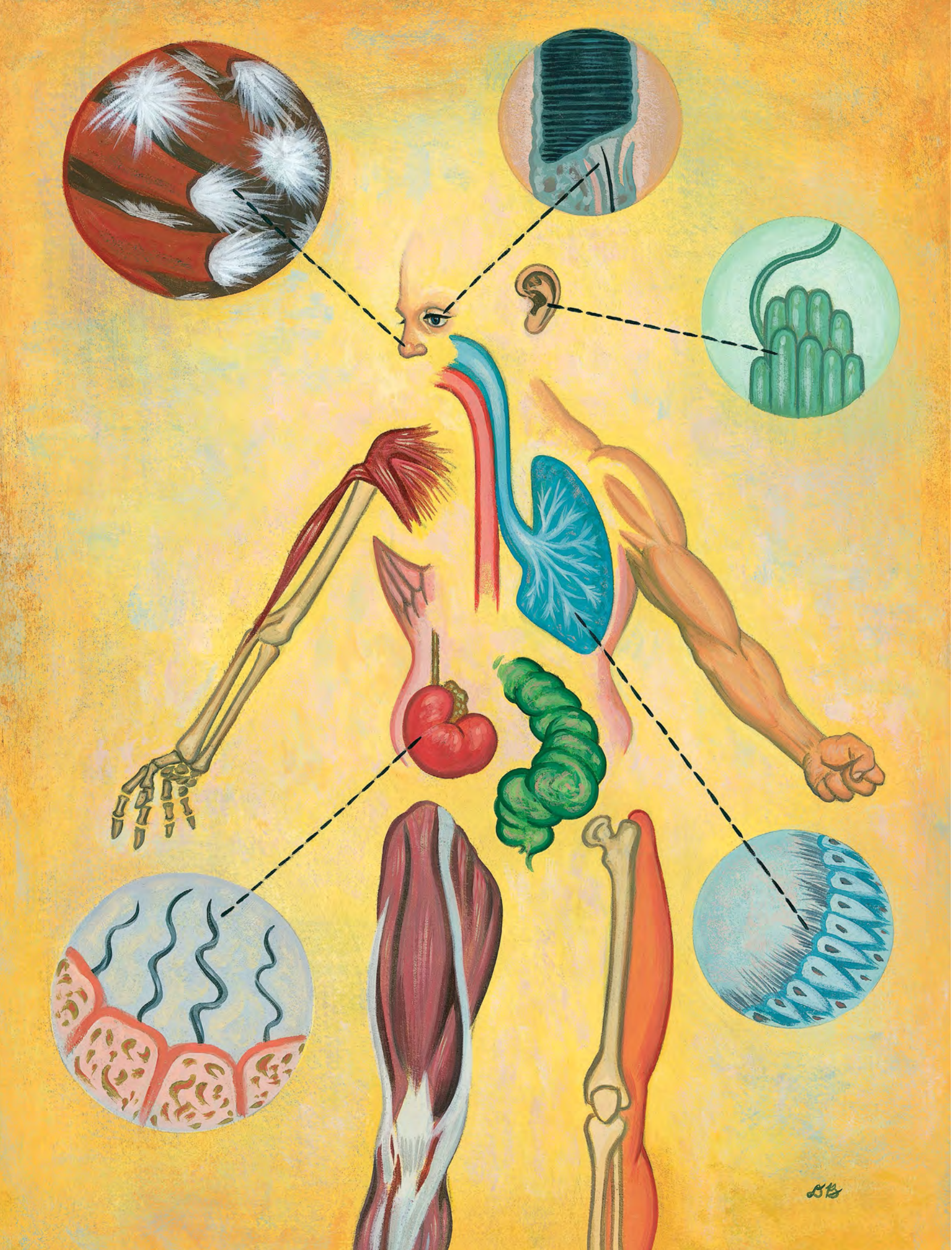


BY MARY BETH GARDINER

Illustration by David Brinley

The Importance of Being CiLiA

Once considered merely a vestige of evolution, cilia are in fact essential to many of the body's organs. As researchers learn more about cilia's role in disease, they're starting to pay this once-ignored organelle much more attention.

On the surface of nearly every cell in the body is a slender protuberance called the primary cilium. Although ubiquitous, the primary cilium was long considered—with a few exceptions—to be a largely useless evolutionary vestige, destined to go the way of the tailbone and the wisdom tooth. But now we know that cilia are functioning organelles, essential to normal development and health. • Some cilia are rigid spikes that act as antennae, gathering sensory information for the cell from the surrounding environment. Other cilia are flexible and whip-like, capable of registering the surrounding fluid's flow and ebb. • Scientists have recently implicated malfunctioning cilia as factors in a number of diseases. One of the most devastating among them is the heritable Bardet-Biedl syndrome (BBS)—a rare disorder, involving multiple organ defects, that results in obesity, retinopathy, polydactyly (more than five digits on the hands or feet), kidney disease, and mental retardation, among other problems. HHMI investigator Val C. Sheffield at the University of Iowa Carver College of Medicine is among the scientists trying to get at the genetic origins of the disorder. So far, at least eight genes have been tied to BBS, several of which Sheffield's lab identified, and all of them have been linked to ciliary function. • But it was another malady affecting the kidney—polycystic kidney disease, or PKD—that fed the current surge of interest in cilia and what they do. It began when a group of scientists saw something in common between a mouse and a single-celled plant.

CRUCIAL RAILWAYS

• **Primary cilia were first** described in 1898. For the next hundred years or so cell biologists largely ignored them, but microscopists continued to document their presence in the cells of most vertebrate organisms. It was generally believed that the nonmotile cilium was either a sensory organelle, because of its presence in the nose and eye, or that it no longer served any purpose. Understanding the role of the motile cilium and the flagellum (a structure nearly identical to the cilium) was easier. They provide movement, as in sperm and in the lungs and trachea of the respiratory tract.

In the 1990s, researchers began to understand more about the internal workings of cilia and flagella, and how cargo moves up and down the microtubular tracks within them. During such intraflagellar transport (IFT), large protein complexes are carried to the ciliary tip and then back to the cell body. "You can think of the IFT particle as the equivalent of railroad cars," says Gregory J. Pazour, a researcher at the University of Massachusetts Medical School. "It carries materials needed to build the cilia and returns with spent materials." Pazour suspects that the IFT particle, which is made up of at least 17 polypeptide subunits, may also carry signals—messages collected by various receptors embedded in the ciliary membrane—back from the tip.

During the late 1990s, Pazour partnered with Joel Rosenbaum at Yale University, Douglas Cole at the University of Idaho, and George Witman at the University of Massachusetts Medical School to purify and sequence subunits of the IFT particle isolated from the unicellular alga *Chlamydomonas*. In October 2000, the *Journal of Cell Biology* published their finding that one of the alga particle's subunits—termed IFT88, or polaris—is encoded by a gene that is homologous to the mouse and human gene *Tg737*. They observed that mutant *Chlamydomonas* lacking the *IFT88* gene are normal—except for the absence of flagella. And, it turns out, mice with defects in *Tg737* die shortly after birth from PKD.

The evidence suggested that IFT is important for primary cilia assembly and that defects in ciliagenesis in the kidney can lead to PKD. So the researchers got the defective mice, publicly available from the Oak Ridge National Laboratory, and looked at their kidneys.

"As we predicted," says Pazour, "the kidney cilia were aberrantly formed. This evidence laid to rest the idea that kidney cilia had no function—that they were vestigial organelles. That was pretty exciting for us."

Intraflagellar transport, known to be essential for the assembly and maintenance of cilia, may have far-reaching effects that researchers are only just beginning to discover.

POLARIZATION MATTERS

• **Within our kidneys,** the dense winding ductwork that carries urine to the bladder is lined with cuboidal epithelial cells. In PKD, the kidney enlarges and fills with strangulating cysts thought to result from an overproliferation of those epithelial cells. Could it be that malfunctioning cilia on those cells play a causative role? It's beginning to look that way.

Ben Margolis, an HHMI alumni investigator at the University of Michigan, is a nephrologist who studies polarization of epithelial cells in the kidney. He's interested in knowing why those cells lining the ducts are specifically oriented so that the apical side, where the cilium is located, is exposed to urine flow while the opposite, or basolateral, side is exposed to blood.

"Cilia must point into the urine space," says Margolis. "The model is that urine flow bends the cilia, which send some kind of signal to tell the cell there's flow, and that this somehow inhibits cyst formation. Nobody is clear exactly how that happens."

Margolis became intrigued with the role of cilia in cyst formation through discussions with fellow University of Michigan researcher Friedhelm Hildebrandt, who studies a cystic disease in children called nephronophthisis, which is a much rarer condition than PKD but one that appears to be cilia-dependent.

Taking a closer look at one of their apical polarity proteins—called Crumbs—Margolis was surprised to see how it distributed itself. "It was on the apical surface and then it seemed to enter into the cilia," he says. "And it wasn't just *in* the cilium; it occurred in discrete punctate spots, which is consistent with the intraflagellar transport process in which seemingly large particles of material are moving along the cilium."

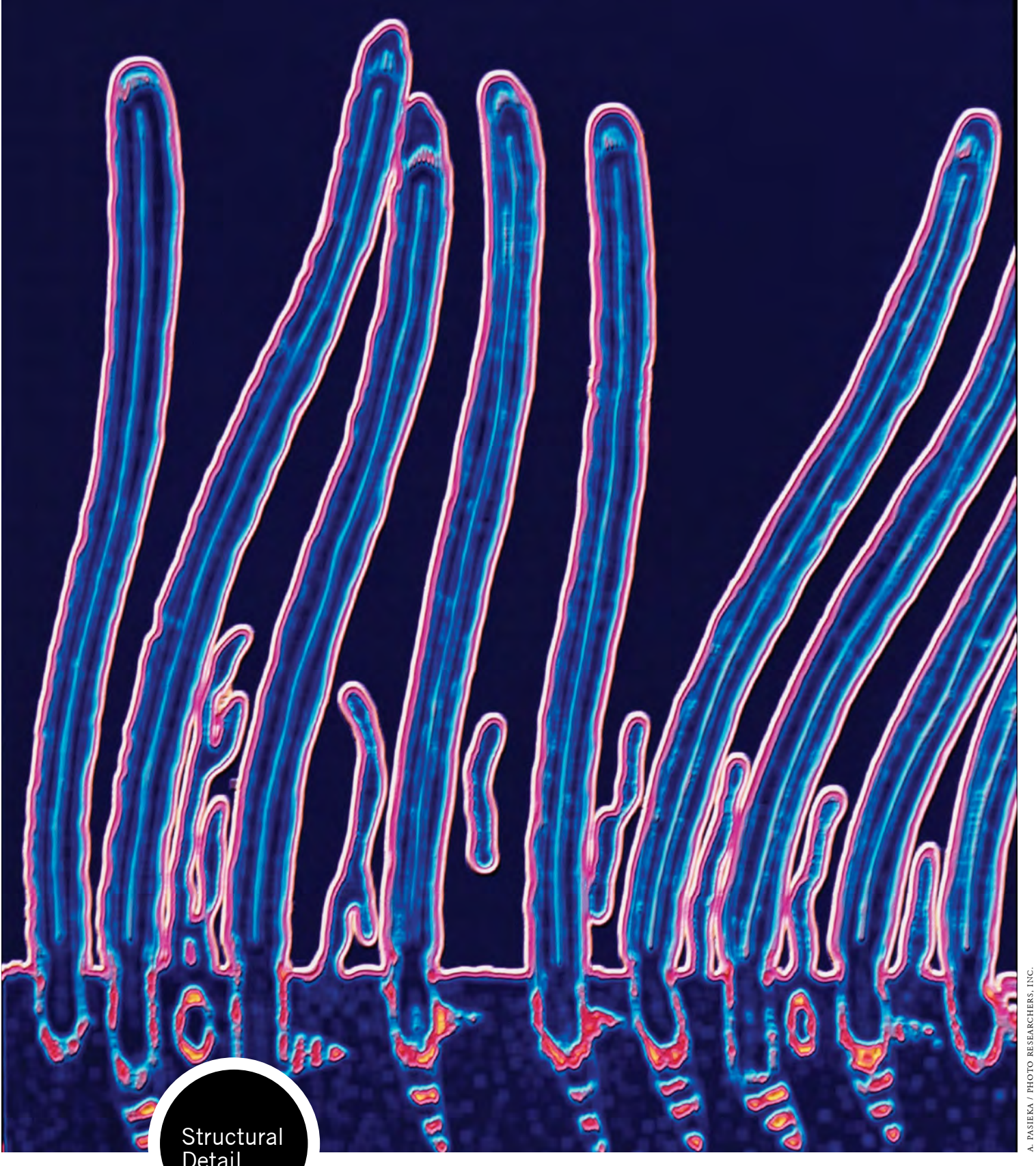
When Margolis knocked out the gene for Crumbs3 in cultured kidney cells, no cilia formed. And he found similar results with another family of proteins—called Par—that controls apical/basolateral polarity. Interestingly, Margolis points out, the *Crumbs1* gene (closely related to the *Crumbs3* gene) is linked to retinitis pigmentosa, a disease associated with defects in intraciliary transport. "We feel that the Crumbs protein can somehow regulate or have an important role in cilia formation," he says. "We're working to identify what that role is."

PINPOINTING THE GENES

• **Iowa researcher Sheffield** first started studying the genetics of BBS in 1993. Analyzing the DNA from three large families of Bedouin Arabs with BBS-like symptoms, Sheffield's lab came up with a surprising result: Each of the families mapped to a different place in the genome. "That told us this was a genetically heterogeneous disorder, which other groups' work has since confirmed," says Sheffield. "To make a long story short, eight genes at eight different loci have been identified, and five of those came out of our laboratory."

He thinks the number of BBS genes won't stop at eight, and the search continues in his and other labs. Meanwhile, there is the question of what unifying disease mechanism might link all these genes. By applying the power of bioinformatics, scientists have identified at least one common thread: cilia.

Sheffield's lab and others compared the sequences of human BBS genes to genome sequences from other organisms—from algae to higher plants, insects, fish, and mice—in search of similarities. They found that BBS genes are conserved in organisms with cilia but not in nonciliated organisms.



A. PASIEKA / PHOTO RESEARCHERS, INC.

Structural Detail

Inside cilia and flagella is a microtubule-based cytoskeleton called the axoneme. The axoneme of primary cilia typically has a ring of nine outer microtubule doublets (called a 9+0 axoneme), and the axoneme of a motile cilium has two central microtubule doublets in addition to the nine outer doublets (called a 9+2 axoneme). The axonemal cytoskeleton acts as scaffolding for various protein complexes and provides binding sites for molecular motor proteins, such as kinesin II, that help carry proteins up and down the microtubules. • At the base of the cilium is the microtubule organizing center, also called a basal body, which is created as the centriole (a microtubular structure essential to cell division) migrates to the surface. The transition zone between basal body and axoneme serves as a docking station for intraflagellar transport and motor proteins. • Cilia and basal bodies have been implicated directly in a number of developmental processes, including left-right asymmetry, heart development, maintenance of the renal epithelium, respiratory function, electrolyte balance in the cerebrospinal fluid, and reproductive fecundity.

Charles S. Zuker, an HHMI investigator at the University of California, San Diego, was one of the first to carry out a whole-genome subtraction study. Zuker's postdoc, Tomer Avidor-Reis, crafted the analysis, which identified nearly 200 conserved ciliary genes encoding both known and candidate ciliary proteins. Using the fruit fly *Drosophila* as a model system, the lab then investigated the function of some of the candidate proteins, at least two of which were suspected to derive from BBS genes.

Another comparative study, this one led by Susan K. Dutcher, a researcher at Washington University School of Medicine, looked at the genes and proteins of humans, *Chlamydomonas*, and a weedy plant. The researchers subtracted all genes found in the weed from the combined genomes of humans and algae, leaving them with a set of 688 genes found exclusively in organisms with cilia or flagella. From this set, they identified a novel BBS gene, *BBS5*, and showed that the protein product from that gene localizes to the "basal body"—the point, underlying the cilium, from which its microtubule railways originate—in the mouse and in the worm *Caenorhabditis elegans*.

"Though none of the precise functions of the BBS gene products are known, it is clear that cilia dysfunction is involved in some of the phenotypic characteristics," says Sheffield. Male patients have infertility problems, for example, and in the BBS mouse model, sperm are missing flagella. Another phenotype of BBS, blindness, is also cilia-dependent. Humans and mice with BBS see fine at birth, but in time the photoreceptor cells within the retina degenerate. A key component of photoreceptors, it turns out, is a structure called a connecting cilium.

"What we think is going on is that there is abnormal transport—intraciliary transport—related to the connecting cilia," says Sheffield. "That eventually leads to the dysfunction of those photoreceptor cells and ultimately to their death."

SECReTS OF CHuBBY MiCE

• **Intraflagellar transport,** a process now known to be essential for the assembly and maintenance of the cilium, may have far-reaching effects that researchers are only just beginning to discover. What if, for example, IFT is responsible for delivering receptor proteins to the membrane that encases the cilium? The University of Massachusetts' Pazour, who sees the cilium as a sensory antenna, says he is putting a lot of energy into learning how proteins are localized to the ciliary membrane and whether any of the IFT proteins play a role. "We're searching for targeting sequences in those proteins," he says, "looking for the 'address' that directs them to the ciliary membrane. If we can get the address, maybe we can work backwards from that point."

