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HHMI BULLETIN

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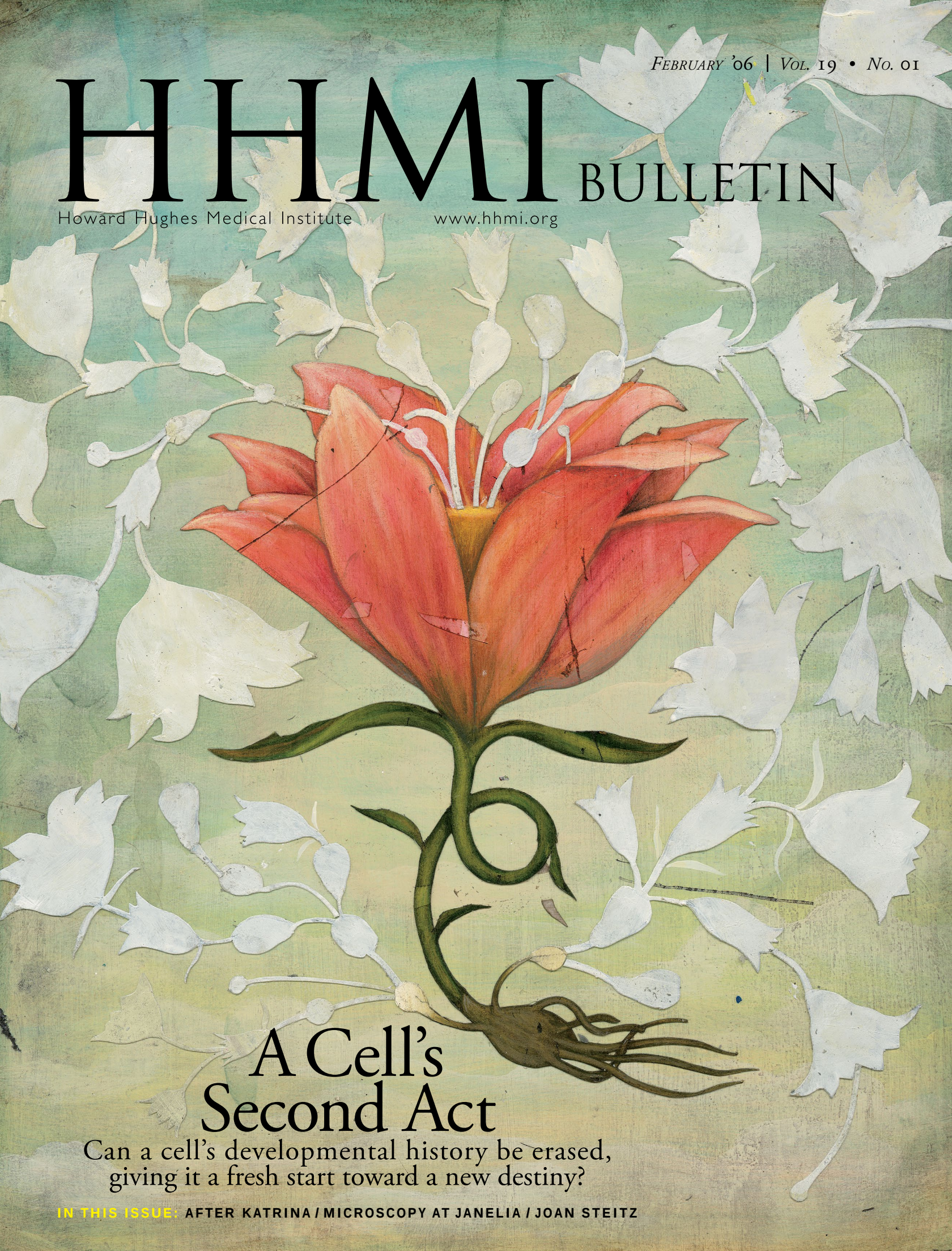
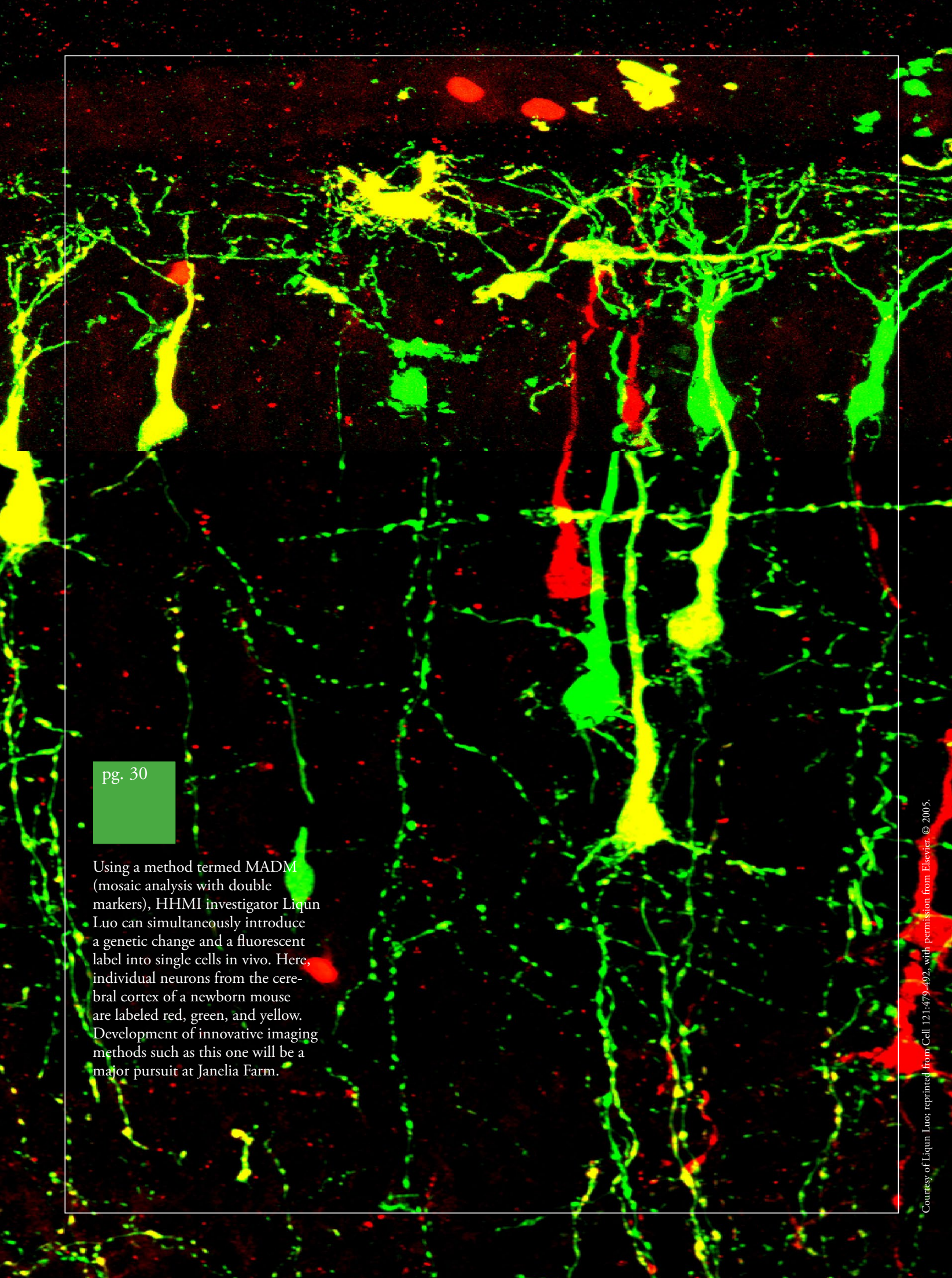


A Cell's Second Act

Can a cell's developmental history be erased, giving it a fresh start toward a new destiny?



pg. 30

Using a method termed MADM (mosaic analysis with double markers), HHMI investigator Liqun Luo can simultaneously introduce a genetic change and a fluorescent label into single cells in vivo. Here, individual neurons from the cerebral cortex of a newborn mouse are labeled red, green, and yellow. Development of innovative imaging methods such as this one will be a major pursuit at Janelia Farm.

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DEPARTMENTS

FEATURES

WWW.HHMI.ORG/BULLETIN
Visit the *Bulletin Online* for additional content and added features.

Cover image: Jason Holley

contributors

(1)

(2)

(3)

(4)

Dan Ferber is a freelance journalist based in Indianapolis and a contributing correspondent for Science, where he covers biology, biotechnology, and biomedical research. His stories about science, technology, and medicine have also appeared in Reader's Digest, Popular Science, New Scientist, and other magazines. In his former life as a microbiologist, he peered through many a microscope. (1)

Author of Alternative Treatments for Arthritis: An A to Z Guide (Arthritis Foundation, 2005) and a contributing editor for Health and Arthritis Today magazines, **Dorothy Foltz-Gray** is currently writing a memoir about being and losing a twin, entitled With and Without Her. (2)

Richard Saltus is a science writer and editor at the Dana-Farber Cancer Institute in Boston. He has been a staff science and medical writer for the Boston Globe, San Francisco Examiner, and Associated Press in Los Angeles. Saltus is a frequent contributor to the HHMI Bulletin, and his freelance articles have also appeared in The New York Times, Science, Popular Science, Science Digest, Harvard School of Public Health Review, and Boston Globe Magazine. (3)

A freelance education writer and editor based in Rockville, Maryland, **Judy Saks** was an education reporter for the Boston Globe and a senior editor of The American School Board Journal. When not at her keyboard, Saks enjoys such endeavors as teaching summer workshops for young writers and moderating focus groups on adult education issues. (4)

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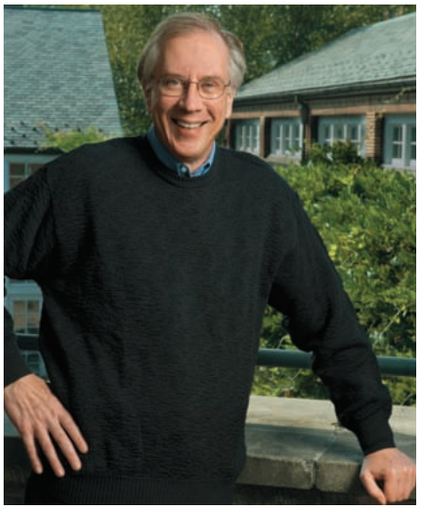
Ferber: Courtesy of Dan Ferber *Foltz-Gray:* Courtesy of Dorothy Foltz-Gray
Saltus: Courtesy of Richard Saltus *Saks:* Courtesy of Judy Saks

Working Backward to Move Forward



Paul Fetters

FOR MORE THAN 20 YEARS AT THE UNIVERSITY OF COLORADO, I spent many hours in the classroom, sharing a lifelong interest in biochemistry with undergraduate and graduate students. Yet, like many of my colleagues, I may have tackled my teaching assignment from the wrong direction. I went forward when I should have been going backward—at least that's the conclusion I've drawn after reading a handbook on "scientific teaching" written by an HHMI Professor.

As Jo Handelsman and her colleagues at the University of Wisconsin–Madison point out in their handbook, *Scientific Teaching: A Guide to Transforming Undergraduate Biology Education*, the idea is deceptively simple: Decide what you want students to understand and determine how you will assess whether they do, in fact, understand the material *before* deciding how to teach it. In my experience, "backward design"—Grant Wiggins and Jay McTighe coined the memorable description in 1998—is rare. Certainly, I wasn't alone in first choosing a textbook, deciding the order in which to cover the chapters, and seeing when and where I could fit in some demonstrations or experiments to enhance the course—without considering the impact the course would have on the same students a year later.

Since the grants program commenced in 1988, HHMI has invested more than $1.4 billion in a variety of educational programs, among them the HHMI Professors initiative pioneered by Handelsman and 19 other teacher-scholars. Our efforts have ranged from research fellowships for medical students and new graduate training programs to research experiences for undergraduates and outreach programs for K–12 students. HHMI and its grantees now have considerable knowledge of what works and what doesn't, and we're placing a renewed emphasis on extending the reach of our programs.

But because we're scientists, we're also experimenting—experimenting with a variety of approaches to engage the community in high-impact teaching. Right now, we're in the midst of evaluating applications for the second group of HHMI Professors, and I'm struck by the number of accomplished research scientists who have applied. We're committed to the broad dissemination of their experiments in education. In another initiative, HHMI and the journal *Science* have begun a collaboration that will bring information about innovative teaching approaches directly to scientists. In January 2006, the editorial staff at *Science* began producing a monthly section about education. We hope it will engage a broad array of scientists—beyond the life sciences and beyond colleges and universities—with a lively selection of articles intended to pique interest and spark discussion.

The challenge, of course, goes beyond how an individual professor structures a course or even how an individual college or university organizes its science curriculum. In fact, we need to think broadly about how we structure science education, with two goals in mind: generating a cadre of creative scientific thinkers as well as an educated citizenry. When we choose those measurable results, applying the rubric of backward design, it becomes clear that we must rethink the nation's approach to science education.

> "We need to think broadly about how we structure science education, with two goals in mind: generating a cadre of creative scientific thinkers as well as an educated citizenry."
>
> THOMAS CECH

A working paper issued by the National Bureau of Economic Research, which pulled together data from a variety of sources, is illustrative. The demographics of U.S.-trained Ph.D.s in science and engineering have changed substantially over the past 40 years. On the positive side, more women and minorities are pursuing careers in science, but, on the negative, the percentage of U.S. citizens receiving Ph.D.s has dropped significantly. In 1966, 71 percent of Ph.D. graduates in science and engineering were men born in the United States, 6 percent were U.S.-born women, and 23 percent were foreign-born. By 2000, the statistical picture had shifted: 36 percent of all science and engineering Ph.D.s were U.S.-born men, 25 percent were U.S.-born women, and 39 percent were foreign-born.