Ciliary membrane proteins also play into Susan Dutcher's favorite

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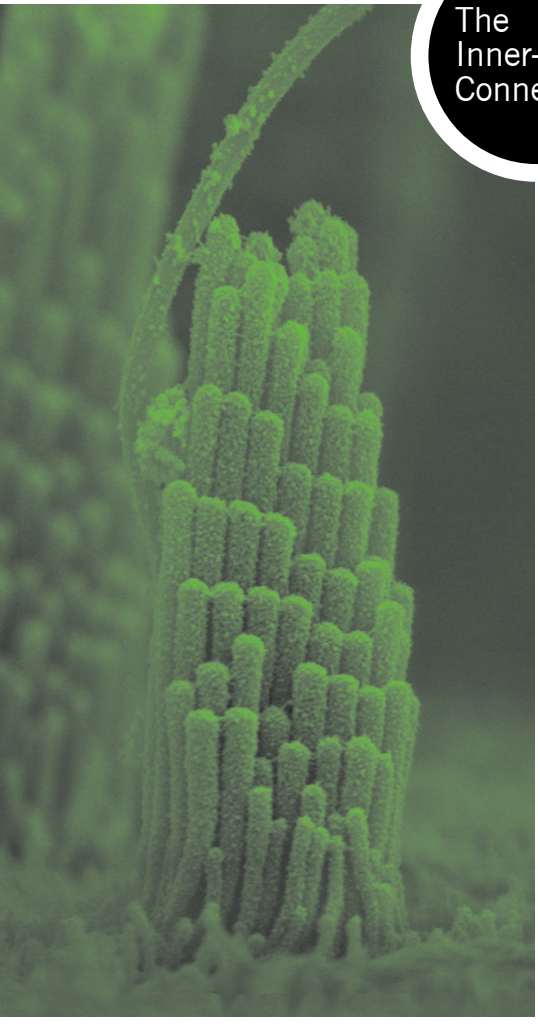

PHOTO COURTESY OF BECHARA KACHAR/NIH; COLORIZED SEM

The Inner-Ear Connection

In mammals, sound is detected by hair cells of the snail-shaped cochlea, which form a long, spiraling ribbon of sensory epithelium. At birth, each hair cell has a single true cilium, called the kinocilium, and each kinocilium is associated with 100 to 200 stereocilia—appendages that bundle together and jut from the top surface of each hair cell into the cochlear fluid. Stereocilia are not true cilia—they are instead microvillae, based on the structural protein actin, with no tubulin (the protein that makes up microtubules) present. Stereocilia vary in height, becoming progressively shorter the farther they are from the long kinocilium at the edge of the bundle. • Sometime after birth, the kinocilium is lost from the hair cell in the mammalian cochlea. Although the precise role of the kinocilium remains unclear, it is believed essential to proper development of the stereocilia. Adding to the mystery is the fact that the kinocilium persists in other parts of the ear and in lower vertebrates. • "We know it's not necessary for [signal] transduction, but we can't say why it survives elsewhere in the body," says HHMI investigator A. James Hudspeth, a researcher at the Rockefeller University who has been studying signal transduction in hair cells for decades. "The only hint we have is that when the hair bundle is formed, the kinocilium develops first, and it always moves to one edge of the cell. It seems to set up the axis along which the stereocilia are subsequently polarized. But the evidence for that is still circumstantial." • Signal transduction in hair cells involves movement of the stereocilia in the direction of the kinocilium—or rather, where the kinocilium was. The mechanical action of the cilia bending somehow triggers a signal necessary to hearing. The long-held suspicion is that spring-like "tip links" extending between adjacent stereocilia physically tug open ion channels when hair bundles are deflected by sound or movement. Hudspeth and several other HHMI investigators are determined to figure out how that happens. • David P. Corey, an HHMI investigator at Harvard Medical School, has identified a protein at the tips of stereocilia—called TRPA1—that is a candidate for the mechanically sensitive channel in hair cells. "Currently, we're carrying out a number of tests, including making a knockout mouse, to see whether it's doing what we think it's doing," he reports. • Corey says TRPA1 is very similar in structure to the nompC (no mechanoreceptor potential, type C) protein discovered in the laboratory of HHMI investigator Charles S. Zuker at the University of California, San Diego. In 2000, Zuker identified the protein, which is also a member of the

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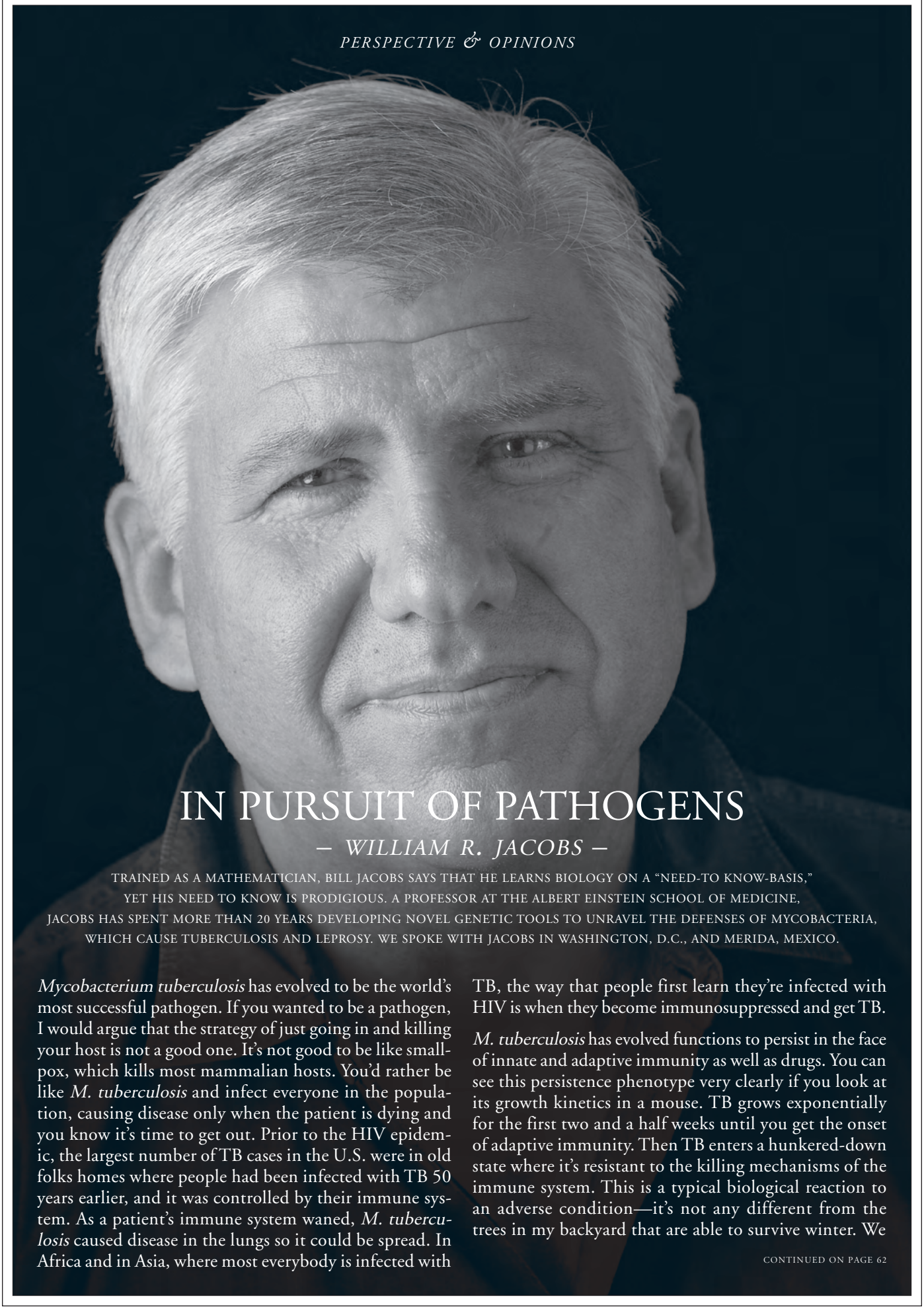

SEAN KERNAN

IN PURSUIT OF PATHOGENS

— WILLIAM R. JACOBS —

TRAINED AS A MATHEMATICIAN, BILL JACOBS SAYS THAT HE LEARNS BIOLOGY ON A "NEED-TO KNOW-BASIS," YET HIS NEED TO KNOW IS PRODIGIOUS. A PROFESSOR AT THE ALBERT EINSTEIN SCHOOL OF MEDICINE, JACOBS HAS SPENT MORE THAN 20 YEARS DEVELOPING NOVEL GENETIC TOOLS TO UNRAVEL THE DEFENSES OF MYCOBACTERIA, WHICH CAUSE TUBERCULOSIS AND LEPROSY. WE SPOKE WITH JACOBS IN WASHINGTON, D.C., AND MERIDA, MEXICO.

Mycobacterium tuberculosis has evolved to be the world's most successful pathogen. If you wanted to be a pathogen, I would argue that the strategy of just going in and killing your host is not a good one. It's not good to be like smallpox, which kills most mammalian hosts. You'd rather be like *M. tuberculosis* and infect everyone in the population, causing disease only when the patient is dying and you know it's time to get out. Prior to the HIV epidemic, the largest number of TB cases in the U.S. were in old folks homes where people had been infected with TB 50 years earlier, and it was controlled by their immune system. As a patient's immune system waned, *M. tuberculosis* caused disease in the lungs so it could be spread. In Africa and in Asia, where most everybody is infected with TB, the way that people first learn they're infected with HIV is when they become immunosuppressed and get TB.

M. tuberculosis has evolved functions to persist in the face of innate and adaptive immunity as well as drugs. You can see this persistence phenotype very clearly if you look at its growth kinetics in a mouse. TB grows exponentially for the first two and a half weeks until you get the onset of adaptive immunity. Then TB enters a hunkered-down state where it's resistant to the killing mechanisms of the immune system. This is a typical biological reaction to an adverse condition—it's not any different from the trees in my backyard that are able to survive winter. We

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GENE THERAPY: STILL A CONTENDER

– KATHERINE A. HIGH –

DESPITE SEVERAL PUBLICIZED INSTANCES OF CANCER IN GENE THERAPY EXPERIMENTS, THE ROLLERCOASTER TREATMENT OF GENE THERAPY IN THE MEDIA, AND MIXED OPINION ABOUT IT ON THE PART OF THE PUBLIC, THE FIELD IS ALIVE AND WELL, SAYS ONE OF ITS LEADERS. PROBLEMS ARE BEING SOLVED, PROGRESS IS BEING MADE, AND FINANCIAL SUPPORT IS COMING FROM A WIDER SWATH OF SOURCES.

SEAN KERNAN

Few scientists are as familiar with gene therapy's promises—and obstacles—as HHMI investigator Katherine A. High, who served last year as president of the American Society of Gene Therapy. Once touted as a revolutionary breakthrough, gene therapy has endured intense scrutiny since 1999, when a teenager died in an experiment. Nevertheless, High remains a vocal advocate for fully exploring the field's possibilities. Her own research involves development of gene-therapy techniques to treat hemophilia. She is an attending hematologist at the Children's Hospital of Philadelphia and William H. Bennett Professor of Pediatrics at the University of Pennsylvania School of Medicine.

HHMI: ***GENE THERAPY IS OFTEN CALLED A MIXED SUCCESS. HAS THAT STATUS BEEN DEMORALIZING FOR PEOPLE IN THE FIELD?***

KH: Gene therapy is as complicated a therapeutic idea as any that researchers have attempted, but if you're intensively involved in the field, you don't feel despair. We're solving problems every day. Five years ago, we could only use gene therapy to cure diseases in mice. Today, we're curing diseases in dogs and cats—and even beginning to treat humans. Researchers in Milan have used gene therapy to successfully treat six kids with a form of severe combined immune deficiency known as ADA-SCID. And China approved the first commercial gene-therapy product, to treat cancerous tumors of the head and neck.

For perspective, consider the history of novel monoclonal antibodies for the treatment of cancer. When I was in medical school in the 1970s there was a lot of hype about them, followed by widespread disappointment during the 1980s when newspapers announced that all the clinical trials were failing. The field then dwindled to fewer scientists, and this core group worked hard to overcome hurdles. Today, monoclonal antibodies are considered a great success, though it's easy to forget that this success was 30 years in the making.

HHMI: ***WHAT ARE THE BIG CHALLENGES IN GENE THERAPY NOW?***

KH: Immune response to the viral vector is one big challenge. Think of this vector as an envelope and the gene product being delivered to the patient as the letter inside. Too often, that patient's immune system rejects the envelope, and the letter within is never even read. So we're exploring ways to create transient immunosuppression—to shut down a patient's immune system just long enough for the viral vector to degrade, thereby allowing the body full exposure to the gene product inside.

Another challenge is immune response to the gene-transferred product itself. In this regard, one interesting strategy is to ask: Can we exploit nature's redundancy? Traditionally, gene therapy meant delivering a healthy gene to replace a mutated one. But now we often ask whether we can achieve the same biochemical effect through a different route.

Hemophilia offers one example. The body has at least two biochemical pathways that produce several important blood-clotting factors. The first pathway produces factor VIII and factor IX, but if a hemophilia patient is missing one of them, we don't necessarily try to replace it. Therapy might instead rev up the second biochemical pathway, which relies on factor VII, by generating extra amounts of that factor, which the patient's immune system will accept.

HHMI: ***WHAT ARE SOME RECENT ADVANCES?***

KH: The ADA-SCID work of Milanese scientists was an important success. These researchers, in clinical trials that began 5 years ago, used a retrovirus to deliver the gene encoding adenosine deaminase (ADA) to six children. Today, all six kids are able to lead normal healthy lives, with no need for treatment and no overt symptoms. This result has been largely ignored in "bad news" stories of gene therapy.

More recently, other researchers have used zinc-finger DNA-binding and -cleaving proteins ["zinc finger" refers to the proteins' shape and composition] to correct errors in a gene that lead to another form of severe combined immune deficiency known as X-linked SCID. That technology for gene correction is promising because it could be adapted into different strategies for fighting many immunodeficiency conditions, including HIV.

And last year scientists published research using a viral vector to loop out the mutant part of the dystrophin protein that's implicated in some forms of muscular dystrophy. Together, these results show that the field is moving forward.

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IF YOU COULD GO BACK TO COLLEGE FOR JUST ONE COURSE, WHAT WOULD IT BE?

HINDSIGHT, AS THEY SAY, IS 20/20. WHILE HHMI INVESTIGATORS ARE HIGHLY EDUCATED, THEY'RE ALSO PERCEPTIVE ENOUGH TO RECOGNIZE WHERE THEIR INSTRUCTION MIGHT HAVE FALLEN SHORT. HERE, FOUR HHMI INVESTIGATORS LOOK BACK AT THEIR UNDERGRADUATE YEARS WITH AN EYE TOWARD COURSES THEY WISH THEY HAD TAKEN.

-Edited by Kathryn Brown-

CATHERINE DULAC
PROFESSOR OF MOLECULAR AND CELLULAR BIOLOGY, HARVARD UNIVERSITY

"Engineering. As a student, I took a lot of theory classes such as physics, math, and biology. That's very important to a scientist's intellectual training, of course. But I don't really know, for example, how a microscope works in terms of its different parts. It occurs to me quite frequently that when I have a piece of equipment from a vendor, I cannot adapt it to my needs. I can adapt my thinking to whatever I see, but if I wish to make a piece of equipment better, I do not know how to do it."

ROBERT J. LEFKOWITZ
PROFESSOR OF MEDICINE AND BIOCHEMISTRY, DUKE UNIVERSITY MEDICAL CENTER

"Math. I took just one year of undergraduate math, and it was a standard introductory course. I wish I'd taken a really good math class, and then another, to get more firmly grounded in that subject—and not just for rigorously analyzing research. Like some art, mathematics has a subtle beauty, which I'm unable to appreciate because I lack that certain aesthetic sensibility. I'm always reading descriptions of books about Newton's work, for instance, and I think to myself, 'Wow, I really wish I could get more into this.'"

MICHAEL B. O'CONNOR
PROFESSOR OF GENETICS, CELL BIOLOGY AND DEVELOPMENT, UNIVERSITY OF MINNESOTA

"Math. Biology is increasingly quantitative. In my own field of developmental biology we now can model tissue patterns—or how molecules diffuse through tissues along gradients—with partial differential equations. Similarly, genomic analysis and statistics call for math. I was originally a chemistry major, but I switched to biology. When I did, I stopped taking a heavy load of math courses. Now I wish I had taken more."

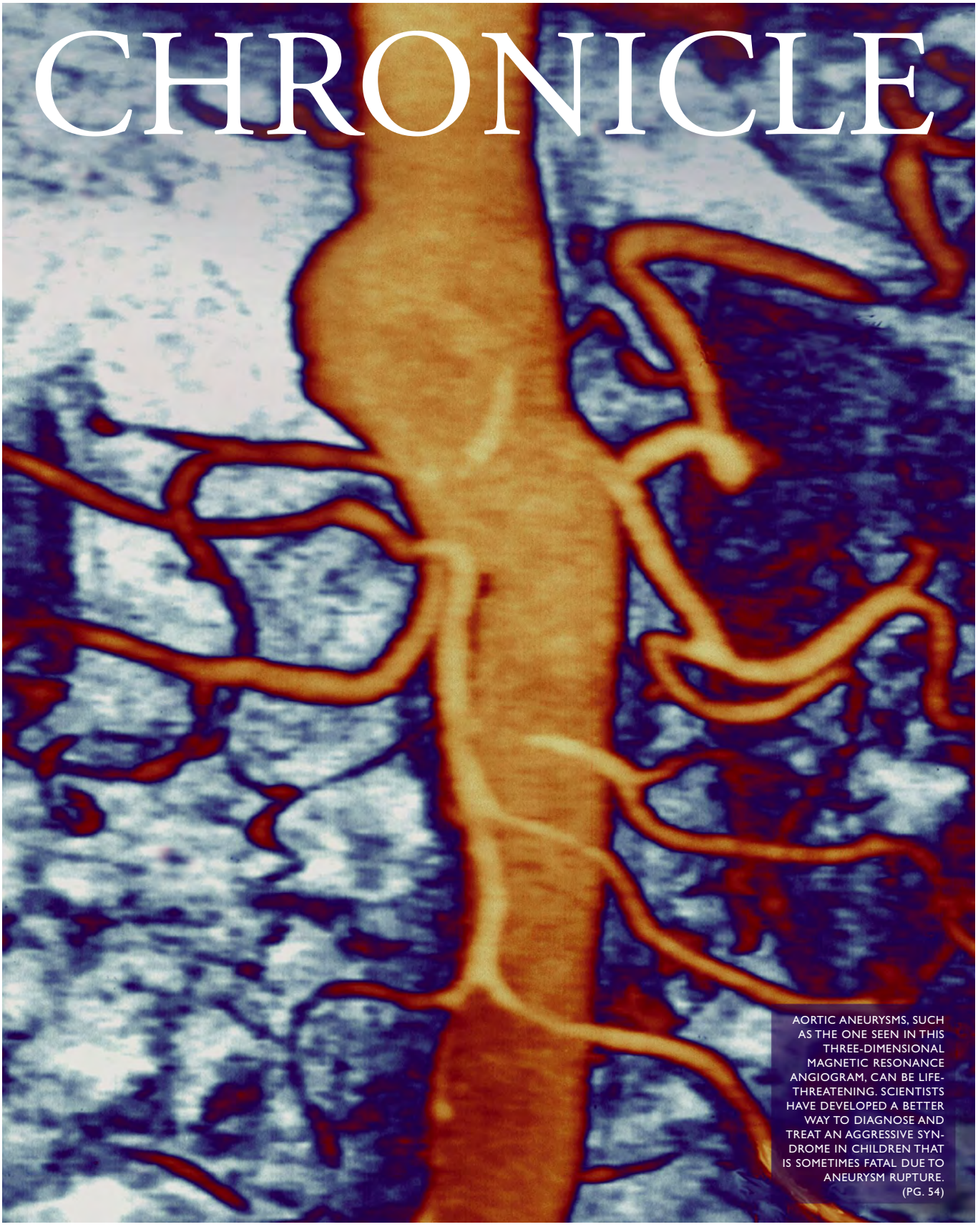
DAPHNE PREUSS
PROFESSOR OF MOLECULAR GENETICS AND CELL BIOLOGY, UNIVERSITY OF CHICAGO

"Finance. To turn lab observations into treatments for people, we have to launch clinical trials and develop products. Researchers often license results to biotech companies, which then license work to pharmaceutical companies. But many of the individuals involved along the way are less interested than are scientists in actually getting this work to the public. So we ourselves must be business savvy. Classes in accounting or economics, for instance, would help us better understand how to translate research results into direct benefits for human beings."