The recent Summit on National Competitiveness—convened by U.S. Reps. Frank Wolf (R-Virginia), Sherwood L. Boehlert (R-New York), and Vernon J. Ehlers (R-Michigan)—was spurred by an outcry from corporations both large and small about the dearth of well-trained scientists and engineers. The summit has called for nothing less than a transformation of the U.S. educational system. High on the list is a doubling of the number of undergraduate degrees in science and mathematics to 400,000 a year by 2015. It's a tall order, particularly in an era when many technical jobs are being exported overseas and federal research funding is being reduced. By defining the goal—and working backward—the nation may, with contributions from organizations like HHMI, move forward.

Thomas R Cech

NOBELIST'S CATHEDRAL

"They stayed true to the baroque architecture—not polluting it with additions from the 19th or 20th centuries—and did a wonderful job."

GÜNTER BLOBEL

Günter Blobel saw a 60-year-old dream come true this past October 30: the reopening of an 18th-century baroque cathedral he had first admired just 2 days before it was reduced to rubble during the final European air raids of World War II.

Dresden's Frauenkirche, or Church of Our Lady, with its cupola, turrets, and enormous stone dome, had been considered an architectural masterpiece and an essential part of the city. But it lay in ruin for decades.

When Blobel, an HHMI investigator at the Rockefeller University, won the 1999 Nobel Prize in Physiology or Medicine, he devoted most of his $1-million award to rebuilding the Frauenkirche as close to its original design as possible, and to reconstructing a Dresden synagogue. He also raised another $2 million in the United States for the project.

The result is "better than I even expected," he says. "They stayed true to the baroque architecture—not polluting it with additions from the 19th or 20th centuries—and did a wonderful job. The colors are the true, vibrant colors that graced the church before, and when you enter, it's full of light."

Another special feature is its cupola, which Blobel compares to the Duomo in Florence. "Now the next generation too can love this skyline along a bend in the Elbe," he says.

They'll have at least one more reason to love it. Although the original Frauenkirche was not built as a concert hall, its superb acoustics drew legendary musicians, including Johann Sebastian Bach and Richard Wagner.

Apparently, that quality has been restored as well. After returning to Dresden to attend a concert of the New York Philharmonic on November 17, part of an extended celebration of the church's reconsecration, Blobel was ready to pass an additional judgment: "The acoustics are outstanding!" —**Cori Vanchieri**

FOR MORE INFORMATION
To learn more about the Frauenkirche, visit http://www.frauenkirche-dresden.org/

Blobel: Matthew Septimus *Frauenkirche:* Aidan O'Rourke

Q. WHAT'S ON YOUR iPOD?

Ask music lovers anywhere, and they'll tell you the hottest gift in town is an iPod or MP3 player. That goes for HHMI investigators as well. After all, what's a lab break for, if not a quick surf to a digital music store? Here's what's playing on the portable audio players of some HHMI scientists.

Evan E. Eichler
ASSOCIATE PROFESSOR OF GENOME SCIENCES, UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

"At the moment, my portable MP3 player holds two albums from U2: *Rattle and Hum* and *How to Dismantle an Atomic Bomb*. There are a few odds and ends, too, including songs by AC/DC, Merle Haggard, and Tom Petty & The Heartbreakers."

Dianne K. Newman
ASSOCIATE PROFESSOR OF GEOBIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

"On my iPod, there's everything from merengue (Juan Luis Guerra) and opera (Jessye Norman) to tango (Astor Piazolla) and rap (C&C Music Factory). There's also '80s music (Pretenders, U2, David Bowie), Latin pop (Chi-Chi Peralta, Ana Belen, Shakira), and old classics (Frank Sinatra). The only unifying theme is that you can run or dance to most of them."

David G. Schatz
PROFESSOR OF IMMUNOBIOLOGY, YALE UNIVERSITY SCHOOL OF MEDICINE

"I've got the full text of *The Shadow of the Wind*, a bestseller in Spain by Carlos Ruiz Zafón, the two most recent Harry Potter books, and hundreds of classical and popular music tracks."

Brenda A. Schulman
ASSOCIATE MEMBER, DEPARTMENTS OF STRUCTURAL BIOLOGY, AND GENETICS AND TUMOR CELL BIOLOGY, ST. JUDE CHILDREN'S RESEARCH HOSPITAL

"My iPod holds an eclectic mix of pop, jazz, R&B, folk, country, and classical music. I listen to different genres, depending on what I'm doing. One constant: I always like a good dance beat when I'm doing lab work or looking at crystal structures."

Kunkel: Jason Grow *Lily illustration:* Getty Images

"I'd take my computer to the greenhouse and work there. It was my dream office."

LOUIS KUNKEL

Botanical Bloodline

In the midst of the civil war, President Abraham Lincoln created the National Academy of Sciences (NAS) as an honorific society of prominent scientists who would—and still do—advise the federal government on matters of science and technology.

Newly elected members by long tradition have penned their signatures in a large book that resides in the NAS building on the mall in Washington, D.C. In 1991, when HHMI investigator and Harvard geneticist Louis M. Kunkel was inducted and signed the volume, he leafed through it in search of family.

"I found my dad's signature," he says. That would be Henry George Kunkel, a clinical immunologist born in 1916 and elected to the NAS in 1967. "But I couldn't find my granddad's name." Louis Otto Kunkel, who had a farm in Bucks County, Pennsylvania, and a lab at the Rockefeller Institute (now University) in New York City, was elected in 1932. "He didn't travel to the induction ceremony," says Kunkel. "In those days it was a much bigger deal to get to Washington."

Though no gene for NAS membership has yet been discovered, the three Kunkels have all shared two other traits: the drives to do outstanding science and to work the soil to bring forth beautiful plants.

"I spent hours and hours with my dad in the garden" at the family home in Yonkers, New York, recalls Kunkel. It had vegetables and flowers of all kinds. When Kunkel was 10 years old he began breeding irises there to obtain a variety of colors and shapes. "We had more than 40 varieties of irises and many more hybrids, only a few of which were kept," he says. His favorite was one his dad bred, nicknamed Big Blue, after its beautiful blue color and size. Looking back, it was the beginning of his interest in genetics.

Kunkel is based at Children's Hospital Boston, where he cloned genes for muscular dystrophy and now studies complex traits such as human longevity and autism. He and his wife and three daughters live west of the city on two acres of land, where he grows many kinds of vegetables and flowers and continues to propagate irises—including Big Blue. He also has 20 of the original irises he grew with his father, including a two-tone lavender iris, which came from his grandfather's farm.

Kunkel says one thing is still missing. "Our other house had a big greenhouse made with 32 old glass DNA sequencing plates," he says. The plant-filled enclosure, 12 feet long and 8 feet high, was a treasured sanctuary. "I'd take my computer to the greenhouse and work there. It was my dream office," says Kunkel.

Has the NAS-botany gene combination been expressed in the next generation as well? There is always the possibility. Kunkel says, "My daughters have been great garden companions over the years and they share the same attitude toward the beauty of nature as I do." Although they have found their own paths to take, only the future will tell whether their names will ever grace the pages of that NAS roster.

There are additional tendrils of hope. "I had my sister's teenage son in my lab two summers ago—and he's very interested in science. But alas, he isn't very interested in flowers."–**Richard Saltus**

A Little Lab Music

What a long, strange trip it's been for Steve Mickelsen, from high school dropout to 37-year-old third-year student at the University of New Mexico School of Medicine. Along the way, he was front man (including a brief stint with purple hair) for two successful bands, developed into an accomplished painter, became a cardiac technician, then a medical student, and most recently an HHMI-NIH Research Scholar.

Mickelsen grew up in Clovis and Albuquerque, New Mexico. After struggling mightily in high school, he decided to pursue music. "I wanted to go to college and I was always interested in science," he recalls. "It's just that schools weren't really set up for me with my dyslexia."

His band, The Bellyachers, got signed to a record label and toured, visiting some of the country's best-known clubs—Hollywood's Whisky A Go-Go and CBGB in New York City, for example. Mickelsen also painted, becoming good enough after 5 years to sell some of his impressionistic work.

Later, inspired by the jazzy music of the beatnik era in the late 1950s, he formed a band called Venus Diablo. During more than one season, viewers of MTV's *The Real World* heard Mickelsen's guitar riffs and what one reviewer called "sweeping baritone vocals."

Critical success in the music and art worlds can still require the oft-dreaded "day job" to make ends meet, so Mickelsen worked as a technician in a cardiac research lab at Lovelace Medical Center in Albuquerque. The cardiac lab stint, however, truly inspired a change of heart. Encouraged by mentor Fred Kusumoto, now at the Mayo Clinic in Jacksonville, Florida, Mickelsen gave academia another—this time, highly successful—try.

He worked hard to overcome the dyslexia by forcing himself to read. "I recognized that there was a vast resource of knowledge, entertainment, and inspiration in books," he says. "With much practice, either I learned to overcome my obstacles or I just became less annoyed and more patient about them." Writing was no longer impossible, though he credits computer spell-check for getting him through college. "Writing and reading still require great effort, but I can now keep up with most of my cohort in medical school."

Mickelsen's creative side was evident in his choice of HHMI-NIH research project at the National Heart, Lung, and Blood Institute. Working with animals, the guitarist went electric, showing that a tiny purposeful cardiac puncture—usually the thing to avoid—could accommodate electrical leads used in correcting arrhythmias (see Lab Book, page 50).

"Many of the conversations I had with musicians when I was working as a tech revolved around the interface between art and science," Mickelsen says of the connections between his various interests. "The creative drive is common to artistic and scientific endeavors. A symphony or a painting is a 'solution' to human emotion like a mathematical equation is a solution to natural phenomena. They are both abstractions that serve to communicate efficiently a kind of beauty and understanding to others." **–Steve Mirsky**

> "A symphony or a painting is a 'solution' to human emotion like a mathematical equation is a solution to natural phenomena."
>
> STEVE MICKELSEN

Douglas Merriam

FEBRUARY '06

upfront

The Key to Making a Perfect Baby. Write that book and you've got yourself a bestseller. One chapter might highlight the work of HHMI investigator Edward De Robertis, who has recently figured out how normal embryos develop. He can show you how to slice a frog embryo in half to yield identical twin tadpoles—and he can name the exact proteins that seesaw up and down during the process. We admit, the book likely wouldn't bump Harry Potter from the top of the charts, but some biologists might want to give it a read.

Solving the Puzzle of the Resilient Embryo

Two types of regulatory proteins working in seesaw fashion ensure normal embryonic formation—even if the embryo is split in half.

Facing off below, identical tadpole twins—generated by cutting an embryo in two equal halves—are indistinguishable from the typical tadpole above.

Courtesy of Hiroki Kuroda/De Robertis Lab

IT WAS IN 1903 THAT BIOLOGIST HANS SPEMANN—USING A LOOP OF HIS BABY daughter's hair and a newt egg—revealed the startling embryological mystery that would persist for more than a century. Testing the adaptability of an embryo, Spemann deftly lassoed the egg with the hair and constricted it so that all nuclear divisions occurred only on one side. Eventually, a nucleus would escape through the constriction to the other side and nuclear divisions would begin there as well. At this point, he would tighten the lasso to completely separate the two sides. To his amazement, both halves developed into identical, perfectly normal, half-sized embryos. This embryonic fail-safe machinery doesn't only reside in amphibians, though. Identical twins often result when such egg splitting occurs in humans.

Now, HHMI investigator Edward M. De Robertis and graduate student Bruno Reversade have made an important advance in revealing the molecular mechanism underlying this remarkable resilience—namely, the "morphogenetic fields" that govern embryonic development. Such fields are gradients of regulatory proteins that guide differentiation of embryonic cells and organize the embryo's overall shape. Although researchers have long known that such fields exist, little was known about the molecular basis of their function.

De Robertis and Reversade, both at University of California, Los Angeles, sought to understand the possible role of the regulatory molecules called bone morphogenetic proteins (BMPs). Other studies have shown BMPs to be key regulators in the dorsoventral (back-to-belly) patterning of embryos whereby dorsal cells differentiate into neural cells, and ventral cells become epidermal cells. Yet no one had been able to demonstrate their role by shutting down the system and eliminating such embryonic "self-regulation."

In their experiments with embryos of the African frog *Xenopus*, the researchers split the embryos into dorsal and ventral halves and used sophisticated molecular techniques to selectively inhibit BMP signaling in each half. They then observed the effects of their manipulations on embryonic development.

The experiments, published in the December 16, 2005, issue of *Cell*, revealed that while the ventral half of the embryo requires specific BMPs for normal development, "It was rather shocking to us that the dorsal part of the embryo developed fairly normally," says De Robertis. Indeed, further experiments revealed that normal dorsal development instead requires a different member of the BMP family, called anti-dorsalizing morphogenetic protein (ADMP).

Importantly, De Robertis and Reversade discovered that the two kinds of proteins in embryo halves are regulated in a seesaw fashion. When the researchers decreased BMP signaling levels, they found that ADMP levels would rise, and vice versa. This compensatory ability is a key to self-regulation in the embryo, according to De Robertis.

Another surprise came when the researchers shut down all the relevant BMP proteins, including ADMP, in *Xenopus* embryos. The entire surface of the embryo became neural tissue. "This is a major transformation of a type you almost never see in embryos," says De Robertis. "It told us that BMPs play a crucial role in the establishment of a self-regulating morphogenetic field for dorsoventral patterning." In fact, when the scientists grafted material from either dorsal or ventral BMP sources into embryos depleted of all BMPs, either of the grafts could restore normal embryo formation.