MORE INFORMATION AT HHMI ONLINE
www.hhmi.org

DULAC: KAY CHERNUSH. *LEFKOWITZ:* SCOTT DINGMAN. *O'CONNOR:* MARY JANE SHIMMEL. *PREUSS:* MARK SEGAL

CHRONICLE

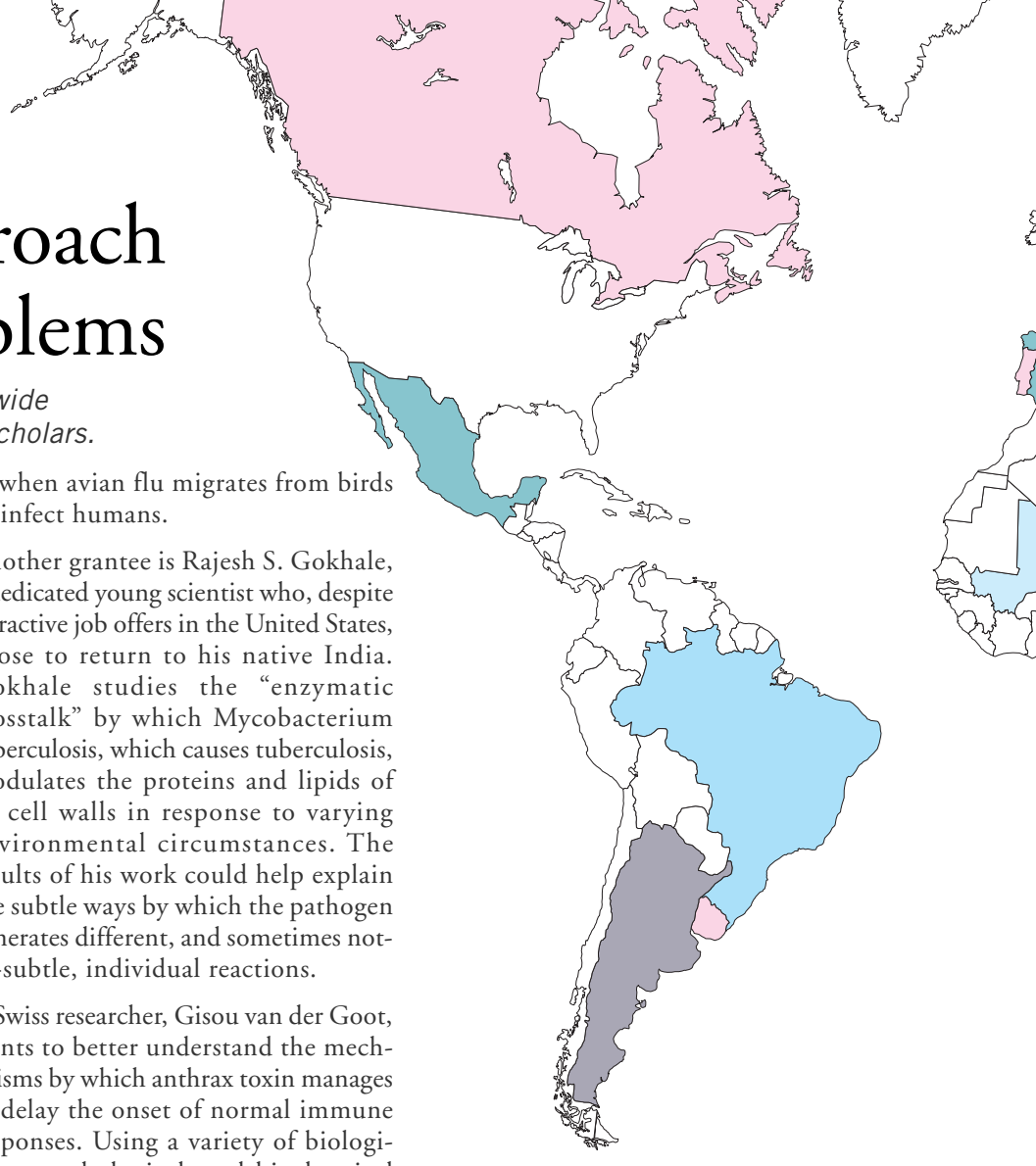


AORTIC ANEURYSMS, SUCH AS THE ONE SEEN IN THIS THREE-DIMENSIONAL MAGNETIC RESONANCE ANGIOGRAM, CAN BE LIFE-THREATENING. SCIENTISTS HAVE DEVELOPED A BETTER WAY TO DIAGNOSE AND TREAT AN AGGRESSIVE SYNDROME IN CHILDREN THAT IS SOMETIMES FATAL DUE TO ANEURYSM RUPTURE. (PG. 54)

ISM / PHOTOTAKE

A Global Approach to Global Problems

Forty-two biomedical scientists worldwide named HHMI international research scholars.



FROM AVIAN FLU TO SARS, TODAY'S headlines regularly highlight new outbreaks of disease around the world. Add the persistent presence of malaria, cholera, tuberculosis, and countless other infectious and parasitic diseases, and the challenges to world health seem truly staggering.

To support researchers abroad who address such challenges, HHMI recently awarded $17.5 million to 42 outstanding scientists in 20 countries. Their research, which in diverse ways tackles the mysteries of the molecular and genetic mechanisms underlying infectious and parasitic disease, could have a great impact on public health around the world. It may lead, for example, to the identification of potential drug targets and the development of vaccines.

Among this distinguished group of HHMI international research scholars is Grant McFadden, a virologist at Canada's University of Western Ontario. His award will support the ongoing study of molecular mechanisms by which viruses disarm host immune systems and cross species barriers—such as when avian flu migrates from birds to infect humans.

Another grantee is Rajesh S. Gokhale, a dedicated young scientist who, despite attractive job offers in the United States, chose to return to his native India. Gokhale studies the "enzymatic crosstalk" by which Mycobacterium tuberculosis, which causes tuberculosis, modulates the proteins and lipids of its cell walls in response to varying environmental circumstances. The results of his work could help explain the subtle ways by which the pathogen generates different, and sometimes not-so-subtle, individual reactions.

A Swiss researcher, Gisou van der Goot, wants to better understand the mechanisms by which anthrax toxin manages to delay the onset of normal immune responses. Using a variety of biological, morphological, and biochemical techniques, including an RNAi screen, she analyzes the molecular systems that govern the delivery and presentation of the toxin and its enzymes in the cell.

Nearly 500 scientists in 62 countries applied for the 5-year awards, from which HHMI selected its 42 winners. They hail from Argentina, Australia, Brazil, Canada, China, Denmark, France, Germany, Hungary, India, Israel, Mali, Mexico, Portugal, Russia, South Africa, Spain, Switzerland, Thailand, and Uruguay. Each researcher will receive $350,000 to $500,000 over 5 years.

Half of these awards are for first-time HHMI grant recipients, and the other 21 are current HHMI international research scholars. Since 1991, HHMI has awarded more than $100 million to support scientists in 32 countries.

These awards underscore the "international scope of science," says Peter J. Bruns, HHMI vice president for grants and special programs. Moreover, "the Institute's support of these scientific leaders in their home countries encourages those nations' most creative researchers, strengthens their research environments, and provides vital educational opportunities for aspiring scientists."

HHMI SCIENTISTS: $57 MILLION TO IMPROVE WORLD HEALTH

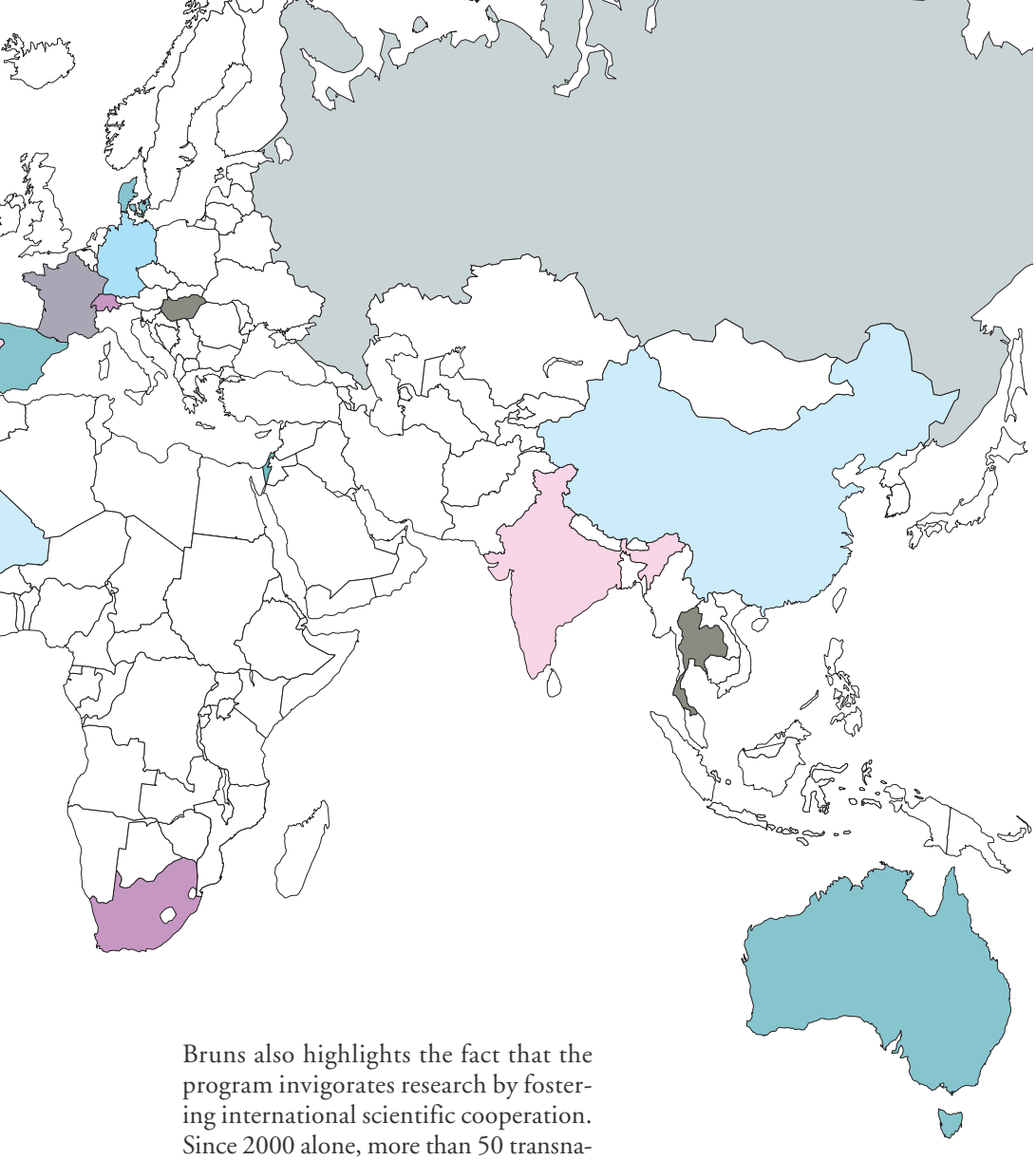
Three HHMI investigators and two HHMI international research scholars at universities in the United States, Canada, and the United Kingdom will lead projects that have been offered grants—aimed at creating effective health tools in developing countries—totaling $57 million.

The grants are part of an international effort launched in 2003 by the Bill & Melinda Gates Foundation in partnership with the National Institutes of Health. This initiative focuses on 14 main scientific and technological challenges that, if met, could have a profound impact on improving health in the world's poorest countries. Key goals are to devise new ways to test the safety of potential vaccines, better understand how the body naturally fights infection, and incapacitate disease-carrying insects.

Among the HHMI awardees is Richard A. Flavell, an HHMI investigator at Yale University. He and colleagues have been offered $17 million to develop laboratory mice whose immune systems are similar enough to humans to allow testing of human vaccines. George M. Shaw, an HHMI investigator at the University of Alabama at Birmingham School of Medicine, will lead a team offered $16.3 million to study how the immune systems of patients with HIV change as they are infected by and respond to the virus, as well as corresponding changes in the virus itself. HHMI investigator Richard Axel and two HHMI international research scholars, B. Brett Finlay and Adrian Vivian Hill, also received support.

FOR MORE INFORMATION

Including brief descriptions of the HHMI scientists' projects, visit www.hhmi.org/news/062805.html.



Bruns also highlights the fact that the program invigorates research by fostering international scientific cooperation. Since 2000 alone, more than 50 transnational collaborations have sprung from HHMI meetings of international research scholars and HHMI investigators.

Two HHMI-supported scientists, for example, one from the United States and the other from Argentina, joined forces to identify two unique protein-forming features of *Trypanosoma cruzi*, the parasite that causes Chagas disease. Parasitologist Mariano Levin, who recently completed a term as HHMI international research scholar, works at the Institute for Research on Genetic Engineering and Molecular Biology in Buenos Aires. Joachim Frank is an HHMI investigator at Health Research Inc., at the Wadsworth Center in Albany, New York.

Levin told Frank of his work on Chagas and his need to know more about the structure of the parasite's ribosome (the site of protein synthesis in the cell). Frank, it turns out, was a pioneer in the analysis of ribosome structures. The partnership formed that day resulted in the solving of *T. cruzi's* unusual ribosome structure. This research was published July 19, 2005, in the *Proceedings of the National Academy of Sciences*.

In a separate initiative sponsored by HHMI's international program, the Institute plans to announce later this year a new round of grants for biomedical research scientists working in the Baltics, Central and Eastern Europe, Russia, and Ukraine. The Institute is also conducting a competition at present that will award 5-year grants to promising Canadian and Latin American scientists who are doing basic biomedical research. ■

FOR MORE INFORMATION

www.hhmi.org/grants/office/international/

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* Indicates renewed support for a previous HHMI international research scholar

PETER SIBBALD

RIGHT _ "THE INTERACTIONS WE'RE DISCOVERING AMONG YEAST GENES THAT ARE CONSERVED IN HUMANS," CHARLES BOONE SAYS, "MAY VERY WELL BE IMPORTANT FOR HUMAN DISEASE."

Uncovering Genetic Combinations

Researchers systematically identify critical gene interactions in yeast.

MOST TRAITS THAT ARE INHERITED, INCLUDING THOSE THAT predispose individuals to certain diseases, are conferred not by single genes but by combinations of them.

That's both good news and bad news, says Charles Boone, an HHMI international research scholar at the University of Toronto. The good news is that genomes have a remarkable capacity to buffer themselves against potential harm from mutations. "Biological systems have evolved to be robust," he explains. "They can withstand all sorts of environmental and genetic perturbations." Most higher organisms have multiple sets of genes performing similar duties, "like backup systems."

The bad news is that all this genetic redundancy presents a challenge to disease detectives. The more genes that contribute to a particular condition, the more avenues researchers must follow to find the responsible genes and possible treatments.

To help meet that challenge, Boone and colleagues turned to baker's yeast, a single-celled fungus containing some 6,000 genes, roughly a quarter of the estimated number in humans. "A large fraction of yeast genes are 'conserved,' meaning that they have structurally and functionally related counterparts in higher organisms," says Boone.

Boone's team devised a method, called synthetic genetic array, or SGA, analysis, to systematically identify gene interactions in yeast. (The study was published in *Science* in 2001.) The approach works by uncovering genetic redundancy.

Boone notes that almost 5,000 of the yeast genes are "nonessential," the functions of most of them being covered by backup genes. If any of those genes are damaged or wiped out by a mutation, the yeast cell normally can function fine. But geneticists have observed that if they combine pairs of mutations, some rare combinations—those involving genes that normally cover for each other—cause the cells to die. They call that phenomenon "synthetic lethality."

Boone and his former graduate student, Amy Hin Yan Tong, teamed with an international group of collaborators in an effort to map every such genetic interaction in yeast. Their approach was to generate double mutants that represent each pairwise combination of all the nonessential genes and then catalog which pairs are lethal.

To combine two mutations in yeast, geneticists first have to cross two strains, each having a single mutation. Boone's team rigged robots to handle all the crosses, thousands at a time.