"We think this finding is important in showing that the embryo is probably patterned by two gradients of BMP—one from the dorsal side and one from the ventral," says De Robertis. "The key to making a perfect baby every time, these experiments tell us, lies in the ability to have a double gradient that will ensure a robust developmental system."

This discovery could also have important implications for efforts to use stem cells to rejuvenate tissues lost to disease or trauma. When cultured in vitro, stem cells tend to differentiate into multiple cell types, as their self-regulatory systems work to produce an embryo. De Robertis suggests it might be necessary to shut down such self-regulation in stem cells to induce them to produce specific tissues. **–Dennis Meredith** ■

> "It was rather shocking to us that the dorsal part of the embryo developed fairly normally."
>
> EDWARD DE ROBERTIS

Hiroki Kuroda

Edward De Robertis (right) and Bruno Reversade

A Life-Altering Chemical

Researchers are beginning to get a handle on dopamine's role in depression and addiction.

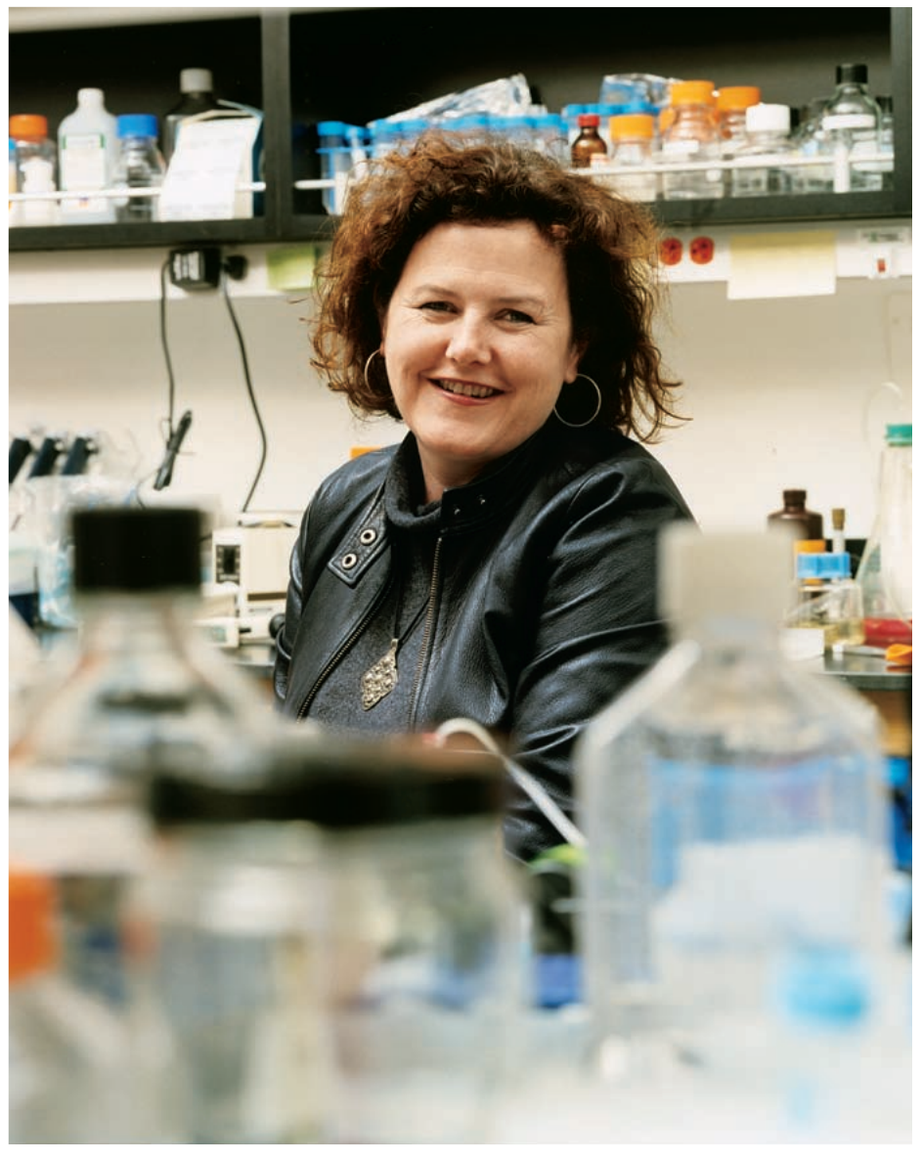
ALTHOUGH LESS THAN 1 PERCENT OF THE BRAIN'S NEURONS PRODUCE dopamine, the neurochemical exerts powerful effects on motivation, reward, learning, memory, sexual desire, and pleasure. "To a large degree, dopamine is what makes us human," says HHMI investigator Li-Huei Tsai. Yet, scientists know relatively little about how this neurotransmitter is so vital for many different behaviors. >> Neurotransmitters such as dopamine and serotonin are molecular messengers released by neurons to communicate information to neighboring neurons. Studies by two independent HHMI research teams are helping to clarify the molecular events that occur after dopamine binds to its receptors. Their findings may lead to new treatment strategies for depression, Parkinson's disease, and addiction.

> "Investigators have begun to focus on the dendrite and its spines as potential sites that are altered during reward and addiction."
>
> ERIN SCHUMAN

In studies published in the July 27, 2005, issue of *Cell*, a research group led by Tsai at Harvard Medical School discovered a molecule that links faulty dopamine signaling in the brain to the neural machinery that breaks down in people with depression. The findings may explain why commonly prescribed antidepressants are ineffective for some people and why, for others, they can take weeks to work.

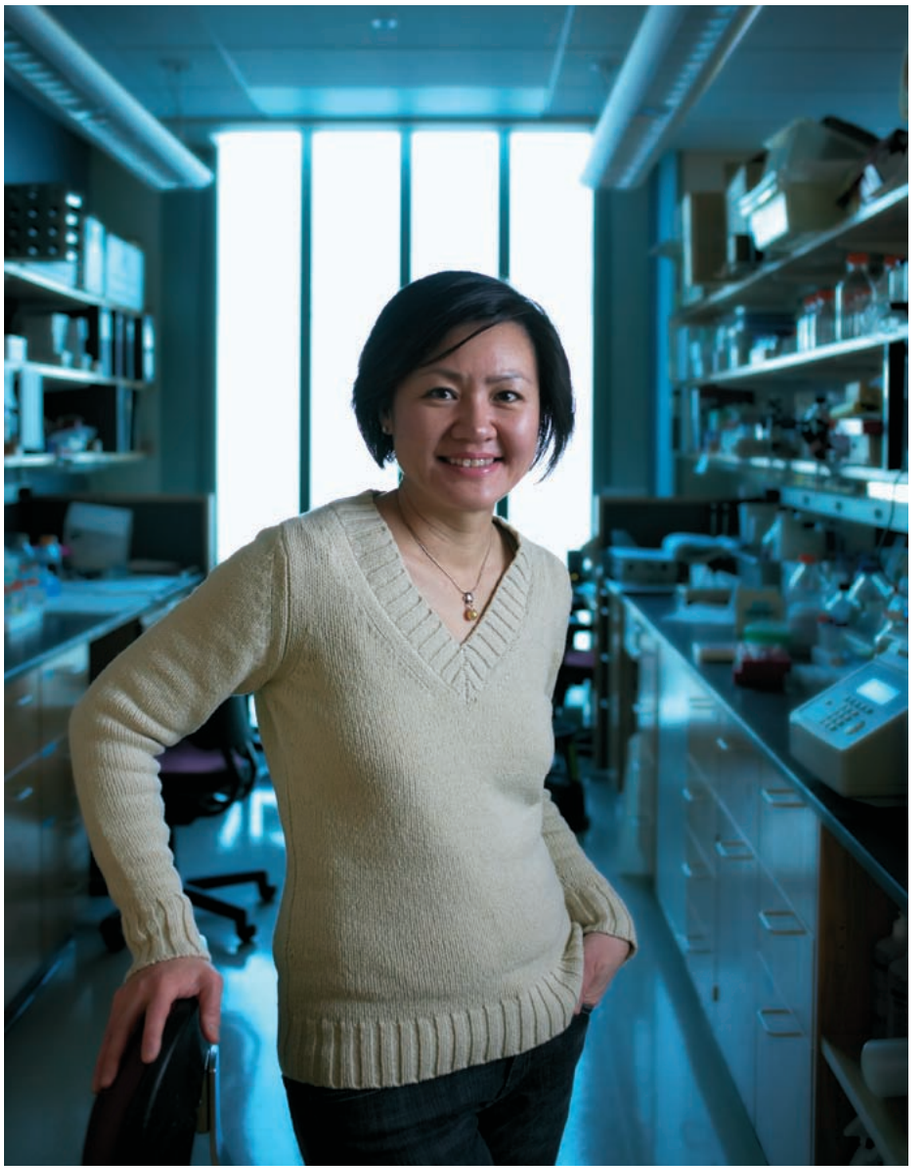
That long lag time has been one of the enduring puzzles in the treatment of depression, says Tsai. Antidepressants work by increasing levels of the neurotransmitters serotonin and/or noradrenaline in the brain. The efficacy of antidepressants, however, may depend more on changes to much later events that occur in the dopamine-signaling pathway.

Tsai and lead author Sang Ki Park wanted to know more about those downstream events—which may involve little-known signaling pathways that are triggered when one type of dopamine receptor, D2, is activated. Park launched



Local stimulation of protein synthesis by dopamine may also modify synapses in the brain during learning, says Erin Schuman.

Misha Gravenor



Li-Huei Tsai believes her lab's findings may lead to antidepressant drugs with improved efficacy.

Sean Kernan

the studies with a broad screen that turned up surprising information: A cell suicide molecule, prostate apoptosis response 4 (Par-4), interacted with a central regulatory segment of the D2 receptor.

The researchers then showed that Par-4 was produced in neurons where D2 receptors function. By knocking out Par-4 in mouse neurons or disrupting its interaction with the receptor, Tsai and Park caused striking behavioral changes in the mice. The knockout mice showed depression-like behaviors in multiple tests, easily giving up when faced with ordinary challenges.

"These are very exciting results for two reasons," Tsai says. "First, they indicate the importance of the signaling pathway mediated by the D2 receptor in depressive behavior. And second, this study pinpoints a specific pathway that implicates Par-4 in this process, which opens new possibilities for developing improved antidepressants."

Synthesize Locally, Act Globally

Approaching dopamine from a different direction, HHMI investigator Erin M. Schuman and her colleague Bryan Smith, both at the California Institute of Technology, discovered how dopamine stimulates the synthesis of proteins in neuronal processes, which may in turn modify synapses in the brain during reward-related learning. The brain's reward circuitry is the top target of addictive drugs.

According to Schuman, scientists knew that dopamine influenced the strengthening of synaptic connections among neurons. This strengthening, or plasticity, causes activation of protein synthesis in the dendrites, which somehow leads to enhanced activity of other kinds of neurotransmitter receptors. However, Schuman says, no one knew how dopamine influences local protein synthesis and triggers plasticity.

Schuman, Smith, and their colleagues introduced the gene for a fluorescent reporter molecule into cultured rat neurons, so that the neurons glow during protein synthesis. When the researchers activated dopamine receptors on the dendrites, they detected the glow in the dendrites, revealing that dopamine activated local protein synthesis and, thus, promoted plasticity.

> "To a large degree, dopamine is what makes us human."
>
> LI-HUEI TSAI

Additional experiments indicated that activation of dopamine receptors triggered immediate enhancement of synaptic transmission in the neurons. "That's a result that people have been seeking for years," says Schuman. "It's a very rapid effect on synaptic transmission that is protein synthesis-sensitive." The findings were published in the March 3, 2005, issue of *Neuron*.

According to Schuman, this research could have implications for understanding and treating drug addiction. "Over the past few years, investigators have begun to focus on the dendrite and its spines as potential sites that are altered during reward and addiction," she says. "This raises the possibility that some of the signaling that goes awry during addiction may have to do with local protein synthesis." **–Susan Gaidos and Jim Keeley** ■

New, Improved Mini Me

The latest mouse models can mimic human disease with greater precision.

KEVIN P. CAMPBELL USED TO STUDY MUSCULAR DYSTROPHY BY analyzing small pieces of thigh muscle from a child with the disease. Today, there's a better option. Campbell can now mimic the biochemistry of muscular dystrophy and test its effects in mice—rather than children struggling with disease—and evaluate tissues that could never be tested before.

>> "With a patient," says Campbell, an HHMI investigator at the University of Iowa Roy J. and Lucille A. Carver College of Medicine, "you never biopsy the diaphragm, but it's a very important muscle because most patients die from respiratory problems. With the mouse, one can test the diaphragm to study the pathogenesis or the response to particular therapies."

An astonishing 99 percent of mouse genes have comparable versions in the human genome, and many of them appear in the same order in the two organisms' chromosomes. "We also have similar reproductive systems, similar physiology, very similar

Mouse models of human cancer help Tyler Jacks explore biochemical pathways regulated by cancer-associated genes.

Flynn Larsen

nervous systems, and so forth," says HHMI investigator Mario R. Capecchi, professor of human genetics at the University of Utah School of Medicine. "For all these reasons, the mouse model is a good representation of human biology."

In the last century, the mouse became the premier mammalian model system for genetic research. Now, the creation of mouse models is "like a cottage industry," says Capecchi. "There are literally thousands of labs all over the world making mutations in mice."

Capecchi pioneered "gene targeting," a technology that has revolutionized scientists' ability to use the mouse to model human disease. This advance of the late 1980s allowed researchers, in their attempts to re-create the possible genetic cause of a specific disease or study the function of a particular gene of interest, to "knock out" the function of that gene or modify its activity.

Since then, researchers have refined gene targeting to create strains of mice with mutations in virtually any gene. They can direct gene mutation so that it occurs in every cell of the body or only in certain tissues or cell populations. And they can control when that mutation occurs—right away, or later in the animal's life span. They can even inactivate combinations of genes, independently of each other, within the same animal.

Mimicking cancer

These advances are critical to faithfully mimic human cancer in the mouse, says Tyler Jacks, an HHMI investigator at the Massachusetts Institute of Technology, because the effects of cancer-associated mutations can depend on the specific type of cell or tissue in which they occur. "Making accurate cancer models requires a good deal of subtlety," says Jacks. "It's important to match the relevant mutations to the appropriate cancer and to pay attention to details ranging from the timing of the mutations to the levels of expression."

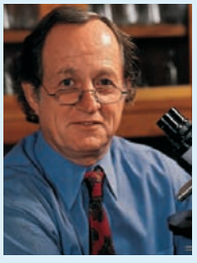
Campbell: Creative Media Group, University of Iowa *Capecchi:* Paul Fetters
Flavell: Harold Shapiro *Heintz:* Robert Reichert

Researchers

KEVIN CAMPBELL
HHMI INVESTIGATOR
UNIVERSITY OF IOWA
COLLEGE OF MEDICINE

Mouse models helped Kevin Campbell's team discover that defective sarcoglycan complex causes constriction of smooth muscle in the vessels of the heart. In addition, looking at brain tissue from their mice, they've found that the dystroglycan protein is a "major player" in the abnormalities in neuronal migration and mental retardation associated with muscular dystrophy.

MARIO CAPECCHI
HHMI INVESTIGATOR
UNIVERSITY OF UTAH
SCHOOL OF MEDICINE



Mario Capecchi has created mouse models to study problems as wide ranging as limb skeletal defects; obsessive-compulsive disorder; and alveolar rhabdomyosarcoma, an aggressive childhood muscle cancer, for which the scientist created the first mouse model.

RICHARD FLAVELL
HHMI INVESTIGATOR
YALE UNIVERSITY SCHOOL
OF MEDICINE

Humanized mouse models are getting big support. Richard Flavell received a $17 million pledge in 2005 from the Bill & Melinda Gates Foundation to develop laboratory mice with immune systems similar enough to humans to allow testing of human vaccines.

NATHANIEL HEINTZ
HHMI INVESTIGATOR
ROCKEFELLER UNIVERSITY

To study neurological function, Nathaniel Heintz introduces large pieces of DNA—called bacterial artificial chromosomes, or BACs—into specific brain cell populations in the mouse. Because the BACs contain a gene and all the regulatory information necessary to express that gene, they can be used to introduce human genes into mice.

> "Today, mice are our test tubes."
>
> NATHANIEL HEINTZ

Jacks studies an oncogene called *K-ras* whose activation has been linked to many different cancers. His group recently developed two mouse models of lung cancer involving *K-ras* that come close to mimicking spontaneous human disease. One strain of mice has an inactive *K-ras* gene in its cells; a second strain has an inactive *K-ras* gene plus a tampered-with version of the tumor suppressor gene *p53*. The genes are engineered in such a way that when triggered—by the introduction of a virus, for example—the oncogenes can be turned on or the tumor suppressor can be turned off, thereby tripping the cellular overgrowth characteristic of cancer. This scenario—mutations in multiple genes, occurring in particular tissues and at particular times in the animal's life span—simulates what we know about cancer initiation in humans.

"That's a powerful tool in the study of lung cancer," says Jacks, "because we are interested in using these models to explore tumor progression, even from the earliest points." In June 2005, his group published a paper in *Cell* identifying a stem cell within the lung as the origin of non-small-cell lung cancers. "We wouldn't have been able to do that without use of sophisticated mouse models to control the initiation of tumor development." **–Mary Beth Gardiner** ■

a cell's second act

RESEARCHERS SET OUT TO UNDERSTAND NUCLEAR REPROGRAMMING TO REVERT ADULT CELLS TO MEDICALLY USEFUL EMBRYONIC STEM CELLS

BY RICHARD SALTUS // ILLUSTRATION BY JASON HOLLEY

Nothing in nature is more elegant—or more humbling to scientists—than the transformation of a single-celled embryo into a unique and complex individual containing, in the case of an adult human, some 10 trillion cells with more than 200 different specialized functions. // Also astonishing, however, is that a cell's career decision isn't necessarily permanent. It can be reversed. When novelist F. Scott Fitzgerald said shortly before his death in 1940, "There are no second acts in American lives," scientists had not yet discovered that committed, specialized human cells could, in effect, go back and start again from scratch.

"Reprogramming"—the erasure of a cell's developmental history—enables it to start over as an uncommitted embryonic cell. The process is central to cloning, which creates an exact copy of an adult organism from the genetic material in one of its cells. A notable example was the birth of Dolly the lamb in 1996, the first mammalian clone. She developed from a fully differentiated adult cell whose nucleus had been turned back in time, developmentally speaking, so that its full set of genes was once again in its original, embryonic state—and capable of giving rise to a new animal genetically identical to Dolly's donor.

First observed and confirmed in a series of experiments from the late 1950s to the mid-1970s, reprogramming is still something of a "black box" to scientists. It appears that unknown factors in the egg's cytoplasm signal the nucleus to erase its specialized genetic program and reactivate previously silenced genes that support the development of a new embryo. But little is known about what these essential factors are or how they turn back the developmental clock.

"It's a wonderful problem and an incredibly fascinating subject," says Allan C. Spradling, a developmental biologist and HHMI investigator at the Carnegie Institution of Washington in Baltimore. "You're talking about one of the most fundamental of the processes that make life possible."

But limited understanding of the process explains why cloning is still an inefficient and error-prone technology. And that hampers the promise of "regenerative medicine"—generating rejection-free repair tissues by cloning a patient's own body cells. The hope is to improve treatments for neurodegenerative diseases like Alzheimer's and Parkinson's, diabetes, heart disease, and spinal cord injuries, for example.

Following the birth of Dolly, pigs, mice, calves, and other animals have been cloned through the transfer of adult-cell nuclei into unfertilized eggs. In addition to nuclear transfer, reprogramming can also be induced by the fusion of certain cells. In 2005, biologist Kevin Eggan and HHMI investigator Douglas A. Melton at Harvard University succeeded in reprogramming human adult skin cells to revert to an embryonic state by fusing them with stem cells removed from embryos. Evidently, the embryonic stem cells contained something that could reawaken the embryonic genes in the skin cells. But there was a problem: The resulting embryonic cells contained a double set of chromosomes, an abnormality that made them unsuitable for practical cloning.

Using his own approach, Yuri Verlinsky, of the Reproductive Genetics Institute in Chicago, reported in the January issue of the journal *Reproductive BioMedicine Online* that he had fused several types of human somatic cells with human embryonic stem cells, and that in some instances the adult nuclei had completely replaced the stem cell nuclei, leaving only one set of chromosomes.

These were patient-specific embryonic stem cells, Verlinsky notes. But when grown in culture, some of the cells had both donor and recipient nuclei, so they'll need further purification before they have any practical value.

These studies and many other lines of research aim to elucidate the factors needed for reprogramming, and eventually free the cloning process from the need for unfertilized eggs or embryos, which are scarce and expensive, and raise ethical concerns.

The ideal, says Melton, who is co-director of the Harvard Stem Cell Institute, "would be if you could take a specialized, differentiated cell from an individual and use some chemical compounds to reprogram it, turning it into an embryonic stem cell" without having to resort to nuclear transfer or cell fusion. "But we are many steps and years away from that."

Dormant Genes Can Be Awakened

Each of our cells contains the full set of DNA instructions, or genome, for making all the proteins that build and operate a human being. According to current estimates, the genome of a human cell carries 25,000 to 30,000 genes on its 46 chromosomes within the nucleus. But the function of any particular cell—skin, nerve, or blood, for example—requires the activity of only a small subset of the genome. It would be not only superfluous but also harmful if a nerve cell made proteins, say, for bone or intestine. So how do cells prevent this from occurring? For many years scientists pondered whether a cell specialized for one role permanently inactivates, or even loses, its genes for making other types of cells—as well as

Kathleen Dooher

the genes that were essentially in its embryonic past.

In 1975, John Gurdon and colleagues at the MRC Molecular Biology Laboratory in Cambridge, England, carried out a series of experiments that built on those first observations, in the 1950s, of nuclear reprogramming. Gurdon transferred the nuclei from adult-frog skin cells into an egg whose own nucleus had been removed. Of all the nuclei placed in the eggs, 4 percent generated fully developed tadpoles, though none of them led to adult frogs. Inefficient and incomplete as the process was, it nailed an important point: Genes in the nuclei of differentiated cells could be reactivated to direct the development of a normal embryo—at least, up to a point. The reprogramming was clearly induced by the cytoplasm of the egg, and it was accomplished within a few hours after nuclear transfer.

Before the work of Gurdon and others, "One might have thought that a cell is locked in—that a blood cell can only be a blood cell—and can never be changed," says Leonard I. Zon, an HHMI investigator and stem cell researcher at Harvard Medical School and Children's Hospital Boston. But the nuclear-transfer experiments suggested that the differentiated cell's chromosomal DNA hadn't been permanently altered; nothing had been discarded, and no part of the genetic sequence had been edited or rewritten.

When Eggan was at the Whitehead Institute in Cambridge, Massachusetts, with Rudolf Jaenisch in 2004, they partnered with HHMI's Richard Axel at Columbia University and brilliantly demonstrated this capacity for the reawakening of genes in even the most specialized of cells. To be attuned to specific odors, mice have hundreds of different types of olfactory sensory neurons: In each nerve cell, just 1 of 1,500 olfactory genes is turned on, while the rest are silenced. The researchers extracted one such cell from an adult mouse and cloned it, through nuclear transfer, to create a mouse that had a full repertoire of smell-sensitive neurons. Clearly, the process had reactivated the entire set of silenced olfactory genes.

That's why reprogramming can occur: The unexpressed DNA is dormant but intact, and can be awakened. These reversible changes in gene activity are due to so-called "epigenetic modifications." Epigenetics, the study of changes in gene silencing that occur without changes in the genes themselves, has become a highly active field—a journal devoted entirely to the subject was just launched in January—and scientists are steadily discovering more about its role in development, reprogramming, and diseases. Most of the events that occur during development, they've learned, are orchestrated by epigenetic modifications triggered by signals from the cell's environment.

"Epigenetics explains many things that happen between development and death," says Jeannie T. Lee, an HHMI investigator at Harvard Medical School and Massachusetts General Hospital. "Not only is epigenetics responsible for setting up a developmental program, it determines whether you are going to get cancer or develop autoimmunity, or get prion diseases such as mad cow."

LEONARD ZON, HHMI INVESTIGATOR, HARVARD MEDICAL SCHOOL AND CHILDREN'S HOSPITAL BOSTON

Inducing Cellular Amnesia

Though epigenetic modification is not well understood, scientists have a broad outline view. "Inside the nucleus of each cell there are proteins that bind to DNA and tell particular genes to be activated or repressed," explains Zon. The accessibility of binding targets on the DNA molecule depends in part on the chromosomes' shape and configuration at any given time. When the chromosomes are densely packed in the nucleus, they offer fewer binding targets. When they're in a more threadlike stretched-out arrangement, chromosomes are 50,000 times longer and that much more accessible to regulatory binding proteins.

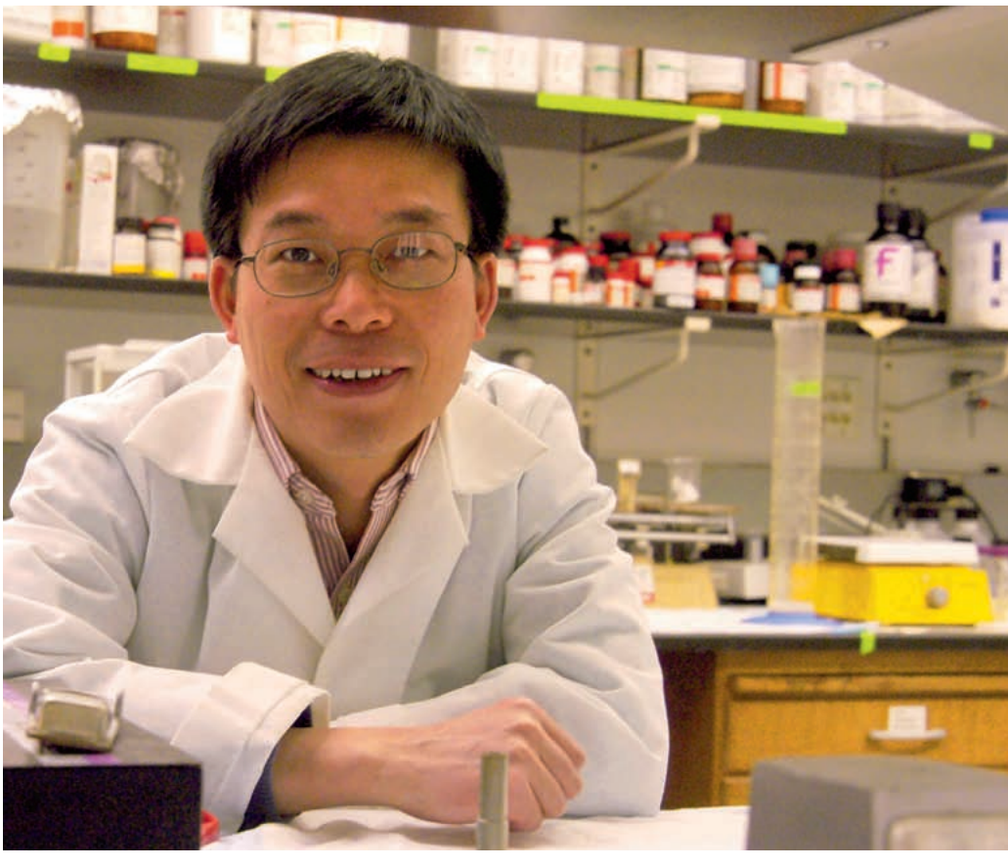
Chromosomal DNA is packaged in a structure called chromatin, which contains small, spool-like proteins, or histones, around which the DNA is wrapped. These histone spools are like scaffolding that determines the configuration of the DNA and its availability for modification by regulatory proteins. Changes in chromatin structure caused by chemical modifications to the DNA or the histones are passed to successive generations of the same type of cell. Zon sums up the big picture like this: "Development from a single cell into a multicellular organism is regulated by environmental signals acting through changes in chromatin, affecting gene expression." The chromatin changes enable genes to be transcribed—that is, expressed—or to be repressed. Appropriate genes are thus turned on or silenced in different types of cells.