"There's a huge number of synthetic-lethal interactions," Boone reports after analyzing only 4 percent of all the possible gene combinations. (The analysis was published last year in Science.) Interactions occur predominantly between genes involved in the same general process, such as DNA replication, chromosome segregation, or secretion. However, Boone says, "some incredibly interesting interactions are those that illuminate connections between different processes, such as interactions that link chromosome architecture to cell morphology."

"Looking at the double mutants really sorts things out, identifying pathways and complexes that cooperate to drive essential cellular functions" Boone says. "So now, instead of just gathering collections of genes, we're actually drawing the scaffold, or wiring plan, of the organism."

Yeast geneticist and Nobel laureate Lee Hartwell says of Boone's research, "Many approaches to interactions measure physical interactions that may or may not be functionally important. The significance of this work is that it is identifying functionally important interactions and has the capacity to do so in a genome-wide manner."

Of course, one of the goals of yeast research is a better understanding of human biology. "We hope to map these networks in yeast, and then, using different technologies, to map relevant parts of them in higher organisms like worms, flies, and mice," Boone explains. And because of the strong kinship between these organisms' genomes, Boone says that mapping their gene networks may paint a picture of similar networks at work in humans. ■

~Paul Muhlrad~

Undergrad Helps Find Possible West Nile Cure

Dogged research—and long hours in the lab—yields big insights.

"AT FIRST, IT WAS LIKE A WHOLE NEW world to me," says Christopher Doane, 21, of his work in Michael S. Diamond's lab. Awarded an HHMI undergraduate fellowship after his freshman year at Washington University in St. Louis, Doane was assigned to generate monoclonal antibodies against a specific protein of the West Nile virus (WNV).

The goal sounded difficult, and even veteran researchers attest to the complexity of the process. Diamond, who researches infectious diseases at Washington University School of Medicine, taught Doane how to create special antibody-producing cells, called hybridomas, to combat WNV—a mosquito-borne pathogen that can afflict humans with anything from mild flu symptoms to brain swelling and occasionally even death.

The laboratory process involves fusing tumor cells with the spleen cells of lab mice previously inoculated with WNV's E protein. If the fusion is done just right, the two cells create a hybridoma and secrete monoclonal antibodies capable of binding to the E protein, thereby neutralizing the virus. To increase the odds of success, a number of fusions are attempted at once in a multiwell plate; bright fluorescence within a well signals a successful binding.

During his first 5 months, Doane achieved little success. He kept at it into the next school year. Then, one day near Thanksgiving, his luck changed.

"The whole well plate lit up," says Doane. Preliminary results suggested he had generated 30 different hydridomas, each secreting antibody against WNV E protein. Doing further screening, Doane and his colleagues observed that one antibody in particular, named E16, had surprising binding ability—it neutralized 10 different strains of WNV, preventing the virus from infecting cells. Further testing showed that E16 prevented infected mice from dying, even when administered up to 5 days after exposure.

Quite an accomplishment for any scientist, much less an undergraduate. Doane had made an antibody that, as Diamond says, "may be a viable treatment option against WNV in humans."

"Potentially such antibodies could be used in the treatment or prophylaxis of disease caused by a specific virus," says Robert B. Tesh, who studies WNV and other viruses at the University of Texas Medical Branch at Galveston. "Since there is yet no vaccine against or treatment for West Nile virus infection, the Wash. U. antibodies are of special interest, but this technology could be used to produce antibodies against other viral diseases as well."

The research was published in the May issue of *Nature Medicine*, with Doane as the fourth author (among 14). The antibody has been licensed to Rockville, Maryland-based MacroGenics, Inc., which is now trying to bring it to clinical trials. Doane stands to collect royalties if the antibody is commercialized successfully. ■

-Doug Main-

BOB HOWER / QUADRANT PHOTOGRAPHY

Of his great grandfather, who helped found HHMI, Christopher Doane says, "I am just amazed a person could help so many people in his lifetime."

SCIENCE ACROSS GENERATIONS—AND ACROSS HHMI

COURTESY OF THE HHMI ARCHIVE

WEISKOTTEN

Christopher Doane's interest in medicine is due in no small part to the influence of his great grandfather, Herman Weiskotten, a nationally prominent physician and medical school administrator in the early 20th century. As fate would have it, Weiskotten was a consultant to Howard Hughes, and when HHMI was founded in 1953 he was named one of the initial four members of its advisory board. Two years later, Weiskotten followed HHMI to its new headquarters in Miami, where he served on the board of directors for the next 10 years, retiring in 1965. Weiskotten died in 1972, before Doane was born.

Though Doane never met his great grandfather, he keeps Weiskotten's picture in his bedroom and talks glowingly about him. He keeps two leather-bound volumes of thank-you notes Weiskotten received at retirement from the deans of major medical schools and medical leaders around the world.

Doane earned his HHMI fellowship without any special consideration due to his great grandfather. And he plans a career in dentistry rather than medicine. Regardless, he still appreciates the link his research represents with Weiskotten's considerable contributions to HHMI. "I am just amazed a person could do so much and help so many people in his lifetime," says Doane, rightfully pleased that circumstances have enabled him to follow a path that his great grandfather first helped define.—D.M.

Not Your Ordinary 'Toons

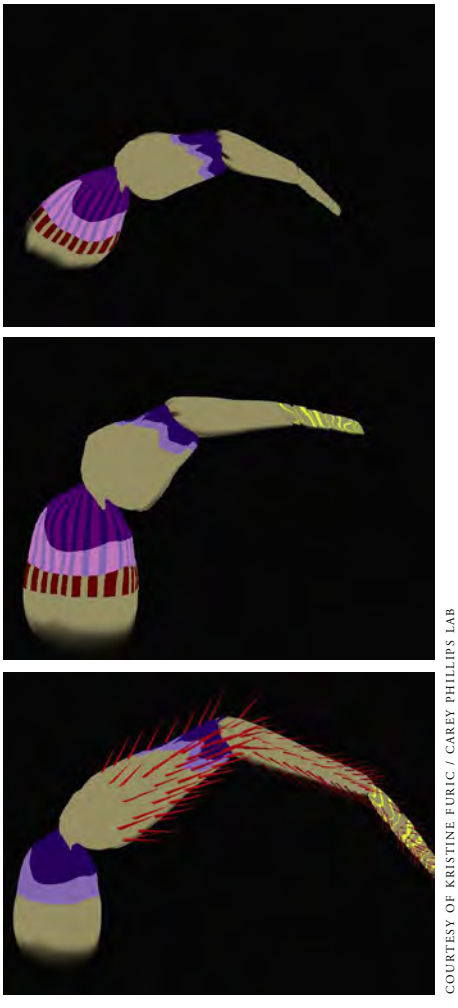
Developing interactive science animations, a scientist at Bowdoin shows that innovations in teaching can be used in research—instead of the other way around.

MIDWAY THROUGH THE ANIMATION, Carey R. Phillips points to his computer screen. "There," he says, as flecks of yellow appear at the tip of an insect's developing leg. "These areas show where genes for limb formation are being expressed at this point in time."

What started as a flat disc of single cells has divided, multiplied, and rearranged itself to create the likeness of a three-dimensional leg bud. As yellow splotches appear briefly, a second segment sprouts from the tip of the growing limb. And as the object telescopes outward to form an eerily lifelike, multisegmented leg, other colored patterns—red, blue, green—emerge and fade. The colorful stains illustrate carefully orchestrated patterns of gene expression, part of the process of ensuring that each segment of the limb develops properly. Students can rotate the developing model, fly through it, or even "meet" other students within the model and discuss it, all in real time.

This virtual reality model is just one of several interactive programs created by Phillips and his students at Bowdoin College that take viewers—via animation and virtual reality—through the insides of organs and cells, as a way to help them visualize biology in action. Based on real-world data that students have gathered during long hours peering through a microscope or from reviewing the scientific literature, the animations simulate complex biological processes such as cell division and cell signaling. The animations help students to visualize difficult-to-grasp scientific constructs.

But viewing these animations is no passive experience. With funding from HHMI, Phillips has been developing new ways to use virtual reality to create worlds in which students interact with the objects under study. In one virtual world, for example, students enhance the virtual model by "painting" new information on it, adding data gathered from their own research or from published findings. With such tools, Phillips says, students can readily experiment with modeled processes under various conditions and can view com-



COURTESY OF KRISTINE FURIC / CAREY PHILLIPS LAB

RIGHT _ ANIMATION CAN BE AN EFFECTIVE TOOL FOR VISUALIZING BIOLOGY IN ACTION. THIS SERIES OF STILLS, TAKEN FROM A STUDENT-GENERATED VIRTUAL REALITY MODEL, SHOWS A SNAPSHOT ALONG THE DEVELOPMENTAL TIMELINE OF AN INSECT'S LEG. BANDS OF COLOR MARK THE CHOREOGRAPHED CHANGES IN GENE EXPRESSION AS THE LEG DEVELOPS FROM A FLAT DISC OF CELLS TO A THREE-DIMENSIONAL LIMB.

HUMAN BRAIN PROJECT

DAVID MCLAIN

CAREY PHILLIPS

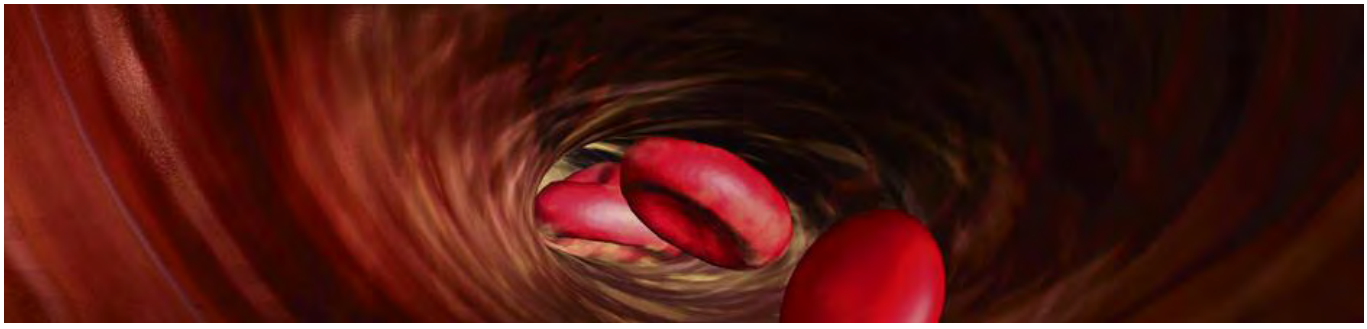
Carey Phillips is in the process of translating his animation techniques to a larger scale. This past year, Phillips and collaborators at the University of Tennessee and the University of Florida received a $4 million grant from the National Institutes of Health to create a visualization tool for the Human Brain Project. They plan to do magnetic-resonance-imaging scans of mama mouse throughout her pups' development, using motion sensor-type technology to track key points in their brains as they grow. The team will then apply the acquired data to build a three-dimensional model of the developing mouse brain, ultimately allowing viewers to observe its tissue layers as they grow. "We will then use the same technology we developed for teaching," Phillips says, "creating tools allowing researchers to log their gene expression data and interactively visualize any combinations of spatial/temporal data stored in the database."

"The NIH award demonstrates very directly," Phillips says, "how innovations in teaching can be used in research, instead of always thinking about it the other way around."

Ultimately, Phillips plans to create a framework that would allow researchers to contribute new findings to the model of how genes, proteins, and other molecules interact in the developing brain. "We'll set up an interface so that people from anywhere in the world can enter their data," he says. "Ideally, it could become a national archive as well as a research tool."—S.G.

FOR MORE INFORMATION

www.bowdoin.edu/news/archives/1bowdoincampus/001189.shtml



COURTESY OF CAREY PHILLIPS

ABOVE_ SEEING HOW TISSUES MOVE THROUGH SPACE AND TIME THROUGH VIRTUAL REALITY MODELS AND ANIMATIONS GIVES STUDENTS A BETTER GRASP OF HOW THE BODY DEVELOPS. EVEN IN THIS CAPTURED FRAME, THE RED BLOOD CELLS APPEAR POISED TO TUMBLE THROUGH A VESSEL.

plex sets of databases in ways that even scientists previously couldn't do.

Phillips discovered a new use for art while doing graduate work in embryology. In a study of RNA in developing eggs, he struggled to understand how the concentrations changed over time. "The concept was difficult for me to grasp, and then, one day, I suddenly got it. By visualizing the development in three dimensions, and then adding a fourth dimension for time, I could see exactly what was happening in my mind's eye from any perspective in the developing embryo."

Phillips built on this revelation when he began teaching embryology and tissue development at Bowdoin. He used animation to help students better understand how tissues move through space and time, not only to change shape but to position themselves to signal each other. Otherwise, "students had a difficult time visualizing that," he says.

Always innovating, Phillips now has a grant from the National Endowment for the Arts to build virtual environments that allow students from any location to create online virtual reality models of Zen gardens, enabling others to log on and experience the spaces. ■

-Susan Gaidos-

Medical and Dental Students Win HHMI Research Awards

MORE THAN 100 STUDENTS FROM 40 MEDICAL SCHOOLS AND ONE DENTAL SCHOOL across the United States will spend their next academic year learning what it's like to do medical research. They are recipients of approximately $3.7 million in new research fellowships and awards from HHMI.

The Institute runs two programs designed to interest medical and dental students in becoming physician-scientists. Both programs enable the students to take a year off from medical school to do mentored research.

This year, 66 medical students received HHMI research training fellowships, which they will use at medical research centers nationwide. Another 42 medical and dental students were accepted as HHMI–National Institutes of Health (NIH) research scholars. They will live and work on the campus of NIH in Bethesda, Maryland, where HHMI's research scholars program is based at a historic building informally known as the Cloister.

"Physician-scientists are essential to the advancement of medical research," says William R. Galey, graduate science education program director at HHMI. "Such individuals are aware of the pressing problems in medicine, so they are in a unique position to help translate new basic science discoveries into treatments for disease."

Since HHMI's research programs for medical students began in 1985, the Institute has supported research training for nearly 1,000 medical fellows and more than 800 research scholars. ■

38%

WOMEN COMPRISE 38 PERCENT OF THE RESEARCH SCHOLARS AND 35 PERCENT OF THOSE RECEIVING FELLOWSHIPS.

24%

MINORITIES UNDERREPRESENTED IN THE SCIENCES ACCOUNT FOR 24 PERCENT OF THE RESEARCH SCHOLARS AND 8 PERCENT OF THE FELLOWS. FIELDS OF RESEARCH INTEREST RANGE WIDELY, WITH A MAJORITY CHOOSING CELL OR MOLECULAR BIOLOGY, GENETICS, IMMUNOLOGY, AND NEUROSCIENCE.

FOR A LIST OF THIS YEAR'S FELLOWS AND RESEARCH AWARDEES

See www.hhmi.org/news/063005.html.

EXtreme Impact

Two HHMI initiatives help disadvantaged students build foundations for careers in science.

DEANNA COCHRAN, 22, IS ONLY THE third person in her large family to go to college. A biology major at Spelman College in Atlanta, Cochran brought a great deal of enthusiasm but limited laboratory experience when she traveled to Philadelphia to spend the summer of 2004 working in HHMI investigator Amita Sehgal's lab at the University of Pennsylvania.

"She came in not knowing a lot about lab work," Sehgal recalls. "She had not done any research. But that's OK," says Sehgal. "Motivation is much more important. If you really want to diversify the scientific workforce, you need to reach out to students who haven't had research opportunities."

After 10 weeks in Sehgal's lab as a participant in HHMI's Exceptional Research Opportunities Program (EXROP), Cochran knew how to design an experiment and how to collect data, analyze it, and draw conclusions. She decided that her future lay in science. And she wanted to spend another summer learning more about clinical and translational research, which she did by returning to Sehgal's lab in 2005.

EXROP pairs undergraduates with HHMI investigators and HHMI professors (a group of scientists who received $1 million each from HHMI to make science more engaging for undergraduates). The summer research program is designed to encourage disadvantaged students, including minorities underrepresented in the sciences, to consider careers in science by involving them in research in some of the top labs in the nation. Students are selected by HHMI professors and directors of HHMI-funded undergraduate science education programs.

Over the past three summers, EXROP has matched 141 undergraduates with 115 HHMI scientists. The students included 52 African Americans, 34 of Hispanic origin, 2 Native Americans, and 36 others of non-Caucasian or multiethnic background. Nearly 80 percent of them now say they plan to study for a Ph.D. or an M.D.-Ph.D. when they finish their bachelor's degrees, and another 13 percent want to go to medical school.

"The EX in EXROP could stand for the EXtreme impact on science that we are aiming to achieve," says HHMI President Thomas R. Cech.

> "If you really want to diversify the scientific workforce, you need to reach out to students who haven't had research opportunities."
>
> AMITA SEHGAL

PAUL FETTERS

RIGHT _ SOME OF THE STUDENTS IN EXROP WHO MET IN MAY AT HHMI'S HEADQUARTERS TO SHARE IDEAS AND EXPERIENCES.

GILLIAM FELLOWSHIPS

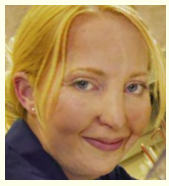

BABAR


BYNOE


LEÓN


RED EAGLE


REZENDE


VAN PROOYEN

BABAR: AMENAH BABAR. BYNOE AND LEÓN: KATHLEEN DOOHER. RED EAGLE: BARBARA RIES. REZENDE: MARC BRYAN-BROWN. VAN PROOYEN: TOM KOCHEL. GILLIAMS AND RED EAGLE: PAUL FETTERS

This past May, HHMI announced the recipients of its first Gilliam Fellowships, named in honor of the late James H. Gilliam, Jr., a charter Trustee of HHMI who spent a lifetime fostering diversity and opportunity in education and science. The fellowships provide support for Ph.D. studies in the life sciences to disadvantaged students, including underrepresented minorities, who participated in HHMI's Exceptional Research Opportunities (EXROP) undergraduate summer research program. These six students received the first Gilliam Fellowships:

IMRAN BABAR is a Native American/Asian who earned a degree in biology at Carleton College in Northfield, Minnesota. He did his EXROP research with HHMI investigator Tyler Jacks at the Massachusetts Institute of Technology, working to discover the role of stem cells in lung tumor formation. He will carry out graduate study in molecular, cellular, and developmental biology at Yale University.