Conversely, Zon explains, "Reprogramming is the undoing of the chromatin changes back to the original conformation of the DNA. It's like a drug that erases the cell's memory of what it has been through."

Epigenetic regulation of gene expression is not a simple switch; many control factors have to operate in concert to achieve the appropriate level of expression. One of these factors is methylation—the addition of a "tag," recognizable by cellular proteins, onto one of the chemical bases, or nucleotides, that make up the DNA code. In methylation, a methyl group—a hydrocarbon unit notated as "CH3"—replaces a hydrogen atom on the base. Methylation generally represses gene expression, whereas removing the tag, or demethylation, allows the gene to be expressed.

Nuclear reprogramming occurs not only in cloning, but as a natural process just after a sperm and egg—both highly committed, differentiated cells—join in conception to create an embryo. For the embryo to take its first steps toward development, many genes in the sperm and egg that had been silenced must be reactivated.

"Very rapidly they demethylate, erasing the entire adult cell program," says David L. Garbers, an HHMI investigator at the University of Texas Southwest Medical Center at Dallas. "It's pretty cool, but none of this is well understood." Last November, Garbers reported on a method of keeping rat sperm precursor cells from differentiating into sperm proper. He maintained them—even after freezing and rethawing them—in a nearly stem cell-like state. "We're only one step removed from pushing these cells back a level to a state that is similar to embryonic stem cells," he says. If that could be done, it might be a model for creating embryonic stem cells without harvesting them from embryos.



YI ZHANG, HHMI INVESTIGATOR, UNIVERSITY OF NORTH CAROLINA–CHAPEL HILL

Karen Tam/AP. © HHMI.

Find the Demethylase

Another question that weighs on the minds of cloning experts is whether some of the inefficiencies and abnormalities that plague the present technology result from incomplete reprogramming of the donor nucleus. Perhaps not all of the essential genes are activated or deactivated when the nucleus's adult program is erased. Jaenisch holds this view and points to an important embryonic gene called *Oct-4* that was shown to be incompletely and randomly reactivated when adult cells were reprogrammed during cloning. This might be one of the reasons, he suggests, that most embryos created through nuclear-transfer techniques die.

Jaenisch and others in the cloning community are carrying out a range of experiments aimed at exploring this issue and, more broadly, trying to identify exactly what factors in the unfertilized egg are responsible for reprogramming.

Zon says the answer lies in the trigger for demethylation. "The big prize is to figure out what the 'demethylase' is. If you could identify it, reprogramming research would take a major step forward."

HHMI investigator Yi Zhang, at the University of North Carolina–Chapel Hill, is hot on the trail of this trigger. Last December, he and his collaborators announced they had discovered in cultured human cells a family of proteins that demethylates not the actual DNA of genes, but sites on the spool-like histones in chromatin that package the DNA. This family of proteins is known as JHDM1.

"We're not sure if members of this protein family can remove methyl groups from DNA itself," Yi says—adding he wouldn't be surprised, however, if this turned out to be the case. If so, it would enable researchers to ask questions that could open the door to "artificially reprogramming" an adult cell without the need for a donated human egg.

"It could be that if you put this demethylase enzyme into the donor cell, it would be all you'd need to carry out reprogramming," he says. "But I doubt that it will be that simple."

Harvard's Melton doesn't suggest waiting until one of these efforts to reprogram adult cells without the need for human eggs or embryos pays off. "The hard fact is that, at this moment, the only way to create an embryonic stem cell from a somatic cell is by nuclear transfer into oocytes," he says. "Taking advantage of this current capability is critical if we hope to realize the extraordinary clinical potential of therapies based on stem cell technology." ■

Searching for Wayward Germ Cells

DEVELOPMENTAL STUDIES SHED LIGHT ON STEM CELL BEHAVIOR

Germ cells, which give rise to sperm and eggs, must navigate from the tail end of an embryo, where they form, to a distant reproductive organ where they will do their work. To discover cues the cells use to guide their journey, Ruth Lehmann, an HHMI investigator at New York University School of Medicine, searches for mutant *Drosophila* embryos whose germ cells get lost along the way. • "Our long-term goal is to determine how germ cells are specified, how they are guided during their migration in the embryo, and how a stem cell population is selected that gives rise to egg and sperm throughout the fly's adult life," says Lehmann. • By inserting a genetic element into the genome of developing fruit flies, Lehmann and her colleagues can stain the germ cells blue and then search for wayward blue cells in thousands of developing fly embryos. Using this approach, Lehmann and her colleagues have identified gene mutations that interfere with discrete steps of germ cell migration. • In 2003, Lehmann's lab identified *trapped in endoderm-1* (*tre1*), a mutant fly whose germ cells get stuck at the back end of the embryo and cannot cross the epithelial cell layers that form its midgut. They have since shown that the germ cells in mutants they call *wunen* and *wunen2* cross the midgut, but then wander around the middle of the embryo in a seemingly random fashion, rarely reaching their proper destination in the developing gonads. • In separate studies, Lehmann's group and Ken Howard's group at University College in London, England, showed that the *wunen* genes encode enzymes that remove phosphates from phospholipids, which float in the spaces between cells and help guide germ cells as they wander through the embryo. Recently, Lehmann's team found that in germ cells Wunen appears to be part of the apparatus that detects those phospholipids and uses the lipids for migration and survival. • "The same phospholipids that could be involved in germ-cell migration have been shown to give the timing signal for lymphocytes migrating out of the lymph node," Lehmann notes. If that is true, Lehmann's group may have stumbled onto a navigation mechanism used by many types of cells as they migrate through different tissues.

–Rabiya S. Tuma

Juliana Thomas


UAG
UAG

Trailblazer Turned Superstar

Joan Steitz

started up the scientific ranks when few women did. Today, Nobel laureates laud her research and scores of scientists praise her mentoring acumen.

BY MARGARET A. WOODBURY

Photographs by Ethan Hill

If anyone had told

HHMI investigator Joan A. Steitz when she was an undergraduate chemistry major at Antioch College in Yellow Springs, Ohio, that she would someday run her own research lab, and that a Nobel laureate would call her a star scientist, she most likely would have exclaimed, "Come on!" It's a phrase she uses often, usually meaning: Get real! >> A Minnesota native who came of age in the 1960s, Steitz says she was always interested in science and got plenty of encouragement from her parents, even though a far different model was everywhere around her. "Women of my day," she recalls, "had six kids and a station wagon." >> "At that time, there were no women professors in the natural sciences at any major university," Steitz says. "Consequently, I never envisioned myself being where I am today: I never thought I would teach undergraduates. I never thought I would mentor graduate students. I never thought I would be on the faculty of a prominent university. I really thought I would be a research associate in someone's lab—a man's, of course."

But Steitz persevered, and now holds high ranks in the world of molecular biology. She is Sterling Professor of Molecular Biophysics and Biochemistry at Yale University and has served as chair of her department and as scientific director of the Jane Coffin Childs Fund for Medical Research. Moreover, Steitz has earned an international reputation for her research on RNA—the chemical that delivers DNA's genetic messages and performs an impressive repertoire of cellular functions. "Joan is looked up to as one who has contributed to a cohesive view of RNA science," says Thomas R. Cech, HHMI president and a Nobel laureate for his work on catalytic RNA. "When I became interested in RNA in the early 1980s, she was already a star. Her work continues to evolve and remains at the forefront."

OPENING DOORS

In the early years, Steitz's decisions clearly reflected her doubts. Thinking her chances of autonomy in a laboratory setting were minimal, she set her sights on becoming an M.D. She was accepted to Harvard Medical School, but summer research in the laboratory of Joseph Gall at the University of Minnesota made her change her mind. Instead, in the fall of 1963 she became the only woman in a class of 10 incoming students in a new program in biochemistry and molecular biology at Harvard's Graduate School of Arts and Sciences. James D. Watson—fresh from winning his Nobel Prize for solving the structure of DNA—became her mentor, and the door to science opened a crack.

Steitz soon discovered, however, that the door could just as easily swing shut. During her first year at Harvard, she approached a male scientist she will only identify as "famous, well-respected, and now deceased." Steitz wanted to work with him for her graduate thesis. But Dr. Famous had other ideas, namely that men belonged in the lab and women at home with those six kids and the station wagon. Steitz recalls running from the room and then dissolving into tears, but she now looks back on the event as one of the best things that ever happened to her: She completed her thesis with Watson, and their professional relationship strengthened into a lifetime bond.

These days, it's hard to imagine Steitz running from anything. (She still counts herself as a bit of a shrinking violet, but only because she dislikes being in the spotlight; despite her status, she rarely grants media interviews and is an intensely private person.) "Joan is extremely competitive in her field, as are all good scientists," says molecular biologist Susan J. Baserga, who was a postdoc in Steitz's lab from 1988 to 1993 and is now a faculty colleague at Yale. "She is not afraid to offend, but her manner is such that she gets done what needs to get done in a way that usually *doesn't* offend."

HHMI investigator Jennifer A. Doudna, who was a junior faculty member in Steitz's department at Yale and is now at the University of California, Berkeley, recalls numerous times when Steitz "called a spade a spade"—times when it might have been easier to let things pass. "If a faculty member wasn't being treated fairly, Joan spoke up in a way that I really respected," says Doudna.

Both Baserga and Doudna are vigorous in their praise of Steitz as a scientist, but it is with particular gratitude that they cite her mentorship in teaching them how to navigate the system and the ins and outs of starting one's own lab. For her part, Steitz recalls what it was like not to have other women around in her early days as a scientist—lonely. So she has made it a priority to give the Basergas and Doudnas of the next generation something she didn't have: a female network within the system.

GIANT STEPS

Steitz characterizes her relationship with RNA as a "personal romance" that dates back to her time in Watson's lab and her initial work on bacteriophage RNA. But her first major leap forward in RNA science came during her postdoctoral years overseas. After finishing at Harvard, the newly married Steitz traveled with her husband, Tom Steitz (also an HHMI investigator at Yale), to Cambridge, England. There, in the division run by two more renowned scientists, Francis Crick and Sydney Brenner, she focused her research on determining the exact point on a strand of messenger RNA (mRNA) that binds a bacterial ribosome to begin the manufacture of a protein. (A ribosome is the machine that does protein synthesis in the cell.) Success came in 1969 when she published a paper in *Nature* showing the

nucleotide sequences in bacteriophage mRNA that act as such start points.

She continued her work on bacterial and bacteriophage RNA upon her return to the United States in 1970, when she joined the Yale faculty as an assistant professor of molecular biophysics and biochemisty (her husband also took a position at Yale). By 1975, her efforts were further rewarded when she published how ribosomes identify the start site on a strand of mRNA: by complementary base pairing.

That discovery remains a highlight of her career—in fact, it tops the list, she says. But she rebuffs a request to rank past accomplishments, preferring to talk about what's currently underway. It's clear this energetic woman would rather get on with her research than rehash "such ancient history." Her cramped office—piled with papers and stacks of books everywhere—is a bit mazelike. She apologizes for the mess, but in pro forma fashion that says, come back next year and it'll look the same.

Dozens of empty champagne bottles line a top office shelf, remnants of an honored lab ritual. Each is savored and signed by a student who has successfully completed a thesis. Steitz has a particular smile that flickers beneath her rosy, yet elegant cheekbones when talking about or with her students. It's something her students notice and appreciate. The smile plays there as a message of encouragement, endorsing their right to think aloud even as they sometimes fumble with their biological formulations.

LEFT *Joan Steitz gives high priority to teaching undergraduates and mentoring graduate students.* BELOW *SnRNAs are part of the spliceosome, which splices introns from mRNA. They are the fourth class of RNAs, essential for gene expression.*

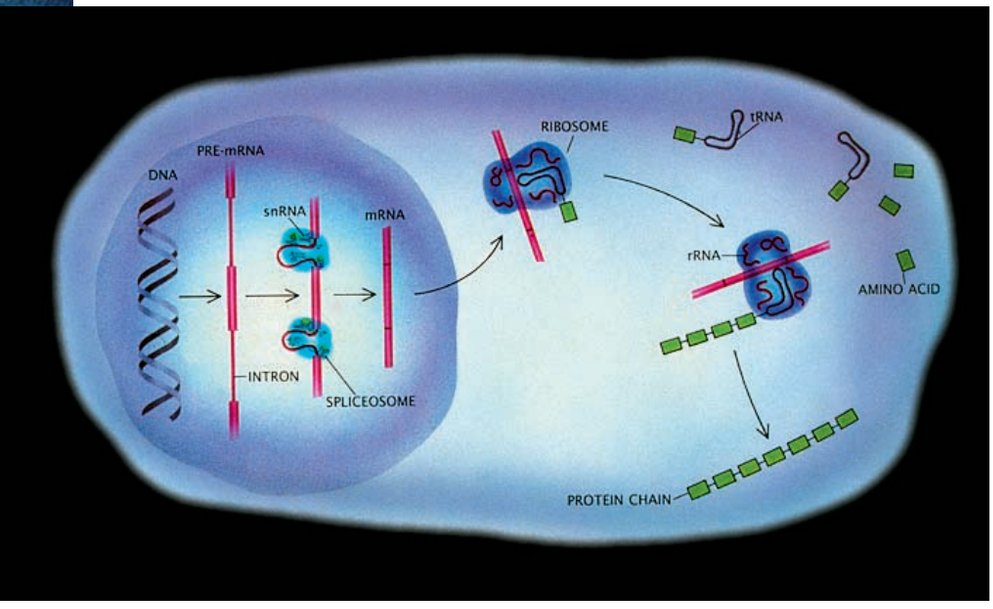


Image by Ian Worpole; reprinted with permission from *Scientific American*, June 1998.

Although Steitz gives top ranking to her work on ribosomes, it was her seminal 1980 paper in *Nature*, "Are snRNPs involved in splicing?", that clinched her scientific reputation. Steitz, like many others in the early 1970s, had turned her attention to eukaryotic cells (a eukaryote is an organism whose genetic material is located within a membrane-bound nucleus). Of particular interest to Steitz was the mystery of why so much RNA was made in the nucleus, but so little—about 10 percent—ever made it out to the cytoplasm to be translated into proteins. As she tried to figure out the answer, experiments by Philip Sharp at the Massachusetts Institute of Technology (MIT), Richard Roberts at Cold Spring Harbor Laboratory, and many others coalesced into the realization in 1977 that the DNA in eukaryotic cells alternates between exons, which contain gene sequences, and introns, which do not code for any protein ("junk DNA").

Yet the discovery of introns did not explain the machinery or how all the noncoding introns were removed from a newly transcribed length of RNA. Steitz kept at the problem, and by analyzing blood samples from patients with an autoimmune disease, she and her student Michael Lerner discovered a novel entity—the snRNP. A snRNP (pronounced snurp) comprises a small length of RNA (about 150 nucleotides long) that is complexed with several proteins. "It turned out," says Steitz, "that the blood samples we analyzed contained antibodies against snRNPs.

"After we discovered snRNPs, we proposed they were involved in splicing [removing the introns from newly transcribed RNA, or pre-mRNAs, as they're now called] and we did the first experiments that showed they were, in fact, involved in splicing," says Steitz. Her lab also determined that it is a particular small nuclear RNA (snRNA), U1, in a snRNP that defines one of the splice sites of an intron via base pairing with complementary pre-mRNA.

Later, other labs coined the term spliceosome for a large assembly made up of several different snRNPs as well as additional proteins. The big mystery—why so little transcribed RNA becomes mRNA—was no longer so baffling.

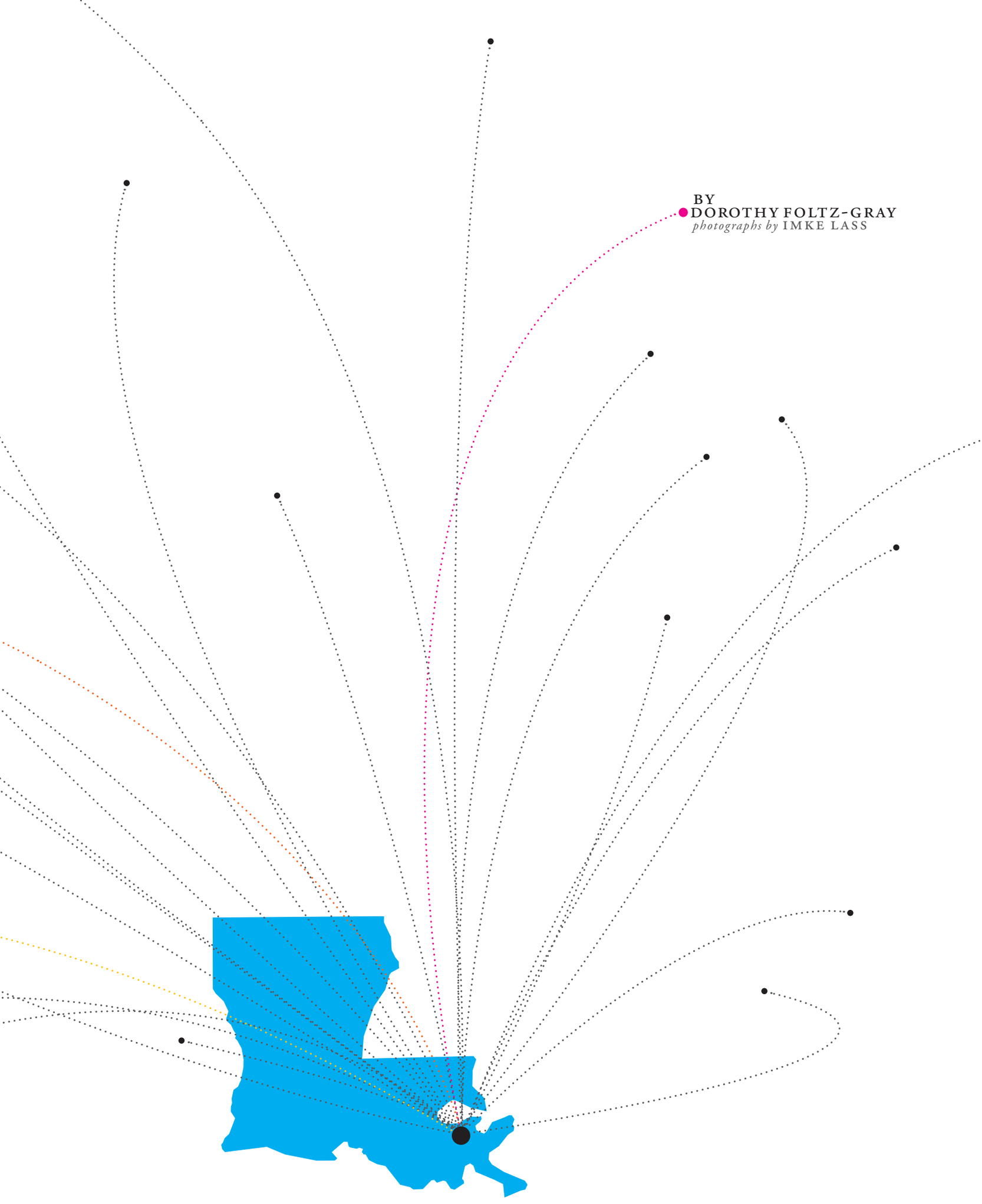
Shortly after Steitz published her celebrated snRNP paper in *Nature*, ribozymes were discovered by Thomas Cech and by Sidney Altman of Yale and Norman Pace of Indiana University. As the name implies, these large molecules of RNA actually have the ability to catalyze a reaction—namely, they can splice or cut strands of RNA. And while it's yet to be proved, Steitz thinks that RNA catalysis is responsible for the cutting and rejoining actions of the spliceosome. "Like a standard enzyme, snRNPs come together and form a spliceosome, do their business, fall apart, and do the whole thing over

CONTINUED ON PAGE 56

A Way Station After Katrina

HHMI responds to help Xavier University retain its displaced faculty, support their professional growth, and ultimately benefit students.

BY
DOROTHY FOLTZ-GRAY
photographs by IMKE LASS



TANYA MCKINNEY // ASSISTANT PROFESSOR OF BIOLOGY, XAVIER UNIVERSITY // UNIVERSITY OF ALABAMA, BIRMINGHAM

McKinney was back at Xavier with the rest of the returning faculty on January 12. Phone service has been in and out, but she's home.

THE MORNING BEFORE HURRICANE Katrina bacteriologist Tanya McKinney and her 2-year-old daughter drove from the city to Mound Bayou, Mississippi, 5 hours away, where her mother lives. McKinney's husband, First Lieutenant Steve L. McKinney of the Louisiana Air National Guard, stayed behind on duty. "I sat in front of my mother's TV, watching Katrina devastate New Orleans," says McKinney, an assistant professor of biology at Xavier University for the past 6 years. "And of course I was terrified for my husband. It was nerve-wracking."

Within a few days, McKinney had word from her husband that both her home and workplace—Xavier is located in the heart of Orleans Parish in New Orleans—were flooded, and that 5 years of scientific research had been destroyed.

McKinney was creating mutant bacterial strains of *Staphylococcus aureus*, a bacterium that is the most common cause of food poisoning. "Although high salt concentrations kill most bacteria, *S. aureus* survives. If we could understand how, then we might be able to better control it."

She was investigating which staphylococcal genes are regulated in various salt concentrations. It was a time-consuming process, but by last summer's end, she finally had identified certain genes and constructed the necessary mutant strains to begin exploring the role of specific proteins. "I was very excited," she says, "but now I have to start over."

Many of McKinney's colleagues at Xavier—one of HHMI's longtime undergraduate science-education grantees—have reported similar losses. Every research scientist with frozen or refrigerated specimens lost all they had, says Elizabeth Barron, the university's vice president for academic affairs, and a lot of scientific equipment was damaged. Most heavily affected were faculty in the departments of biology and chemistry and the College of Pharmacy, says Tuajuanda Jordan, former associate vice president for academic affairs. "They will have to start over, though if their computers were not submerged, they should be able to save their data." Throughout the campus, however, floodwaters rose up to 6 feet, submerging the first floors of 39 buildings and destroying any computers situated there. The school also lost its central power plant.

Yet, Xavier stood to lose its most precious resource of all—its faculty. Without salaries or homes, most would be forced to find employment elsewhere. People at HHMI quickly grasped that problem, and within days of the storm, Hanna H. Gray, chairman of the Board of Trustees, conferred with Institute staff to see what they could do to help.

Providing Safe Harbor

IT IS NO SURPRISE THAT HHMI ZEROED IN ON XAVIER, a historically black university that has received $7.6 million in HHMI grants since 1988. HHMI selects grantee institutions based on their success in sending students to medical school or graduate science programs—an area in which Xavier excels, says Peter J. Bruns, HHMI vice president for grants and special programs. "Xavier puts more African American undergraduates into medical school than any other college or university of any size in the country," he says. "And it ranks in the top 50 of all U.S. universities for graduating chemistry and physics majors."

By September 16—fewer than 3 weeks after the storm—HHMI President Thomas R. Cech had sent a letter to HHMI investigators across the country, asking them to consider including Xavier science faculty on their research teams. Just

as rapidly, HHMI's undergraduate grants staff—Director Stephen Barkanic and his colleagues, Program Officer Patricia Soochan and Program Assistant Mary Bonds—set up a structure for matching Xavier faculty with their scientific hosts. The Institute would fund the sabbaticals, for 9 months at $5,600 per month, of any Xavier science faculty who participated. It also extended offers to members of the staff and students who accompanied them, offering $3,600 and $2,000 per month, respectively, and would pay for expenses such as relocating computers or purchasing special supplies.

Within a week, 200 HHMI investigators had offered places for as many as 360 faculty and 80 students in their labs and institutions. Xavier administrators were scattered around the country—President Norman C. Francis set up an office in his sister's home in Grand Coteau, Louisiana, Vice President Barron was in Gatlinburg, Tennessee, and Jordan worked from HHMI headquarters in Maryland. Yet getting word to the now far-flung faculty, staff, and students was relatively easy because of an online faculty registry that Xavier already had in place for hurricane emergencies. HHMI posted investigators' offerings on the Xavier Web site, updating it as opportunities opened or closed. The first faculty member signed up on September 22, and by October 10, Jordan and the HHMI team had been in touch with 75 Xavier science faculty who asked for support. Ultimately, 62 faculty, one staff member, and two students were placed.

HHMI also was flexible about placements—approving some, for example, outside the HHMI investigators' offerings. Such was the case for McKinney. When her former graduate school adviser Janet Yother, professor of microbiology at the University of Alabama, invited McKinney to work in her Birmingham lab, McKinney loved the idea. But sorting out the finances would take time. That's when she learned about the HHMI program. "Other people had offered me opportunities that weren't in my field, where I could make a contribution," says McKinney. "But HHMI was flexible enough to say, 'If you have someone in mind to work with, just send us your work plan.'"

ASHLEY FORNERETTE
SENIOR, BIOLOGY-EDUCATION, XAVIER UNIVERSITY
TEXAS TECH UNIVERSITY, LUBBOCK

Fornerette is back in class. The sidewalks near Xavier are piled high with debris from houses that have been cleared of their ruined contents. It's a daily reminder, she says, of how hard it was to see her childhood memories thrown onto the sidewalk in a mountain of unidentifiable junk. "Sure it's garbage now, but before Katrina it wasn't."

Deep Loyalties

ALTHOUGH MCKINNEY COULDN'T RESUME HER research on staphylococci at the Alabama lab, she has nonetheless found the work there stimulating and has been productive. Since early October, she has been attempting to isolate and identify an enzyme from *Bacillus circulans*, which degrades the capsule around *Streptococcus pneumoniae*, a bacterium that causes pneumonia. Her collaboration with Yother has also introduced McKinney to new and effective procedures. For instance, a graduate student is showing her how to use a phage display system, an easier and less expensive way to screen protein interactions than any she has used before. "I'm going back to Xavier a better teacher and researcher," she says.

RAY LANG // ASSOCIATE PROFESSOR AND CHAIR OF COMPUTER SCIENCES AND ENGINEERING, XAVIER UNIVERSITY // WASHINGTON UNIVERSITY, ST. LOUIS
Lang finally returned to his house when utilities were restored—on New Year's Day. He started work at Xavier on January 12, where he'll continue the research he began in Sean Eddy's lab in St. Louis.