MEISHA BYNOE was born and raised in the West Indies and earned a bachelor's degree in biology and music at MIT. She conducted research in the lab of HHMI investigator Richard Locksley at the University of California, San Francisco, where she helped develop assays to identify certain macrophages or immune system cells. She entered Yale University's graduate program in microbiology this fall.

LUIS LEÓN, who is Hispanic, supported himself while earning a bachelor's degree in biochemistry at the University of Washington. He did EXROP research in HHMI investigator Robert Siliciano's lab at the Johns Hopkins University School of Medicine, where he investigated how immune cells transcribe the HIV-1 virus, which causes AIDS, during the virus's asymptomatic latent phase. He has completed his first year of graduate studies in immunology at Harvard University.

ALEXANDER RED EAGLE, a Native American, graduated from the University of California, Los Angeles, with a bachelor's degree in biochemistry. He is currently a medical student in Stanford University's Medical Scientist Training Program and will defer his Gilliam Fellowship until 2006, when he enters the Ph.D. part of his training. He conducted EXROP research in the Yale University lab of HHMI investigator Arthur Horwich, studying a protein that, when misfolded, can lead to congestive heart failure or neurodegenerative disorders.

NAIRA REZENDE, who earned a bachelor's degree in biology from Hunter College, is from Belo Horizonte, Brazil. She did summer research in HHMI investigator David Schatz's lab at Yale University School of Medicine, where she worked to inhibit or overexpress DNA repair genes involved in developing immune system memory. This fall, she begins study for a Ph.D. in biochemistry, cell, and molecular biology at the Joan and Sanford I. Weill Medical College of Cornell University.

NANCY VAN PROOYEN, who grew up in Arkansas and Colorado, earned a bachelor's degree in biochemistry and molecular biology at Reed College in Portland, Oregon. She performed her EXROP research in the lab of HHMI investigator and Nobel laureate Eric Kandel at Columbia University College of Physicians and Surgeons, where she studied kinesin, a molecular motor protein. Van Prooyen has completed her first year as a graduate student at the Johns Hopkins University.

JAMES GILLIAM, SR. (LEFT) AND LINDA J. GILLIAM SHARE A LIGHT MOMENT WITH GILLIAM FELLOW ALEXANDER RED EAGLE.

INVESTING IN THEIR BRAINS

Growing up in Mount Morris, Michigan, a city 45 minutes north of Detroit where nearly everybody works for General Motors, Cochran never even thought about being a scientist. "It's hard to want to be something you've never seen," she explains. "I had no idea until I got to Spelman that there were black people in every walk of life, including black women in math and science."

It wasn't easy for Cochran to attend Spelman. Scholarships saw her through her freshman year, and student loans financed her sophomore year. As a junior, she moved off campus, sharing an apartment with a friend because it cost too much to live in the dormitories.

Now Cochran's younger sister attends Spelman too. Their mother has always been determined that these two young women get an excellent college education. "She says her house and her BMW are invested in our brains," Cochran remarks with a grin.

Deanna Cochran spent her first EXROP summer studying how caffeine affects the sleeping behavior of *Drosophila*. Comparing the sleep cycles of fruit flies that had been fed various amounts of caffeine with those of a caffeine-free control group, she gathered data on disruption of the normal sleep cycle and how the flies returned to normal.

"*Drosophila* are great model organisms for sleep research," she says, "and studies of sleep in flies have already been used to dispel many myths about what happens during sleep. Examining how chemicals can affect the normal sleep cycle, for instance, can help us understand the tasks that the brain performs while in its resting state."

As valuable as the EXROP learning experience is to the undergrad, it is a

two-way street. "We learned a lot from each other because we think entirely differently," says Karen Ho, a postdoc in Sehgal's lab who mentored Cochran. "She thinks physiologically. I think molecularly. So we read the same paper and get entirely different things out of it."

Cochran agrees. "Now I can see the relevance of all that boring molecular biology," she says with an infectious grin.

"Deanna is fantastic," Ho continues. "She wonders about everything. She's made me wonder again too."

PAUL FETTERS

ABOVE _ THIRTY-TWO ADDITIONAL EXROP PARTICIPANTS—SCIENTISTS IN THE MAKING—DURING A MEETING AT HHMI HEADQUARTERS THIS PAST MAY.

MENTORS' LEGACIES

Antonio Perez was another EXROP student. He spent the summer between his sophomore and junior years at Harvard University, working in HHMI investigator Louis M. Kunkel's Harvard lab. There he studied the potential of a specialized group of cells, called muscle side population (SP) cells, to play a role in muscular-dystrophy therapy.

Kunkel, who explores the molecular and genetic basis of human neuromuscular diseases, discovered the mutation that causes Duchenne's muscular dystrophy. Kunkel is also strongly committed to engaging young people, having mentored more than 50 undergraduates over the years. "What we're doing here is training the next generation of scientists," he says of the EXROP program. "My legacy will be the people I've trained."

> "What we're doing here is training the next generation of scientists."
>
> LOUIS KUNKEL

For Perez, a Hispanic student majoring in science and history, that summer was such an exceptional research opportunity that it turned him into a fixture in Kunkel's lab for the rest of his undergraduate years. It also formed the basis of a paper published in the Proceedings of the *National Academy of Sciences*, on which he was second author, and of his senior thesis.

Perez continued working in Kunkel's lab throughout the academic year after his EXROP experience, and during the following summer he mentored a new EXROP student there. By then, Perez had turned his attention to bone marrow cells, working to see if he could cajole them into engrafting into and repairing damaged muscle cells.

Perez, who started Harvard Medical School this fall, has his eye on a career in medical research. He says he discovered the role that teaching plays in research by mentoring as well as being mentored, and he has already learned what every scientist knows—that "science isn't a 9 to 5 job. You're thinking about it all the time."

ONWARD AND UPWARD

Alexander Red Eagle, a Native American from California, spent his EXROP summer studying protein folding in the lab of HHMI investigator Arthur L. Horwich at Yale University School of Medicine. He focused on a protein that, when misfolded, can lead to congestive heart failure or neurodegenerative disorders. "My HHMI experience was a real confidence booster," Red Eagle recalls. "I learned to tackle big ideas by breaking projects down into one question at a time."

Red Eagle's exceptional research opportunity didn't end with EXROP. He was one of the first EXROP alumni to receive a Gilliam Fellowship for Advanced Study from HHMI.

Starting this fall, the Institute will award up to five Gilliam Fellowships annually to outstanding EXROP students who want to pursue Ph.D.s in the biological sciences. The fellowships, which pay for up to 5 years of graduate school, are named for the late James H. Gilliam, Jr., a charter member of HHMI's Board of Trustees. They honor his commitment to fostering diversity in the scientific community.

Red Eagle is now completing the medical school part of an M.D.-Ph.D. program in genetics at Stanford University. He deferred his Gilliam Fellowship until he starts the Ph.D. portion of his studies in 2006. ■

-Jennifer Boeth Donovan-

Boosting Science Education, One Step at a Time

Santiago Fund helps provide resources and inspiration at a Philippines high school.

MARIA ZELDA ILASIN-NICANOR, WHO teaches biology and chemistry at Nueva Ecija High School in the Philippines, has become the first Nestor V. Santiago–HHMI Teacher of Science. Voted the school's outstanding science teacher of 2004, Ilasin-Nicanor has taught there since 1985.

The Santiago Fund was established in 2000 by Nestor V. Santiago and his siblings to improve science education at Nueva Ecija, the high school from which he graduated. Santiago, who served as HHMI's vice president and chief financial officer, died in 2003. In his honor, Trustees, officers, and staff of the Institute as well as colleagues outside HHMI contributed $107,060 to the fund to help endow the salary of a highly qualified science teacher at the school, which is located in Cabanatuan City, some 70 miles north of Manila.

A graduate of Nueva Ecija herself, Ilasin-Nicanor went on to earn two masters' degrees, one in biology education and the other in science and mathematics education. She has been a demonstration teacher of biology and served as trainer/coach of a student science project that took first place in the 2002 regional Intel-Philippines Science Fair. In 2000 she received a certificate of recognition from her congressional district for outstanding improvisation of secondary school science equipment and devices.

COURTESY NUEVA ECIJA HIGH SCHOOL

ABOVE _ MARIA ZELDA ILASIN-NICANOR, AT RIGHT, ACCEPTS A CERTIFICATE NAMING HER THE FIRST NESTOR V. SANTIAGO-HHMI TEACHER OF SCIENCE, AT A CEREMONY AT NUEVA ECIJA HIGH SCHOOL IN THE PHILIPPINES.

But Ilasin-Nicanor is most proud of her impact on students. "As a teacher, I feel that the greatest rewards are not certificates of merit recognizing my achievements," she said at a ceremony in April 2005 when she was named the Nestor V. Santiago–HHMI Teacher of Science. "Rather, they are the moments when students thank me for being part of their lives and serving as an inspiration to them."

Despite such successes, things could be a lot better at Nueva Ecija. Crowded conditions, shortages of resources, and a resulting decline in the quality of education at the school have increasingly prompted affluent students and well-qualified teachers to seek other institutions. One of the Santiago Fund's major aims, then, is to help encourage good teachers to stay and provide educational opportunities for the school's talented but needy students.

For example, the fund already has enabled Nueva Ecija to set up a chemistry laboratory, says Ilasin-Nicanor. And while this is merely one step in the right direction—biology, physics, and earth-science labs are still needed—it is making a difference.

Teodoro V. Santiago, Nestor's brother, who is chief of the division of pulmonary and critical care medicine at the Robert Wood Johnson Medical School, visited Nueva Ecija High School last November. He found that "The boost in morale of the teachers and students was very palpable, just knowing that someone cares." ■

-Jennifer Boeth Donovan-

Modern-Day Bioethical Issues: The DVD

AS A RESOURCE TO HELP INTRODUCE AND TEACH BIOETHICS, HHMI has produced *Ethics in Biomedical Research*—a DVD that explores some of modern-day science's most profound ethical issues, competing moral arguments, and the conflicts they engender. First released last summer as a beta-test version to HHMI investigators and others, the new version offers revisions of the original three videos—*Animal Studies, Genetic Alteration, and Scientific Integrity*—in response to user comments and suggestions. An overview, which briefly reviews the history of bioethics in research and clinical medicine, was added as well.

The DVD's "Companion Resources" section provides succinct summaries of each video, along with PDF files of important documents cited in the overview. To aid deeper exploration into bioethics, the section also directs viewers to a newly created Web site (www.hhmi.org/bioethics) that offers case studies, discussion questions, Web links to other bioethics resources, and bibliographies. ■

THE DVD IS AVAILABLE FREE OF CHARGE

See HHMI's online catalog at www.hhmi.org

Protein Disposal: Gumming Up the Works

Researchers discover a class of molecules that can prevent proteins from being degraded.

FOR A CELL, DESTROYING PROTEINS IS AS ESSENTIAL AS building them, says Rati Verma, a researcher in the Caltech laboratory of HHMI investigator Raymond J. Deshaies.

The job of mincing proteins is performed by enzymatic machines called proteasomes. "Proteasomes affect almost all biological processes in the cell," Verma says. By their deliberate destruction of regulatory proteins, they orchestrate activities from cell division to cell death.

Ubiquitin is the protein that hands down the death sentences. "Ubiquitin is the most highly conserved protein in eukaryotes [organisms whose cells contain a distinct nucleus]," Verma says, "with only three amino acid differences between yeast and mammals." A chain of ubiquitins gets attached to doomed proteins, marking them for destruction and ushering them to the gates of proteasomes.

Last year, the Caltech researchers and colleagues discovered a class of small molecules that can block proteasomes from degrading proteins. The finding could open new avenues for treating diseases.

Few researchers considered proteasomes likely candidates as drug targets. Because proteasomes control so many processes, most researchers thought that perturbing them with drugs could only wreck havoc with the body, Deshaies says. "Even I initially felt that by inhibiting the proteasome you would just kill everything."

Then, 2 years ago a proteasome inhibitor called bortezomib received quick approval for treating multiple myeloma. "Everyone in the proteasome field started paying attention once the drug was fast-tracked by the FDA," Verma says.

BELOW _ RATI VERMA (LEFT) AND RAYMOND DESHAIES FOUND A NEW WAY TO THINK ABOUT HOW A CELL DESTROYS PROTEINS.

JOE TORENO

Randall W. King, Deshaies' colleague at Harvard University, initiated the hunt for inhibitors by screening close to 110,000 different small molecules for their ability to prevent proteasomes from destroying proteins. Three of the compounds showed promise as proteasome inhibitors.

"What was different about what Randy did," says Deshaies, "is that the target was not defined at the outset of the screen. The 'target' was an entire system—a large group of proteins." That left open the possibility that compounds King identified blocked protein degradation at any of a number of steps. It was up to Verma and Deshaies to determine which one.

They analyzed King's compounds further using purified proteasomes and ubiquitin-tagged proteins. Verma and Deshaies systematically narrowed down the possible steps of the protein death march that the inhibitors were blocking. Their first experiments eliminated the two most obvious enzymatic possibilities: removal of the ubiquitin chain and degradation in the core of the proteasome.

The final answer was a surprise. Verma discovered that the inhibitors coated the ubiquitin chain, rendering it unrecognizable to the proteasome's ubiquitin receptors, thereby halting protein destruction before it could even begin.

Small molecule inhibitors rarely work that way, she notes. Most, like bortezomib, clog the tight spaces at the reactive cores of enzymes. But a small molecule disrupting the broad surface contacts between proteins is like the fairy-tale pea poking the princess through a stack of mattresses.

Deshaies cautions that the compounds (which they named ubistatins) are still unsuitable as drugs. In fact, some of their chemical properties suggest that they may never enter the pharmaceutical pipeline. Instead, he says, "our research offers a proof in principle that the ubiquitin chain-receptor interaction is an Achilles' heel of the ubiquitin-proteasome system that is potentially inhibitable by small molecules." With that established, he says, pharmaceutical researchers might be able to accurately measure the binding of purified ubiquitin chains and ubiquitin chain receptor and then "screen a library of half a million compounds to find one that inhibits that binding."

Such a compound could treat diseases besides cancer, he says. Proteasomes play a key role in regulating inflammation, and proteasome inhibitors might have potential in treating inflammatory conditions, such as rheumatoid arthritis, or as anti-tissue rejection drugs for transplant patients. ■

-Paul Muhlrad-

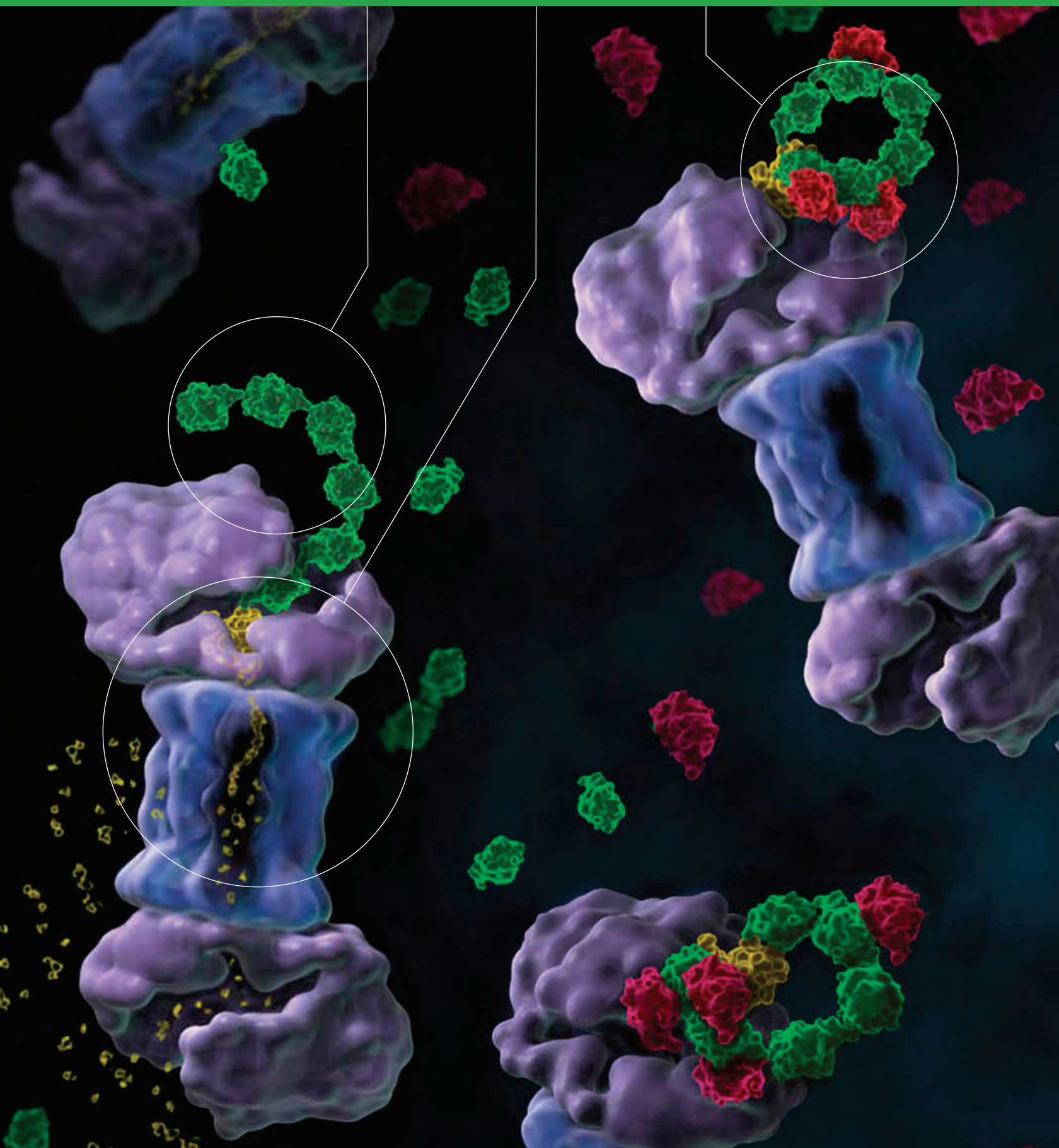
DEGRADING PROTEINS

In this artist's conception, by medical illustrator Graham Johnson, a proteasome (lower left) degrades a protein.

1. A chain of ubiquitin molecules (green) ushers the protein (yellow) to the entryway of the proteasome (lavender).

2. The proteasome then unfolds the protein and feeds the amino acid chain into its digestive core (blue) where it degrades the protein into amino acids.

3. Ubistatin molecules (red) can bind ubiquitin chains, blocking them and their attached proteins from entering the proteasome.

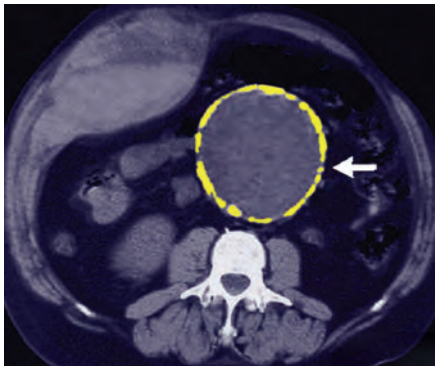


Childhood Threat Now Recognizable

A NEWLY DEFINED, POTENTIALLY FATAL condition is now easier to diagnose and treat because of the work of HHMI investigator Harry C. Dietz and his colleague Bart L. Loeys at the Johns Hopkins University School of Medicine. By connecting the physical and genetic dots, they drew a clear picture of an aggressive syndrome that can cause death in early childhood from aneurysm rupture.

The researchers studied 10 families with a history of aortic aneurysm who had certain physical characteristics: wide-set eyes, cleft palate or split uvula (the soft tissue that hangs at the back of the throat), and a twisted arrangement of the body's blood vessels with rapid swelling of the aorta. There were additional findings reminiscent of those seen in a connective tissue disorder known as Marfan syndrome—but there were enough differences to make the researchers think they were dealing with a different condition.

Dietz and Loeys looked for mutations in the genes for the type I and type II receptors for transforming growth factor beta (TGFb). Signaling through these receptors controls the proliferation, movement, activity, and death of cells. TGFb and related molecules were targeted because work in the Dietz lab and elsewhere had demonstrated a link between altered TGFb activity and Marfan syndrome and because in animal models this signaling cascade has been shown to influence the development of many of the tissues altered in the newly described syndrome.



COMPUTED TOMOGRAPHY (CT) SCANS CAN HELP WITH EARLY IDENTIFICATION OF ANEURYSMS. IN THIS COLOR-ENHANCED CT SCAN OF AN ABDOMINAL AORTIC ANEURYSM, THE FRONT OF THE BODY IS FACING UP AND THE SPINE IS IN WHITE. AT CENTER IS A SEVERELY ENLARGED ARTERY (YELLOW).

The researchers found that mutations in either of two genes—TGF-b receptor 1 or TGF-b receptor 2—can cause the syndrome. These genes are the two halves of the receptor that binds TGFb. With colleagues from Belgium, Canada, Germany, and the United States, Dietz and Loeys published their findings in the March 2005 issue of Nature Genetics.

The syndrome, now called Loeys-Dietz syndrome (LDS), is different from Marfan syndrome and other forms of inherited aortic aneurysm in that it causes aneurysms in much smaller aortas. "For most conditions," Dietz says, "the aorta has to reach about 5 centimeters in diameter before rupture is likely. In this new aneurysm syndrome, many tears occur at smaller dimensions."

In addition, the aneurysms are not limited to the aorta. "We've seen or heard about many patients with aneurysms throughout the arterial tree, so we're very aggressive," Dietz says. "We do full-body CT scans to look for aneurysms." Prompt surgical repair of the aneurysm saves lives.

LDS is fairly common, says Dietz, although he says the syndrome is still too new for a solid estimate. Since defining LDS about a year and a half ago, the researchers have seen more than 40 families with the condition.■

-Cori Vanchieri-

GARO / PHOTO RESEARCHERS, INC.

IN BRIEF

UNDERSTANDING NATURAL KILLERS COULD LEAD TO NEW HEPATITIS TREATMENTS

Researchers have discovered that natural killer T (NKT) cells, the immune system's sentinels, patrol the blood vessels of the liver for invaders or signs of tissue damage and demonstrate a dogged behavior not seen before in other T cells. The new studies show that NKT cells crawl along vessel walls, even upstream against blood flow. They halt only when they receive a chemical signal to unleash an immune-system assault on invaders or damaged tissue.

"In general, these NKT cells could have an important inflammatory role, particularly in the case of chronic hepatitis," says Dan R. Littman, an HHMI investigator at New York University School of Medicine, who led the studies.

The findings offer a new way of thinking about this important class of immune cell, which is responsible for the inflammation and cell death in the liver due to hepatitis. Hepatitis can be a reaction to viruses, parasites such as malaria, or other infections. Learning to "call off" the NKT cells' attack could offer a treatment for hepatitis and associated complications.

The researchers published their findings online on April 5, 2005, in *Public Library of Science Biology*.

GENE KEEPS NEURAL CELLS ON CORRECT DEVELOPMENTAL PATH

Embryonic stem cells with identical genomes grow into distinctive tissues, such as heart, bone, and brain. At one time, scientists believed the differences among cell types arose from various sets of genes switched on inside developing cells. Then, studies showed that adult neurons lack a protein that permanently turns off neuronal genes in the rest of the body's cells.

Now, it turns out that precursor nerve cells contain that same repressive protein after all. In fact, the protein directs the complex gene network that transforms an embryonic stem cell into a mature nerve cell, say HHMI researchers. Their study, published in the May 20, 2005, issue of *Cell*, may be among the first to track a set of genes from stem cell to differentiated neuron.

Led by Nurit Ballas, a postdoctoral fellow in the lab of Gail Mandel, an HHMI investigator at the State University of New York at Stony Brook, the study may advance stem cell research aimed at understanding spinal cord injury repair or replacing defective brain cells in neurodegenerative diseases.

VIRUS USES TINY RNA TO EVADE THE IMMUNE SYSTEM

In the latest version of the hide-and-seek game between pathogens and the hosts they infect, researchers have found that a virus appears to cloak itself with a recently discovered gene-silencing device to evade detection and destruction by immune cells.

The report by HHMI researchers, published in the June 2, 2005, issue of *Nature*, may be the first to show how a virus uses the gene-silencing machinery for its own infectious purposes.

In people, plants, and worms, hundreds of tiny RNA molecules can silence specific genes by interfering with larger messenger RNAs (mRNAs). That interference prevents mRNAs from making proteins. Scientists do not know which genes are

Wiggle in a Worm's Snout

Researchers discover a neurotransmitter that controls important behaviors in the roundworm.

MUTANT WORMS WITH A CURIOUS NOSE wiggle recently helped researchers discover a new brain-signaling chemical. Mark J. Alkema, who headed the team from HHMI investigator H. Robert Horvitz's laboratory at the Massachusetts Institute of Technology, found that the compound, called tyramine, regulates several behaviors and body movements in the nematode *Caenorhabditis elegans*.

The scientists initially set out to study a signaling molecule in worms called octopamine, which is related to the mammalian stress hormone norepinephrine. Octopamine is made from tyramine. "We initially thought that tyramine was just a precursor" and lacked its own signaling function, says Alkema. But when he and his colleagues identified and deleted the gene responsible for making tyramine, the mutant worms displayed several behavioral quirks not observed in mutants lacking octopamine alone.

Among those quirks was an incessant nose wiggle. Normally, worms sweep their snouts from side to side as they forage for food. But when gently prodded—say, with an eyelash—they move backward and the nose wags stop. Alkema speculates that the suppression of these head movements may serve as a survival technique to avoid being trapped by fungal predators. Mutant worms that lacked tyramine continued to wiggle their snouts, however, even when retreating from an eyelash tickle. (This work was published in the April 21, 2005, issue of Neuron.)

Following up on this observation, the researchers discovered that a single neuron in worms, called the RIM neuron, contains tyramine but not octopamine. When they used a laser beam to remove the RIM neuron from healthy worms, the worms wiggled their noses when retreating from a touch, just like the tyramine-deficient mutants did. "That was the real proof that tyramine has a distinct role in *C. elegans* behavior," says Alkema.

"Tyramine-receptor proteins have been identified in several mammals, including humans," he adds. "However, the role of tyramine in human behavior is still unclear." ■

-Paul Muhlrad-

BIG PICTURE

Specific neurotransmitters—such as acetylcholine, glutamate, GABA, dopamine, serotonin, norepinephrine, and octopamine—are key signaling molecules for neurons in the brain and nervous system. Disruptions in neurotransmitter functions can trigger a multitude of neurological diseases and psychiatric disorders. Most drugs used to treat these disorders influence brain function by altering the levels or actions of specific neurotransmitters. Thus, the discovery of a new neurotransmitter is a substantial contribution.

hushed by the micro-RNAs in people, but the new study bolsters growing evidence that the little molecules can play important roles not only in normal human cells but in infected cells as well.

The work was led by HHMI investigator Don Ganem at the University of California, San Francisco.

THERAPEUTIC APPROACH FOR NEURODEGENERATIVE BRAIN DISORDERS

Researchers have found that an enzyme that helps cells dispose of unwanted proteins may actually protect against a class of inherited brain disorders that includes Huntington's disease. The researchers say their findings suggest that drugs or compounds that activate the protective protein, called ataxin-3, might be a possible therapy for neurodegenerative diseases caused by proteins whose sequences are abnormally long due to excessive repetition of the amino acid glutamine.

The research team, led by Nancy M. Bonini, an HHMI investigator at the University of Pennsylvania, published its findings in the April 1, 2005, issue of *Molecular Cell*.

NEW TECHNIQUE MAY LEAD TO GAINS AGAINST CERVICAL CANCER

Researchers may be on the verge of exploiting the vulnerabilities of a virus that causes cervical cancer, thanks to a newly developed technique that enables scientists to mass-produce human papillomavirus (HPV) in the laboratory.

HPV, which exists in more than 100 forms, is the most prevalent sexually transmitted infection and can also occur nonsexually. Although the infection is usually harmless, certain types of HPV are responsible for nearly all cases of cervical cancer, and other types contribute to about a quarter of head and neck cancers and some skin cancers.

Using the new technique, scientists can quickly produce a thousand times more infectious virus per culture dish than they could previously. Researchers are hopeful that this advance could lead to new antiviral drugs and to vaccines that would trigger the immune system to attack at an earlier stage in the HPV life cycle.

The researchers, led by HHMI investigator Paul Ahlquist at the University of Wisconsin–Madison, published their new technique on June 15, 2005, in the early, online edition of the *Proceedings of the National Academy of Sciences*.

LUNG CANCERS MAY BEGIN IN NEWLY DISCOVERED CELLS

The most common form of lung cancer may begin in a group of newly isolated lung stem cells, according to researchers led by Tyler Jacks, an HHMI investigator at the Massachusetts Institute of Technology.

Working with a mouse model, the researchers isolated a novel type of lung cell that can divide into fresh copies of itself and into the two more specialized kinds of cells deep in the lung. Their experiments show that, at the earliest stage of tumor development, the stem cells appear to be the first of the lung cells that respond to a cancer-causing mutation. The newly identified cell type fulfills all but one of the strictest criteria that scientists look for in defining adult stem cells.

"They may be the cells that we have to eliminate in cancer in order to obtain durable cures for the disease," says Jacks. "Along the way, we need to know how these cancer stem cells become different from normal stem cells."

Jumping Gene Provides Clues to Human Immune System

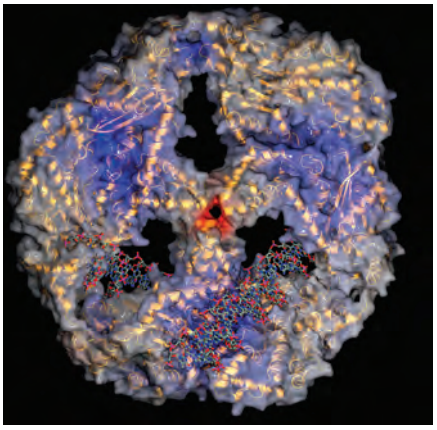
NANCY L. CRAIG HAS BEEN CHASING transposons—mobile DNA that can jump from one location in the genome to another—since the 1980s. One particular transposon, aptly named Hermes for the Greek god of travel and communications, led Craig, an HHMI investigator at the Johns Hopkins University School of Medicine, to a surprising discovery. She found the first clear evidence of an evolutionary link between this jumping gene and the immune system's machinery for recognizing foreign invaders.

To recognize any of a million different antigens, the immune system uses a series of breaking and joining reactions in the DNA to build the right antigen-receptor protein. Craig likens it to ordering dim sum at a Chinese restaurant: As numerous platters pass by, offering a vast array of choices, each diner selects different delicacies to create a preferred meal.

Scientists had suspected that the immune system's à la carte process, known as V(D)J—variable (diversity) rejoining—recombination, may have evolved from an ancient transposable DNA element. In any event, many features of V(D)J recombination share similarities with bacterial and viral transposons. But no known transposon exhibited one of the critical characteristics of the V(D)J mechanism: When a piece of DNA is chopped out during V(D)J recombination, one cut end of the double-stranded DNA left behind is sealed up and forms a hairpin loop. This hairpin loop is important for repair of the donor DNA.

Craig's team found that *Hermes* shares the mechanism of DNA cleavage with V(D)J recombination. When *Hermes* is snipped out of the donor DNA, the frayed end of the leftover DNA temporarily folds back on itself, forming that very kind of hairpin loop. She published the finding with colleagues from the University of California, Riverside, and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), in the December 23, 2004, issue of *Nature*.

A new crystal structure subsequently revealed that the Hermes transposase, the enzyme required for movement of the transposon, and the V(D)J recombination enzymes also share structural similarities in their active sites. Craig and NIDDK's Fred Dyda and Alison B. Hickman published the structure July 24, 2005, in the preprint online edition of *Nature Structural & Molecular Biology*.



FRED DYDA AND ALISON HICKMAN, NIH

A STRUCTURAL MODEL OF THE HERMES TRANSPOSASE, THE ENZYME REQUIRED FOR MOVEMENT OF THE "JUMPING GENE," SHOWS DNA DUPLEXES OCCUPYING THE TWO ACTIVE SITES THAT EXECUTE DNA BREAKAGE AND JOINING DURING TRANSPOSITION. THESE BASIC REGIONS OF THE MOLECULE ARE WELL-SUITED TO INTERACT WITH ACIDIC DNA (BLUE, RED, AND GREEN HELICES). THE GOLD RIBBONS ARE THE AMINO ACID BACKBONES OF THE ENZYME.

Craig sees *Hermes* as a tool that might, for example, lead to transgenic strategies to sterilize and contain disease-carrying insect populations. Ultimately, it might benefit humans by improving gene therapies. ■

-Cori Vanchieri-

The study is published in the June 17, 2005, issue of *Cell*.

IDENTIFYING BLOOD STEM CELLS IS A SLAM DUNK

Researchers have developed a simple technique to identify hematopoietic, or blood-forming, stem cells (HSCs) based on a set of characteristic markers, called SLAM receptors, that the cells display on their surface. The distinctive stem cell code marks the first time researchers have identified specific stem cells by looking at surface markers drawn from a single family of genes.

The new technique will enable scientists to determine where stem cells are located in blood-forming tissues and to trace the developmental routes HSCs take as they mature into blood cells. If the researchers' studies in mice apply to human blood-forming stem cells, the technique may enable safer stem cell transplants by improving purification of stem cells before transplantation.

Sean J. Morrison, an HHMI investigator at the University of Michigan, led the research team, which published its findings in the July 1, 2005, issue of *Cell*.

RESEARCHERS IDENTIFY GENE'S ROLE IN SUPPRESSING LONGEVITY

Researchers have determined that a gene present in mouse cells limits the number of times that a cell can divide. The gene is involved in senescence, a cellular process thought to ensure that aging cells do not pass on harmful mutations.

The researchers say the gene, known as *SIRT1*, suppresses longevity and may play a role in regulating the aging process. But they caution against interpreting the results too broadly, because dividing mouse cells in culture are an imperfect model of how aging affects human cells.

There is, however, some indication from the new studies that suppressing *SIRT1* could prove important in lab techniques used to generate large numbers of normal cells for research. In this context, the *SIRT1*-deficient cells hold an advantage over other highly proliferative cell types, such as cancer cells, because although they divide indefinitely, they otherwise appear normal.