In fact, McKinney never considered not returning. Since college, where she had few black science professors, she has wanted to be a role model for minority students. "At Xavier I can mentor and encourage science students, and also do my research there with funding and equipment. It's too valuable an asset to be lost or diminished. I want to help rebuild it."

Vladimir Kolesnichenko, an assistant professor of chemistry, and his wife, Galina Goloverda, an associate professor of chemistry, have similar feelings about Xavier. The couple left New Orleans the day before Katrina's arrival with their 15-year-old son, Igor, and with friends who didn't have a car, driving to Lafayette, Louisiana. A close friend in Iowa City, Iowa, Ronita Lebeau-Meyerdirk, who knew they were camping near Lafayette, called campground after campground until she found them, whereupon she invited the family to her home. Kolesnichenko and Goloverda were eager to return to Iowa City, where they had lived from 1996 to 1998. They knew professors at the University of Iowa—both of them had worked there—and their son had local friends and could easily slip into school.

"Lou Messerle [an associate professor of chemistry], whose interests are very close to ours, said we were welcome in his lab," says Goloverda, an organic chemist. "As soon as I learned about HHMI's program, I wrote to Dr. Jordan, explaining Messerle's offer. So she made it happen."

Darrell Eyman, an associate professor of chemistry at Iowa, convinced the university's administration to allow Kolesnichenko, a materials chemist, to develop and teach a new graduate-level class in nanochemistry. Jordan again intervened constructively, by ensuring that Kolesnichenko received the difference between the Iowa salary and the higher HHMI stipend.

Assured of income, the two scientists could get back to work. They were collaborators at Xavier, Kolesnichenko developing nanocrystals and Goloverda wrapping them into the shells of organic compounds that make the particles soluble and stable in water. "We target magnetic nanoparticles that can respond to an external magnet passed over the body, carrying drugs to a specific site," she says. "The idea was not ours, but we are trying to improve the drug delivery."

They say their work and their colleagues at Iowa have been a gift during a very difficult time. "We dig through publications and do experimental work in the labs," says Kolesnichenko, who divides his time between teaching and lab research. "Our work here gives us the opportunity to get new ideas that might be applied to our research at Xavier."

Like McKinney, these two scientists have no doubts about returning to New Orleans. "We have never felt like it was just a job," says Goloverda. "It was always a mission, where you have something to offer and the students are happy to accept it. Some of them never before had a good opportunity to learn, and they are ready. That makes our efforts very rewarding."

A Good Place During Personal Loss

RAY LANG, ASSOCIATE PROFESSOR AND CHAIR OF computer sciences and engineering, never considered quitting Xavier either. "I felt very lucky to get a job there," he says, "and after 12 years I'm very invested."

The Gulf Coast's perennial hurricanes were almost ho-hum for Lang, a New Orleans native. Despite dozens of tree-snapping storms, he'd never felt the need to evacuate. But the night before Katrina hit, he and his partner Alex Sanabria realized that the hurricane was going to be a monster—and that it was too late to leave. Six days after the storm, the couple was evacuated by

boat. After 16 hours with tense and sometimes unruly mobs at the New Orleans airport, and a ride in a cargo plane to Austin, Texas, they finally flew with their cairn terrier Caesar to the home of a friend in St. Louis, Missouri.

When Lang heard about the HHMI program, he combed the offerings for a spot and found HHMI investigator Sean R. Eddy, an associate professor of genetics at Washington University in St. Louis, who praises both the HHMI program and his guest researcher. "Ray has research experience in an area of computational linguistics that we use extensively in DNA- and protein-sequence analysis, so it was a really great fit," says Eddy.

Lang's feelings are mutual. The placement and new colleagues have provided him with both expanded opportunities and a distraction from personal grief: Sanabria, ill with liver disease before the storm, became weakened by the stress of Katrina and the move and died 6 weeks after the pair arrived in St. Louis. Says Lang: "We'd been together 26 years."

Lang was in a good place, however, at that difficult time. "Everyone at Washington rolled out the red carpet for me. I began by giving a presentation about my work. Since then, I've been writing a compiler [a program that translates code for a computer] to describe the secondary structures of RNA, an area somewhat connected to what I was doing at Xavier. And working here has been a fabulous opportunity to make the transition into computational biology, which uses techniques from mathematics, statistics, and computer science to solve biology problems like the alignment of gene sequences."

Confidence in Xavier's Future

THESE XAVIER FACULTY ARE ACQUIRING new methods and directions and continuing to do research in the face of huge losses—exactly what HHMI and Xavier administrators hoped the program would offer. "We are a tuition-dependent university, so right now we have no income," explains Vice President Barron. "Being able to pay the salaries of the faculty allows us to keep more of our teachers. But most significant is that HHMI found opportunities that allow faculty to grow, which will enrich our students' research experiences as well."

Xavier senior Ashley Fornerette, 21, a biology-education major, is certainly realizing that benefit. After the hurricane, Xavier biology professor Ray "Trey" Brown asked Fornerette to join him in his lab at Texas Tech University in Lubbock, and she was delighted to do so. Although Brown is not returning to Xavier, Fornerette—who plans to go to graduate school in environmental toxicology—wants her Xavier degree. "At Xavier, you can get to know your professors," she says. "You're not just a name in a grade book. Xavier has molded me."

Among its 4,121 full-time students pre-Katrina, 3,118 said they planned to return when the campus reopened on January 17. Two-thirds of the faculty said they would return; 50 of them are living temporarily in mobile homes or trailers on campus. About one-third had to be laid off, though Xavier administrators hope to rehire many of them next September. HHMI will continue to fund about 56 faculty at Xavier until September 2006.

Of course, challenges remain. Reconstruction alone will cost between $30 million and $40 million, says Xavier President Francis, but he is confident of Xavier's survival. "We have a dedicated faculty overall. The science faculty teaches as a team, and they know what the students have been taught the semester before. It's a very managed process, and it works. That's why we have the record we do. We teach students what academic life is about, and we graduate people who are ready to excel." ■

Paul Fetters

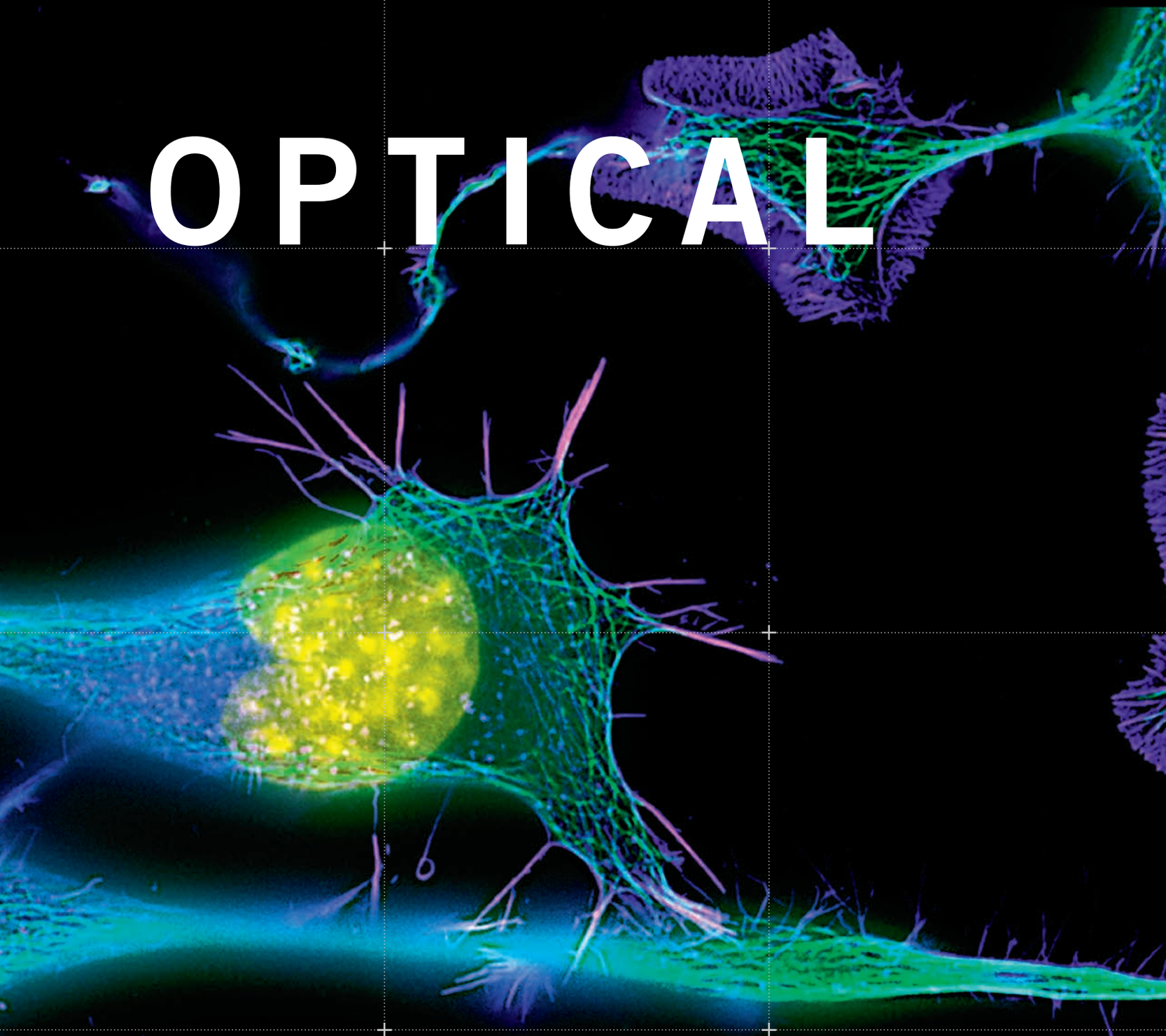
The Storm Brought Her Home

Tuajuanda Jordan grew up in Forestville, Maryland. So after she and her 15-year-old twins were evacuated to Dallas after Hurricane Katrina flooded New Orleans, the three decided to head back to Maryland, to her parents' home, about 20 miles from HHMI headquarters. • *Learning that Jordan,* Xavier University's associate vice president for academic affairs, was in Maryland, Stephen Barkanic, director of HHMI's undergraduate grants program, offered her office space and use of Institute facilities to do her work for Xavier. Meanwhile, her kids, Jordan and Patrice Starck, quickly became ensconced at the Bullis School in Potomac. Not wanting to disrupt their education any further this year, Jordan planned to return to her job at Xavier and commute to Maryland on weekends to be with them. However, she realized this arrangement just wouldn't work. • *When word got out that Jordan* intended to stay in Maryland, Peter Bruns, HHMI vice president for grants and special programs, offered her a position as HHMI's senior program officer in charge of science education. "I was taken aback," she says, "and happy that they had enough faith in my ability to make me an offer like that on the spot." • *The decision to leave* Xavier after 11 years wasn't easy, though. "I don't know anyone who's worked long-term at Xavier who isn't absolutely committed to the institution," Jordan says. "Writing my letter of resignation was one of the hardest things I've had to do in my adult life." On the other hand, her relationship with the school may continue on a different level. "There's lots of science education going on at Xavier," she says, "so I expect to work with the university again."

ASPIRATIONS

At Janelia Farm, researchers will link mathematics, physics, engineering, and computing in the pursuit of better cellular pictures. BY DAN FERBER

OPTICAL



MOUSE NEUROBLASTOMA CELLS WERE STIMULATED TO DIFFERENTIATE IN VITRO, AND THEN LABELED FLUORESCENTLY. THE IMAGE, CAPTURED WITH AN EPIFLUORESCENCE MICROSCOPE, SHOWS ACTIN (BLUE) AND MICROTUBULES (GREEN) IN TWO SUCH CELLS DURING THE EARLY STAGES OF NEURITE OUTGROWTH (DNA IS YELLOW). INDIVIDUAL CHANNELS WERE SUBSEQUENTLY OVERLAID AND COLORIZED. PHOTOGRAPH COURTESY OF TORSTEN WITTMANN

IN THE WORLD OF BIOMEDICAL RESEARCH,

there are big-idea guys and detail guys, and Nikolaus Grigorieff is a detail guy. He doesn't just sweat the details—he thrives on them. For the problems he addresses, details are everything. Grigorieff, an HHMI investigator at Brandeis University, has been appointed one of the first group leaders at HHMI's new Janelia Farm research campus, which opens later this year. He uses electron microscopy to visualize tiny three-dimensional protein structures inside cells. That means grappling with a sea of details. A single molecular machine may contain dozens of proteins, each with hundreds of amino acids. To understand how it works, you need to "open it up, see what's inside, and see how those bits and pieces fit together," Grigorieff says. And that's where microscopy comes in.

Since Theodor Schwann first peered inside animal cells in the 1830s, curious biologists have sought to identify cellular components and comprehend how they work together. But until recently, light microscopes could not distinguish objects much smaller than a mitochondrion—an organelle about one-fiftieth the diameter of a typical cell. Electron microscopes have long helped scientists like Grigorieff make out smaller objects, such as molecular machines and small organelles—but only in dead, chemically fixed cells. To better understand such objects, biologists tried to obtain sharper images of them and see them in action. But until the last decade, they had little luck.

Now that's changing. "Every 20 years or so there's a big technical advance that really changes the way biomedical research is done," says Gerald M. Rubin, vice president of HHMI and director of Janelia Farm. In the late 1950s, x-ray crystallography allowed biologists to see the atomic structure of proteins, which carry out most of the work of the cell; in the 1970s, cloning and DNA sequencing led to new insights into evolution, gene regulation, and the biochemical workings of individual proteins.

At Janelia Farm, HHMI has focused a good deal of its efforts on developing new microscopy methods and new computing methods to analyze images. Nationally, the organization is investing tens of millions of dollars each year in microscopy. That's because imaging, Rubin says, "is the most important technology that we need now."