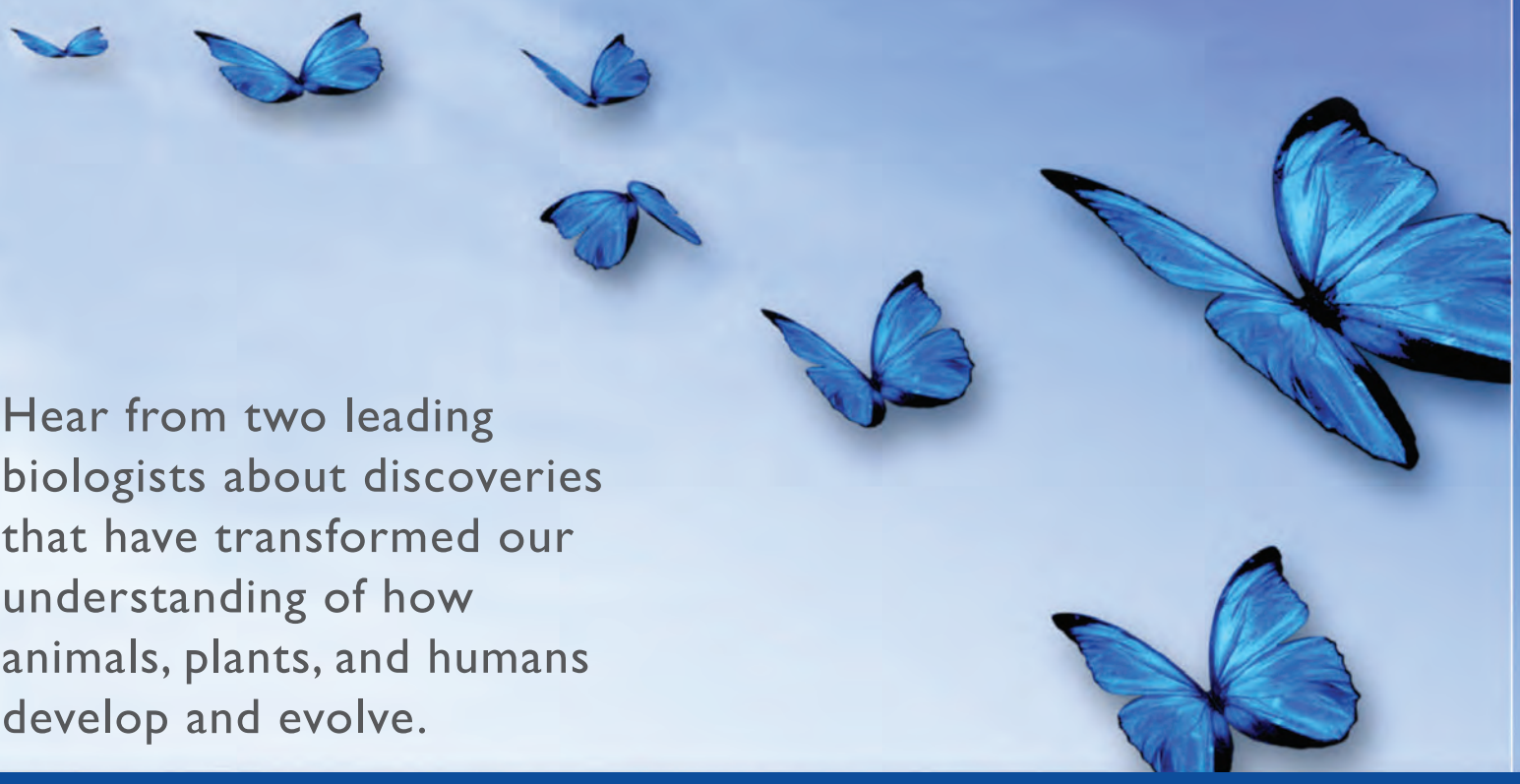
The research team, led by Frederick W. Alt, an HHMI investigator at Children's Hospital, Boston, and Harvard Medical School, published its findings in the July 2005 issue of *Cell Metabolism*. Alt and his colleagues plan to explore potential roles for *SIRT1* in tumor suppression and other functions.

RANDOM GENE EXPRESSION MAY DRIVE HIV INTO HIDING

Random fluctuations in gene expression can influence the fates of cells infected with human immunodeficiency virus (HIV) far more than previously thought, according to new research from the laboratory of HHMI investigator Adam Arkin at the University of California, Berkeley. By combining experimental and computational studies of HIV's replication cycle, the researchers found evidence that the virus may become latent in some cells by harnessing the random molecular behavior of the cell.

HIV can hide in cells for years before reappearing to make new virus. Latency is considered one of the biggest reasons why drug therapy fails to eradicate HIV from patients. The findings, published in the July 29, 2005, issue of *Cell*, could help scientists design new and more effective treatments to slow or halt the progression of HIV infection.

HOWARD HUGHES MEDICAL INSTITUTE 2005 HOLIDAY LECTURES ON SCIENCE



Hear from two leading biologists about discoveries that have transformed our understanding of how animals, plants, and humans develop and evolve.

Evolution

Constant Change and Common Threads

THE SPEAKERS

Sean B. Carroll
HHMI Investigator,
University of Wisconsin–Madison

David M. Kingsley
HHMI Investigator,
Stanford University School of Medicine

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SPOTLIGHT

INSERM Taps International Research Scholar for Major Award

KENT KALLBERG

INSERM, the French National Institute of Health and Medical Research, has awarded its 2005 Prize for Fundamental Research in Physiology/Physiopathology to **PASCALE F. COSSART**, an HHMI international research scholar at the Pasteur Institute in Paris, France. INSERM awards the prize annually in recognition of originality and excellence in basic physiology research.

Cossart's work focuses on understanding *Listeria monocytogenes*, a sometimes deadly bacterial pathogen found in uncooked meat, raw vegetables, and foods like cold cuts and soft cheeses that are tainted during processing. Her classic genetic studies, beginning in 1986, and her more recent physiological studies have helped to uncover how **Listeria** breaches three barriers in humans—the intestinal barrier, the placental barrier, and the blood-brain barrier—and have opened the door to identifying new virulence factors.

■ **James P. Allison**, an HHMI investigator at Memorial Sloan-Kettering Cancer Center, won the 2005 William B. Coley Award for Distinguished Research in Basic and Tumor Immunology from the Cancer Research Institute. The award recognizes Allison's work on the anticancer immunotherapeutic agent anti-CTLA4.

■ **Richard Axel**, an HHMI investigator at Columbia University College of Physicians and Surgeons, received an inaugural Louise T. Blouin Foundation Award, presented on May 2, 2005, in New York, for exceptional contributions to the art and science of creativity.

■ **David P. Bartel**, an HHMI investigator at the Massachusetts Institute of Technology, is corecipient of the 2005 Grand Prix Scientifique from the Foundation Louis D. in France. He shared the award with Ronald Plasterk of the Netherlands Institute for Developmental Biology. Bartel's work focuses on how RNA molecules can act as catalysts and regulate gene expression in plant and animal cells.

■ **Walter "Skip" Bollenbacher**, professor of biology and director of the HHMI undergraduate program at the University of North Carolina (UNC) at Chapel Hill, accepted the 2005 Pirelli Multimedia Award for best educational multimedia product on behalf of the media group at UNC's Institute for Science Learning. The HHMI-supported project, titled "MicroArrays MediaBook," describes the principles of genomics and the technological advances spawned from the field.

■ **Derrick T. Brazill**, a professor on the steering committee of the HHMI undergraduate program at Hunter College, was one of three biologists honored with a 2005 Presidential Early Career Award for Scientists and Engineers. The awards went to a total of 20 young scientists and engineers whose work is supported by the National Science Foundation.

■ **Patrick O. Brown**, an HHMI investigator at Stanford University School of Medicine, received the 2005 Curt Stern Award from the American Society of Human Genetics. The award recognizes Brown's work over the past 10 years in developing and enhancing microarray technology for clinical and basic science applications.

■ **Barbara A. Burke**, coordinator of the HHMI-supported undergraduate faculty-student mentor program at California State Polytechnic University, Pomona, received the 2004 Presidential Award for Excellence in Science, Mathematics and Engineering Mentoring during a May 16, 2005, ceremony at the White House.

■ **Jill G. Conley**, director of HHMI's international program, was elected to the Council on Foreign Relations. The council works to help society better understand the world and the foreign policy choices facing the United States and other governments.

■ **Ronald M. Evans**, an HHMI investigator at the Salk Institute for Biological Studies, was selected to receive the 2005 Grande Médaille D'Or (Grand Gold Medal) by the French Academy of Sciences. The award recognizes Evans' research discoveries illuminating how hormones and drugs control the body's metabolism, development, and reproduction.

■ Two HHMI investigators and an HHMI professor were recently elected to the American Philosophical Society, the country's first learned society, founded by Benjamin Franklin. The investigators are **Elaine Fuchs** and **Roderick MacKinnon**, both at the Rockefeller University. The professor is **Richard M. Losick** at Harvard University.

■ **Stephen P. Goff**, an HHMI investigator at Columbia University College of Physicians and Surgeons, was awarded the inaugural M. Jeang Retrovirology Prize by the Ming K. Jeang Foundation. The prize recognizes the achievements of a midcareer retrovirologist.

■ **David Haussler**, an HHMI investigator at the University of California, Santa Cruz, won the 2005 Classic Paper Award from the Association of Artificial Intelligence for his 1986 paper titled "Quan-

tifying the inductive bias in concept learning."

■ **Richard P. Lifton**, an HHMI investigator at Yale University School of Medicine, was selected as a Distinguished Scientist of the American Heart Association by the American Heart Association/American Stroke Association. The designation recognizes his seminal research that has "importantly advanced our understanding and management of cardiovascular disease or stroke."

■ **Richard M. Locksley**, an HHMI investigator at the University of California, San Francisco, was elected to the 2005 class of fellows of the American Academy of Arts and Sciences.

■ A team of high school science teachers supported through an HHMI grant to the Fred Hutchinson Cancer Center has won the 2005 Toyota Tapestry Award, administered by the National Science Teachers Association. **Laurie Matthews**, **Jessica Casbere**, and **Marcy McCorriston** won the award for their design of a hands-on project targeted to tenth graders that leads the students through a molecular-based investigation of cancer cells.

■ **Eva Nogales**, an HHMI investigator at the University of California, Berkeley, won the 2005 Early Career Life Scientist Award from the American Society for Cell Biology.

■ **Sam Silverstein**, director of the HHMI precollege program at Columbia University College of Physicians and Surgeons, won the 2005 Bruce Alberts Award for Excellence in Science Education, given by the American Society for Cell Biology. The award recognizes the HHMI-funded summer research program for high school science teachers in New York, which Silverstein founded and directs.

■ **Joan A. Steitz**, an HHMI investigator at Yale University School of Medicine, was selected to receive the 2005 E.B. Wilson Medal by the American Society for Cell Biology. The society's highest honor for science, the award recognizes significant and far-reaching contributions to cell biology over the course of a career.

■ **Roger Y. Tsien**, an HHMI investigator at the University of California, San Diego, won the 2005 J. Allyn Taylor International Prize in Medicine from the Robarts Research Institute, an independent center for medical research in Ontario, Canada. The award recognizes Tsien for his outstanding contributions in the area of molecular imaging of cell signaling. Tsien also received the 2005 Distinguished Scientist Award from the San Diego Chapter of the American Chemical Society.

■ **Darren Wells**, a teacher supported by the HHMI precollege education program at Timilty Middle School in Boston, won a 2005 Presidential Award for Excellence in Mathematics and Science Teaching. Wells currently teaches eighth-grade science.

■ **Eric Wieschaus**, an HHMI investigator at Princeton University, received the 2005 Wilbur Lucius Cross Medal. Each year, Yale University honors a small number of outstanding alumni with the award, in recognition of distinguished achievements in scholarship, teaching, academic administration, and public service.

SPOTLIGHT

Chicago Lauds Insulin Expert

STEVE ROESS

On July 15, 2005, the University of Chicago honored HHMI investigator DONALD F. STEINER by holding the Donald F. Steiner Symposium: Exploring Pancreatic Beta Cells, Insulin Biology and Protein Processing. The university organized the symposium as a celebration of the researcher's 75th birthday and of his research career. Steiner's body of work, which includes the discovery of proinsulin, has contributed greatly to our understanding of insulin biology, insulin secretion, protein processing, and diabetes.

SPOTLIGHT

Rapoport Selected for Max Delbrück Medal

GINTARAS SEKMOKAS / HARVARD MEDICAL SCHOOL

TOM A. RAPOPORT, an HHMI investigator at Harvard Medical School, was unanimously selected to receive the 2005 Max Delbrück Medal by the major biomedical research institutions in Berlin, Germany, including the Humboldt-Universität, the Charité, the Free University, the Max Planck Institute, and the Max Delbrück Center for Molecular Medicine. Rapoport will receive the award in Berlin in October, when he will present this year's Berlin Lecture on Molecular Medicine.

Rapoport's research centers on the mechanisms by which proteins are transported across cellular membranes and on how cellular organelles form and maintain their characteristic shapes. Understanding those mechanisms is relevant to identifying the basis of diseases in which proteins are misdirected, misfolded, or degraded. Rapoport, a professor of cell biology, joined the faculty at Harvard in 1995.

"I think our discovery reveals another important function of the mitochondria, and that is in immunity."

Researchers have discovered a surprise lurking inside mitochondria, the power plants that are present in every cell. It turns out that these powerhouses also contain a protein that triggers the immune system to attack viral invaders. According to the researchers, the new role makes perfect biological and evolutionary sense because it fits well with another function of mitochondria as executioners of a biochemical cascade that causes programmed cell death, or apoptosis. "This is the first protein known to be involved in the immune response that is found in mitochondria," said Zhijian "James" Chen, an HHMI investigator at the University of Texas Southwestern Medical Center. Chen and his colleagues reported the discovery on August 25, 2005, in an immediate early publication of the journal *Cell*.

"When we did the standard analysis of the effects of this ERM knockout, the most glaring phenotype was a very rapid sterility that arose in all the males."

Kenneth M. Murphy, an HHMI investigator at Washington University School of Medicine in St. Louis, reporting the identification, with colleagues, of a master genetic switch that regulates the self-renewal of sperm-producing stem cells. Mice that were genetically engineered to lack the switch quickly exhausted their sperm-producing stem cells, rendering them incapable of producing sperm. The researchers said their finding offers new opportunities for exploring how spermatogenesis is regulated in mammals. Published in the August 18, 2005, issue of *Nature*, the work is summarized at www.hhmi.org/news/murphy.html.

"It may be the normal cognitive function of the brain that leads to Alzheimer's later in life. This was not a relationship we had even considered."

Randy L. Buckner, an HHMI investigator at Washington University in St. Louis, discussing his lab's finding that the areas of the brain that young, healthy people use when daydreaming are the same areas that fail in people who have Alzheimer's disease. Published in the August 24, 2005, issue of the *Journal of Neuroscience*, the work is summarized at www.hhmi.org/news/buckner5.html.

WEB EXTRA

Discovering MicroRNA's Role in Cancer

Three papers published this past June—the result of collaborations involving several HHMI investigators—show explicit links between tiny RNA molecules and cancer. Turns out these tiny gene regulators may have a disproportionately large effect.

LOOK FOR THIS ARTICLE IN THE BULLETIN ONLINE:

www.hhmi.org/bulletin/sept2005

FAST FACTS

HHMI'S JANELIA FARM RESEARCH CAMPUS

In June, HHMI announced the selection of seven group leaders for its Janelia Farm Research Campus (see page 28), slated to open next year in Ashburn, Virginia. At Janelia Farm, small research groups will tackle large-scale biomedical problems in a highly collaborative environment. The scope of that environment? Here are some relevant fast facts.

WHO
When the campus is fully operational, there will be 20 to 30 group leaders and a permanent research staff of about 300 scientists.

WHAT
As HHMI's first freestanding research campus, Janelia Farm will provide a setting in which small research groups can explore fundamental biomedical questions in a highly collaborative, interdisciplinary culture.

WHEN
The $500 million campus will open in 2006. The first group leaders will open their labs in the summer of 2006. Recruitment will continue thereafter, and it is anticipated that all group leaders will be in their labs by 2009. A visiting scientist program will begin in 2007.

WHERE
Janelia Farm is in Loudoun County, Virginia, about 8 miles north of Dulles Airport and just under 30 road miles west of Washington, D.C. Its parklike 689-acre campus lies on the southern bank of the Potomac River.

WHY
The plan for Janelia Farm grew out of an acknowledgment by HHMI leadership that while most biomedical problems are handled well in a university setting, there are some that might be better addressed in a place where small groups of researchers with different skills can work together without the barriers typically encountered at a university.

Look for news of further progress at Janelia Farm in future issues of the *HHMI Bulletin*.

ASK A SCIENTIST

Q.

SUBMITTED FROM CALIFORNIA

WHAT IS MUSCLE MEMORY AND HOW DOES IT WORK?

A.

BY C. SUBAH PACKER

"Muscle memory" is the terminology used by muscle physiologists to describe the phenomenon of skeletal muscle activity that is learned and becomes essentially automatic with practice. For example, walking is automatic and takes no real cognitive effort for healthy adults but is initially learned and takes great concentration on the part of a toddler. With constant practice, the neural networks and motor neuron/muscle group pathways become fast and effortless, requiring no conscious thought to achieve the fluid sequence of motor activity that produces optimal walking behavior. Indeed, we rarely actually "think" about walking, and thinking about it might actually slow down walking or interrupt the coordination of the walking pattern. However, if an individual becomes ill and is bedridden for any length of time, walking must be relearned. This same phenomenon of learning and "memorizing" sequences of skeletal muscle activities or behaviors occurs for various tasks besides walking and is much of what athletes are doing when they are training for a particular sport.

The cellular mechanism responsible for this memory is not completely understood. Clearly, the biochemistry of synapses (chemical messages) between the motor nerves and muscle cells is up-regulated when used repeatedly. However, exactly how this occurs is under investigation. In addition, the synaptic transmission is just one component of a complex series of events involved in motor function.

RESEARCHED BY C. SUBAH PACKER, ASSOCIATE PROFESSOR IN THE DEPARTMENT OF CELLULAR AND INTEGRATIVE PHYSIOLOGY AT INDIANA UNIVERSITY SCHOOL OF MEDICINE.

The scientific process starts with a question. When a scientific inquiry piques the interest of a high school or college student, and answers can't be found in class or in a textbook, students can turn to HHMI's Ask a Scientist Web site. There, working scientists field a wide range of biomedical questions. "We want to help satisfy people's honest curiosity about the world around them," says Dennis Liu, program director of the Institute's public science education initiatives. "We want to answer the questions that fall outside the curriculum."

TO SEE OTHER QUESTIONS, VISIT ASK A SCIENTIST

www.hhmi.org/askascientist

continued from page 15
[PERPETRATOR GENE]

genetic analysis. "Gallia spent a week in his lab running all the genotypes. She came back with the data and sure enough, we had mapped the gene!" Ginsburg recounts. "We were pretty excited about it. She started analyzing more markers and narrowing the genetic location. When we got down to a manageable region of the genome, she went through the genes there, one by one, sequencing them in the blood samples from the TTP patients to see if we could find a mutation." Levy finally pinpointed the gene and found it to be mutated in all the children who had TTP. The gene, called *ADAMTS13*, encoded a protease—an enzyme that cleaves protein. At practically the same time, Sadler's team identified *ADAMTS13* as the blood protease that cleaves VWF in particular.

Sadler and Ginsburg have learned much about *ADAMTS13* since their discoveries 4 years ago. The general idea, says Sadler, is that *ADAMTS13* regulates the activity of VWF in the blood. VWF recruits circulating platelets into a thrombus around a broken blood vessel. "If you don't have *ADAMTS13*, or if your own immune system destroys your *ADAMTS13*," Sadler explains, "these platelet thrombi grow out of control and you end up with TTP."

Sadler's group is currently trying to understand why some people produce autoantibodies to *ADAMTS13* and to figure out what causes the vast differences in the severity of the disease in different patients. "We'll treat many of our patients with plasma exchange and they get better," he says. "Their antibody goes away, their *ADAMTS13* comes back, and they're fine. But other patients may return to the hospital in a week or a few months with another episode. And each episode can be devastating. If we can identify patients with a high likelihood of relapse, and give them more intensive therapy up front, then I think we can save some lives." Ginsburg's lab is working toward a better understanding of childhood TTP. They recently developed a "knockout" mouse, lacking the *ADAMTS13* gene, which mimics the human condition.

A commonsense treatment for TTP would be to administer recombinant *ADAMTS13*, Sadler explains. "That would be perfect for kids with congenital *ADAMTS13* deficiency." It might also help adults with the acquired form of the disease.

> "If you don't have *ADAMTS13*, or if your own immune system destroys your *ADAMTS13*, these platelet thrombi grow out of control and you end up with TTP."
>
> EVAN SADLER

Tragically, both researchers concede, the ultimate obstacle to a better treatment for TTP may not be scientific, but economic. "It's one of these rare examples where we have an opportunity to take a basic research finding straight to the bedside," Ginsburg says. "And it just isn't going to happen because of the financial realities of the pharmaceutical industry."

"We're talking about a disease that, at most, strikes maybe 1 in 100,000 people," Ginsburg says. "And pharmaceutical companies are not interested in developing a drug unless the market is a bare minimum of half a billion dollars a year. Otherwise, it's not worth their effort."

Meanwhile, the multiple strokes Jennifer Chamberlin suffered as a result of TTP took her sight and hearing, but her illness has been in complete remission for more than 4 years. Her doctors, including Sadler, credit rituximab, an immunosuppressive drug that kills antibody-making white blood cells and has shown remarkable effectiveness in treating autoimmune diseases. "We may be able to apply what we have learned about *ADAMTS13* to identify high-risk patients like Mrs. Chamberlin, who could benefit from immunosuppression with rituximab at the time of first diagnosis," Sadler says. Ginsburg concurs: "That would be a real improvement." ■

-Paul Muhlrad-

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[WILLIAM JACOBS]

have to figure out how to kill the hunkered-down bacteria, to tear apart how TB evades the immune system.

To prove that a phenotype—a property like virulence or drug resistance—is caused by a specific genotype, you have to isolate a mutant, clone a genotype, and transfer it. I call this Koch's corollary, after Robert Koch, the man who discovered the cause of TB in 1882. Yet, for years we couldn't fulfill Koch's corollary. We didn't know the mechanism of attenuation for BCG—a live tuberculosis vaccine developed by two French scientists that has been given to half the world's population—until more than 70 years after it was introduced. We used drugs like isoniazid for more than 40 years without knowing their targets of action. We did not know the genetic basis for any TB virulence factor.

Over the years, my lab, in collaboration with Barry Bloom, has developed a fairly complete set of genetic tools to do genetics in mycobacteria and complete Koch's corollary. Every time I have had a problem overcoming a genetic obstacle, I went to mycobacteriophages—viruses that infect bacteria—to find solutions. In fact, our first big breakthrough came with the introduction of foreign genes into mycobacteria using chimeric phage vectors that I designed. Using a system based on this original chimeric vector, we can now systematically knock out every gene of *M. tuberculosis*. Recently, in collaboration with Graham Hatfull, an HHMI professor at the University of Pittsburgh, and David Fidock at Albert Einstein, we have developed an efficient complementation system for *Plasmodium falciparum*, the parasite that causes malaria, using genes from Bxb1 (alias the Bronx Bomber), a phage I isolated from my backyard in the Bronx.

I have always been interested in problems that affect the developing world. I went to Barry Bloom's lab because he wanted to make a recombinant BCG as a vaccine vector that could maybe protect against TB, malaria, and other diseases affecting the developing world. We have gone back now and remade BCG from virulent *M. tuberculosis* and *M. bovis*. Derivatives of these strains have shown promise in mice against *M. tuberculosis*. To get it into the clinic, we're going to have to prove that they are safe in humans. Toward that goal, we have demonstrated safety in guinea pigs, immunocompro-

mised mice, and recently in monkeys. Further studies are under way.

My life changed the first day I took a plane to Madras, India, on my way to visit a leper colony. I saw masses of humanity like I had never seen before in the U.S. I wish that I could take President Bush and his advisers with me to a leper colony because what we need in the U.S. is to be exposed to the rest of the world's problems. After all, 75 percent of the world's population is threatened by infectious diseases. ■

-Interview by Avice Meehan-

continued from page 39
[KATHERINE HIGH]

HHMI: ***WHAT IS THE STATUS OF YOUR OWN RESEARCH?***

KH: We have cured hemophilia in lab mice and dogs by injecting them with the gene for Factor IX wrapped in a vector called adeno-associated virus (AAV). Over the past few years, however, humans in clinical trials have expressed therapeutic levels of Factor IX for only a few weeks following vector infusion. That's probably because the patients' immune systems killed the cells hosting the inserted gene. To overcome this problem, we've been working on transiently suppressing the immune response to the viral vector AAV.

HHMI: ***EXPENSIVE GENE-THERAPY RESEARCH NEEDS INDUSTRIAL SUPPORT, BUT DOESN'T INDUSTRY'S IMPATIENCE ADVERSELY AFFECT THE FIELD'S PROGRESS?***

KH: Early safety studies have been slow and time-consuming, and that pace indeed tests the patience of some companies, which exist, after all, to make money for shareholders. But as safety issues are resolved, testing proceeds more quickly—and we are now entering that era.

Meanwhile, we're witnessing the dropout of small biotech companies in gene therapy—and the entry of willing replacements. This shortfall in resources must be addressed by the disease foundations and by federal support for early development of novel therapies.

HHMI: ***WHAT IS MOST CHALLENGING ABOUT BEING A LEADER IN GENE THERAPY?***

KH: It's in imparting a sense of the field's momentum to outside people such as foundation officers and NIH institute directors, who often remain unaware of our progress after having been saturated with negative media coverage of gene therapy. Poor public perception works against us, translating into reluctant funding agencies and clinical trial participants.

During the year I served as president of the American Society of Gene Therapy, the best thing I did was to lead a stakeholders' conference aimed at answering the question: What's slowing down gene-therapy research? We asked scientists to share 15-minute stories of their recent research, sketching what worked and what didn't. From that, we identified several of the field's key hurdles. One of them is the financing of clinical trials, a lost middle ground when pharmaceutical companies want to pick up phase III projects and NIH wants to fund early-stage, or phase I, research. Another challenge is the complex regulatory process governing gene-therapy protocols.

Despite these hurdles, though, there is no question that gene therapy ultimately will succeed. We have to walk before we can run. But we're going to get there. ■

-Interview by Kathryn Brown-

CONTINUED FROM PAGE 27
[ALTERNATIVE SPLICING]

"When we knock these out, they don't seem to just globally screw up splicing—they're very specific alternative splicing events, which was a little bit surprising," Graveley says. "So we definitely know that there are some exons that are highly regulated."

And this, Zipursky says, is a system that is in some ways random. Since his initial discovery of the gene, Zipursky has found that *Dscam*'s role is to enable the extending processes of a neuron's dendrites and axons to distinguish between themselves and their neighbors—and he thinks that by producing so many forms of the protein, the fruit fly has actually minimized the need for elaborate control.

Each neuron expresses 10 to 50 forms of *Dscam* at any given time, and Zipursky's group has shown that each form has unique recognition properties. What is important, he says, is that cells express forms of *Dscam* that are different from one another. With more than 38,000 to choose from, odds are—even if they're randomly selected—one cell's *Dscam* forms won't be the same as those expressed by a neighboring cell.

"The fly has invested in making many, many different types of forms," Zipursky concludes, "but it hasn't invested in the detailed control that would be necessary to make specific forms in specific neurons. That would require a tremendous investment in genetic regulation."

But in mammals, Black says, "the regulation gets very complicated." His lab has been teasing out the precise ways in which specific splicing factors, and the sequences they bind to, orchestrate that regulation. "For most exons that have been analyzed, what we've found is there are multiple regulators," he says. Some of these are so globally expressed that additional regulators must be around simply to counteract them when they're not needed. In some cases, a single molecule can activate splicing in one cell type while repressing it in another. The finely tuned balance of these splicing factors determines how each molecule behaves and how a transcript is ultimately spliced. "There's this combinatorial system of regulation similar to transcriptional regulation," Black says.

The average mammalian gene has eight or nine exons, and with most human genes undergoing some form of alternative splicing, virtually all of these are candidates for elaborate control. Most of the molecules and their interactions remain to be elucidated. In that light, the goal of being able to predict splicing from a gene sequence seems attainable, but that knowledge is still tantalizingly out of reach. "We're not very good at predicting what splicing patterns are going to be, let alone how those splicing patterns will be regulated," Black says. "People are certainly making progress, but we don't know enough about the mechanism." ■

CONTINUED FROM PAGE 36
[THE IMPORTANCE OF BEING CILIA]

"When you look at these kids with BBS and you look at Val Sheffield's BBS4 mouse, it looks like they are probably overeaters, that they lose their appetite control," says Dutcher. "One of the things we're doing is to ask whether the leptin receptor, which is involved in appetite control, is actually localized to ciliary membranes." If that is the case, she reasons, if the cilia don't get built, the leptin receptor isn't present and the "hunger satisfied" message is never received.

Meanwhile, the "tubby" (*tub*) genes tantalize the Dutcher lab. Tubby is a mouse mutant discovered almost 15 years ago that displays some of the same characteristics—obesity and retinopathy, primary among them—as seen in BBS. Yet the role of the tubby genes in the overweight mouse remains unclear. *Chlamydomonas* has three tubby genes, and through its comparative genomics study, the lab found that two of the three tubby genes encode ciliary proteins. Now, the researchers are asking what those proteins do in the alga and how in the world that function might relate to what happens in mammals.

Sheffield, who is a physician as well as a researcher, is also very interested in understanding cilia's contribution to his chubby mice. "It's intriguing, really," he says. "None of us would have thought that cilia would be involved in something like obesity. I'm still trying to figure out how that one happened." ■

CONTINUED FROM PAGE 36
[THE IMPORTANCE OF BEING CILIA, INNER-EAR CONNECTION SIDEBAR]

TRP family of ion channels and is now called TRPN1, in the sensory bristle of the fruit fly *Drosophila*. Interestingly, the bristle's composition is that of a true cilium: tubulin-based rather than actin-based. Corey has found TRPA1 in mouse hair cell kinocilia as well, perhaps suggesting further evolutionary conservation across species. ● Teresa Nicolson, an HHMI investigator at Oregon Health & Science University, studies mutant zebrafish that swim in circles—a classic sign of inner-ear dysfunction. When she learned of Zuker's finding in the fly bristle, she thought it might be the same transduction channel as the one in hair cells. She was excited to find a version of the TRPN1 channel expressed in zebrafish hair cells. ● When her lab knocked out the gene in zebrafish, they saw deafness and balance defects in the mutants. In addition, electrical potential in the channels disappeared. "The results suggest that the TRPN1 channel could be the transduction channel in zebrafish hair cells," says Nicolson. "Now we are trying to show it is actually in the right place to be doing that job." ● Nicolson's lab also has a bead on the tip link protein. Her candidate, cadherin 23, has a very long extracellular domain that could serve to physically link the stereocilia tips. Labeling experiments have localized cadherin 23 to the tips of the bundles, and mutants lacking the protein have no tip links. They are also deaf.

COURTESY OF SEAN CARROLL

A CLOSE-UP VIEW OF A BUTTERFLY WING EYESPOT REVEALS THE COLOR PATTERN FORMED BY ROWS OF SCALES. EACH SCALE IS THE PRODUCT OF A SINGLE CELL.

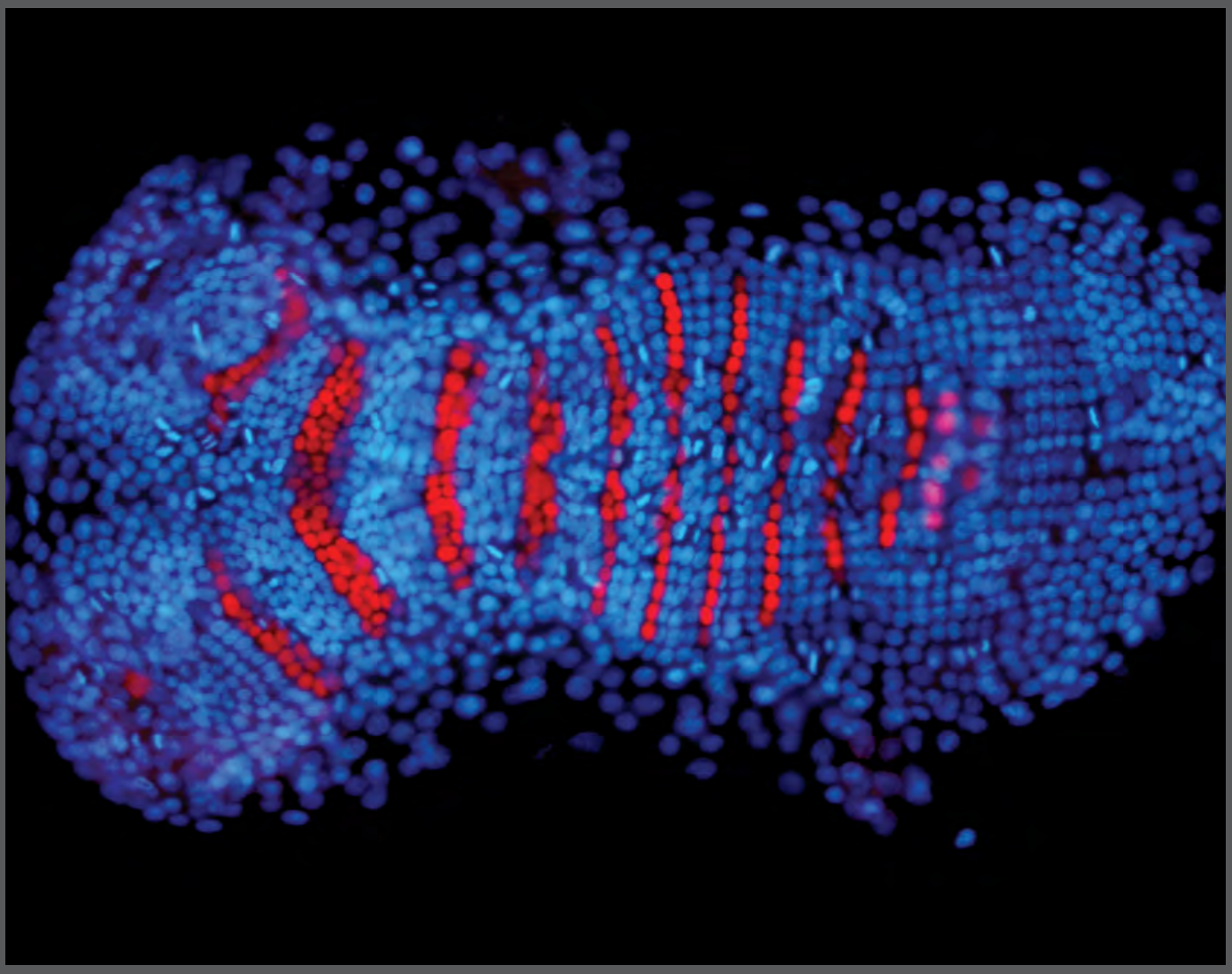
THE BIRTH OF EVO-DEVO

A new discipline forms at the interface of embryology and evolutionary biology.

"Almost immediately after the first sets of fruit fly genes were characterized came a bombshell that triggered a new revolution in evolutionary biology. For more than a century, biologists had assumed that different types of animals were genetically constructed in completely different ways. The greater the disparity in animal form, the less (if anything) the development of two animals would have in common at the level of their genes. One of the architects of the Modern Synthesis, Ernst Mayr, had written that "the search for homologous genes is quite futile except in very close relatives." But contrary to the expectations of *any* biologist, most of the genes first identified as governing major aspects of fruit fly body organization were found to have exact counterparts that did the same thing in most animals, including ourselves. This discovery was followed by the revelation that the development of various body parts such as eyes, limbs, and hearts, vastly different in structure among animals and long thought to have evolved in entirely different ways, was also governed by the same genes in different animals. The comparison of developmental genes between species became a new discipline at the interface of embryology and evolutionary biology–evolutionary developmental biology, or "Evo Devo" for short.

The first shots in the Evo Devo revolution revealed that despite their great differences in appearance and physiology, all complex animals–flies and flycatchers, dinosaurs and trilobites, butterflies and zebras and humans–share a common "tool kit" of "master" genes that govern the formation and patterning of their bodies and body parts....The important point to appreciate from the onset is that its discovery shattered our previous notions of animal relationships and of what made animals different, and opened up a whole new way of looking at evolution."

A molecular biologist and HHMI investigator at the University of Wisconsin–Madison, Sean Carroll *researches the way new animal forms have evolved. His studies of a wide variety of animal species have dramatically changed the face of evolutionary biology. For more information about his work, visit www.hhmi.org/research/investigators/carroll_bio.html*



COURTESY OF NIPAM PATEL

Body Patterning Gets an Early Start

DURING EMBRYONIC DEVELOPMENT OF THE AMPHIPOD CRUSTACEAN *PARHYALE HAWAIENSIS*, CELLS ARE ORGANIZED INTO A PRECISE PATTERN OF ROWS AND COLUMNS, FORESHADOWING THE SEGMENTED BODY OF THE ADULT. IN THIS IMAGE, PARTICULAR CELL ROWS THAT EXPRESS THE *ENGRAILED* GENE GLOW RED, WHILE ALL NUCLEI ARE HIGHLIGHTED IN BLUE.

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