Limited Vision

THE HUMAN EYE can readily distinguish objects as small as 100 micrometers across, about the width of a human hair. A typical human cell is about 10 micrometers in diameter and therefore invisible to the naked eye. As of the early 1990s, even state-of-the-art light microscopes could distinguish only objects larger than 0.2 micrometer in diameter—half the wavelength of blue light—which meant that smaller but important structures like ribosomes and spliceosomes were impossible to see in living cells. Biologists' vision was restricted by the light microscope's resolution—its ability to create a sharp image by distinguishing between two adjacent points. Optics dogma dictated that resolution was limited to about half the shortest wavelength of light used, so many scientists thought it could get no better.

Cellular structures were also difficult to view because they're usually transparent, making it hard to distinguish them from the watery cytoplasm in which they sit. Phase-contrast microscopes made that easier by using interfering light waves to distinguish cellular structures from background. And biologists developed a plethora of chemical stains and fluorescent antibodies that bound to specific cellular structures, making them visible. But cells usually had to be killed first, and scientists then had to surmise what the structures did when the cells were alive.

To see molecular machines and small organelles, scientists used transmission electron microscopes, which utilize electromagnetic coils to focus electron beams instead of glass lenses to focus light. But electron beams destroy biological tissue, so researchers could only see into dead, chemically fixed cells. And electron microscopists like Grigorieff who wanted to determine the atomic structures of protein complexes could not do it. So biologists filled textbooks with descriptions of what they could see, saying little about what they couldn't.

Freeze Frame

IN A DARK, high-ceilinged room near Grigorieff's laboratory, Carsten Sachse pours liquid nitrogen into a stainless steel sample holder on the side of an electron microscope, a foot-thick gray column taller than an NBA star. It hisses and boils off an icy steam.

He points to a computer monitor next to the microscope, to an image of gray fibers packed tightly side by side. They're lab-grown fibrils of amyloid beta peptide, the molecule in the brain suspected of causing Alzheimer's disease, magnified 59,000 times. The regular packing of the fibrils means that Sachse, a visiting graduate student who works with Marcus Fändrich at the Leibniz Institute for Age Research, in Jena, Germany, may well be able to use electron microscope images of similar preparations to determine the three-dimensional atomic structure of amyloid peptide packed into fibrils. Understanding that could be key to blocking tissue damage in Alzheimer's patients. "It looks very promising," he says.

Electron microscopy, Grigorieff says, can help elucidate the molecular structures of complexes too big to analyze by crystallography or nuclear magnetic resonance yet too small to see with a light microscope. "EM is a good technique to bridge the gap to high resolution," Grigorieff says.

But not just any electron microscope. Grigorieff's $2 million microscope contains a 300,000-volt field-emission electron gun to accelerate electrons through relatively thick samples, while ensuring they march in lockstep—a property needed to enhance contrast. It

has specialized CCD cameras—tricked-out large-format digital cameras, essentially—to capture electron images and diffraction patterns. The researchers run recently developed algorithms on high-powered clusters of computers to turn huge amounts of electron microscopy data into three-dimensional images. And the darkened room where Sachse works contains a $400,000 climate-control system that prevents even the smallest drafts and shifts in temperature—all to keep their samples extraordinarily steady, which they must do to obtain good data.

Such attention to technology has paid dividends for Grigorieff. Working in collaboration with HHMI investigator Melissa J. Moore of Brandeis University and former postdoc Melissa Jurica, the group got the first-ever glimpse of the three-dimensional structure of the spliceosome, a molecular machine that splices newly formed RNA to form messenger RNA, which in turn encodes the correct amino acid sequence of the protein. The researchers obtained thousands of electron microscope images of single frozen spliceosomes and then reconstructed the complex's three-dimensional shape on a computer. The result: a cylinder on a hollow ovoid domain with an armlike extension that seems optimized to perform the contortions necessary to cut and splice a threadlike RNA molecule.

Grigorieff's team has also determined the structures of other cellular machines, including, in collaboration with HHMI investigator Axel T. Brünger of Stanford University, a molecular machine called NSF that helps nerve cells export packets of molecules that enable them to signal their neighbors, and, with Thomas Walz of Harvard Medical School, a soccer-ball-shaped delivery structure called a clathrin coat. "We need to know what these molecules look like," Grigorieff says.

For particularly complex molecular machines, it's often necessary to combine methods. David A. Agard, an HHMI investigator at the University of California, San Francisco, uses a technique he's optimized called cryoelectron tomography that takes images of particles from different angles and then assembles those images into a three-dimensional model. He's used the method to visualize the centrosome, an organelle that manages the cell's internal skeleton. To show how the centrosome carries out that task, he uses single-particle electron microscopy and x-ray crystallography to view its components at atomic resolution.

AT JANELIA FARM, Nikolaus Grigorieff says, he'll try to push single-particle cryoelectron microscopy methods to "routinely get to such high resolution that you can build an atomic model."

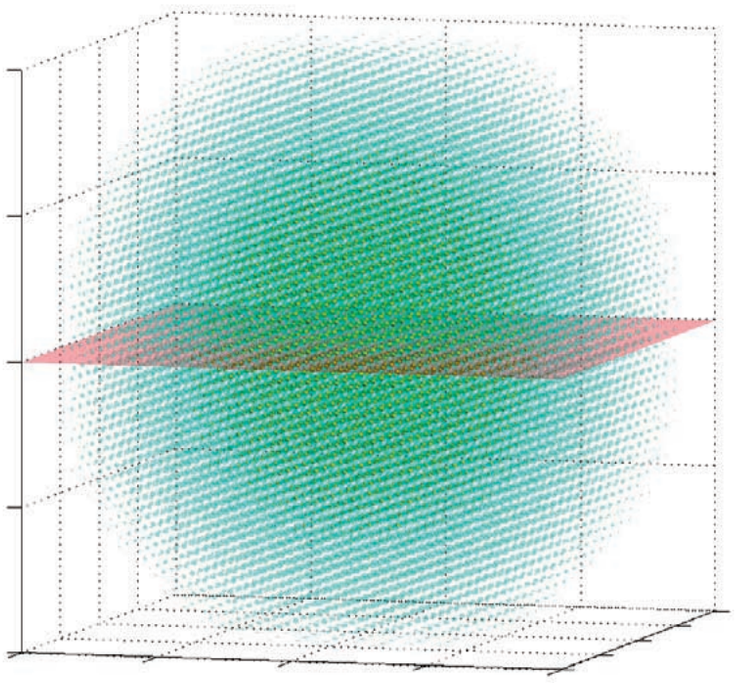


Illustration courtesy of Eric Betzig

A SPHERICAL REGION OF ILLUMINATION WITHIN AN OPTICAL LATTICE MICROSCOPE MAY CONTAIN THOUSANDS OF POINTS OF LIGHT FOR MASSIVELY PARALLEL, RAPID IMAGING OVER A LARGE VOLUME WITHIN A CELL.

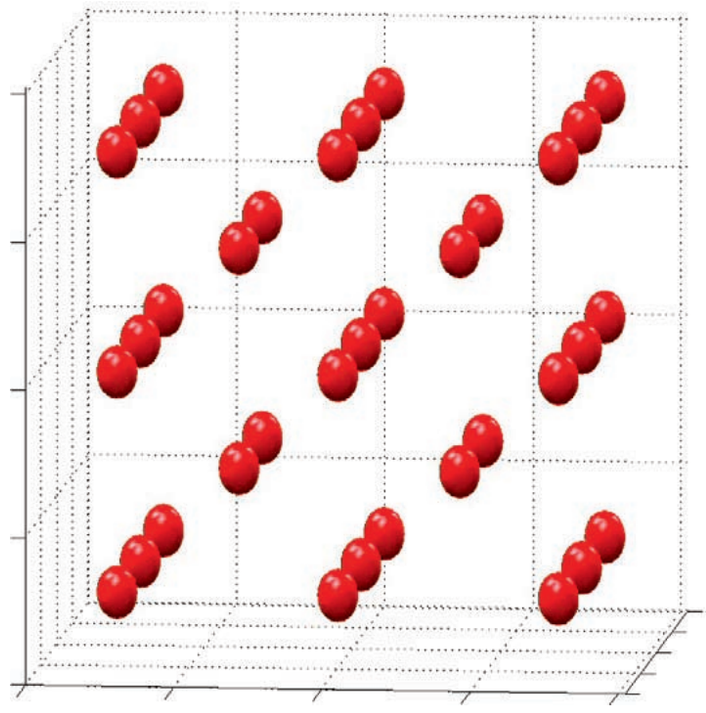
Grigorieff and others also use electron microscopy to visualize two-dimensional crystals of membrane proteins in a lipid bilayer, much like that in the cell. He and others have even used electron microscopy to visualize membrane proteins in atomic detail. In December, Walz reported using this technique, called electron crystallography, to determine the three-dimensional structure of a cellular water-pore protein called aquaporin; the resolution was high enough to spot discrete lipid molecules clinging to the side of the protein. Electron microscope images of single protein complexes (as opposed to crystals) have lower resolution, but they can distinguish parts of proteins, such as helices and loops. At Janelia Farm, Grigorieff says, he'll try to push single-

particle cryoelectron microscopy methods to "routinely get to such high resolution that you can build an atomic model."

Going Live

AT JANELIA FARM, other scientists will press light-microscopy methods to observe cells or even entire brains at high resolution. Janelia Farm group leader Eugene W. Meyers, who wrote programs that dramatically sped the sequencing of the human genome, will design new computing methods to assemble three-dimensional reconstructions of working brains from two-dimensional microscope images. Group leader Karel Svoboda, currently an HHMI investigator at Cold Spring Harbor Laboratory, will use two-photon excitation microscopy, a form of fluorescence microscopy developed in the 1990s, to observe living neurons in mouse brains. "We need new imaging methods to figure out how the brain works," Rubin says.

A CLOSER VIEW WITHIN AN OPTICAL LATTICE MICROSCOPE REVEALS INDIVIDUAL, TIGHTLY FOCUSED EXCITATION POINTS (RED) ARRANGED IN A PERIODIC ARRAY.



KAREL SVOBODA AND OTHER researchers use two-photon excitation microscopy to peer into opaque tissues like the brain—an ability akin to Superman's x-ray vision.

Janelia Farm biologists will make use of more than a decade of extraordinary advances in light microscopy. Until the early 1990s, some biologists examined cell shape and behavior by using light microscopy, while others tried to understand their protein of interest by localizing it in cells that had been killed and fixed, says Doug Murphy, a cell biologist at Johns Hopkins School of Medicine in Baltimore who will move to Janelia Farm to direct its shared light microscopy facility. "Now we don't just want to see where it is, but how it's behaving."

New fluorescent probes make that possible. In the mid-1990s, HHMI investigator Roger Y. Tsien of the University of California, San Diego, began working on a naturally fluorescent jellyfish molecule called the green fluorescent protein (GFP). Since then, he and others have developed GFP variants that glow red, yellow, and many other colors. Biologists quickly learned to tag proteins by fusing them with GFP or its cousins. Today biologists can follow several differently colored proteins simultaneously in live cells and in real time. "To see molecules zip around inside living cells and tissues—that's been huge," Svoboda says.

Xiaowei Zhuang, an HHMI investigator at Harvard University, makes movies of single fluorescently tagged influenza viruses invading cells. Others had used electron microscopes to spot influenza virus in membrane-bound compartments called coated vesicles and endosomes, indicating that cells engulfed the viruses in a process called receptor-mediated endocytosis. But no one knew whether the virus homed in on existing pits in the membrane or induced the cell to create new ones.

Zhuang's movies showed a red virus surrounded by a green coat of clathrin molecules, which coat membrane pits. The pits then bud off to become coated vesicles. "We actually saw pits grow right outside a virus," she says. That means the virus most likely persuades the cell to take it up by receptor-mediated endocytosis—a result she confirmed by statistical analyses. Until Zhuang made her movies, no one knew influenza viruses used this trick to invade cells. Drugs that target key parts of the process could one day help block viral infection.

Svoboda and other researchers use two-photon excitation microscopy to peer into

Illustration courtesy of Eric Betzig

opaque tissues like the brain—an ability akin to Superman's x-ray vision. Optically, brain tissue resembles milk, he says. The interface between fat and water "acts like little mirrors" that scatter light and make the substance appear white, which makes it impossibly murky under older fluorescent scopes.

In two-photon excitation microscopy, however, a new type of laser emits bright, synchronized pulses of infrared light that are focused on tiny volumes in the cell. Tagged molecules fluoresce only when they simultaneously absorb two such photons. Outside of the zone of focus, that's rare, so the method dramatically reduces background. Recently, Svoboda's team watched single nerve endings fire by combining the method with a second fluorescence method called fluorescence resonance energy transfer (FRET).

FRET works like this: One fluorescent dye emits light of a specific color that excites a nearby dye to glow a different color. By tagging one protein with the donor dye and a second protein with the acceptor dye, scientists can see when and where the two proteins interact. Svoboda chose proteins that would come together only when an enzyme called Ras was activated. It turned out that activation of single synapses in the hippocampus activates Ras, which then stimulates the synapse to reshape itself—a phenomenon that underlies learning.

Optical Advances

RECENT ADVANCES IN fluorescence microscopy occurred only because microscope designers focused on developing and adapting new technologies. To see GFP and its cousins, for example, they built new lenses from materials that allow for brighter samples and greater contrast, created thin-film interference filters that transmit only a specific color of light, and replaced film cameras with low-noise CCD cameras to record very dim fluorescence. Two-photon excitation microscopy required new microscope objectives that were transparent to infrared light, and mode-locked pulsed lasers, developed in the 1980s, to create very short, very bright pulses of photons.

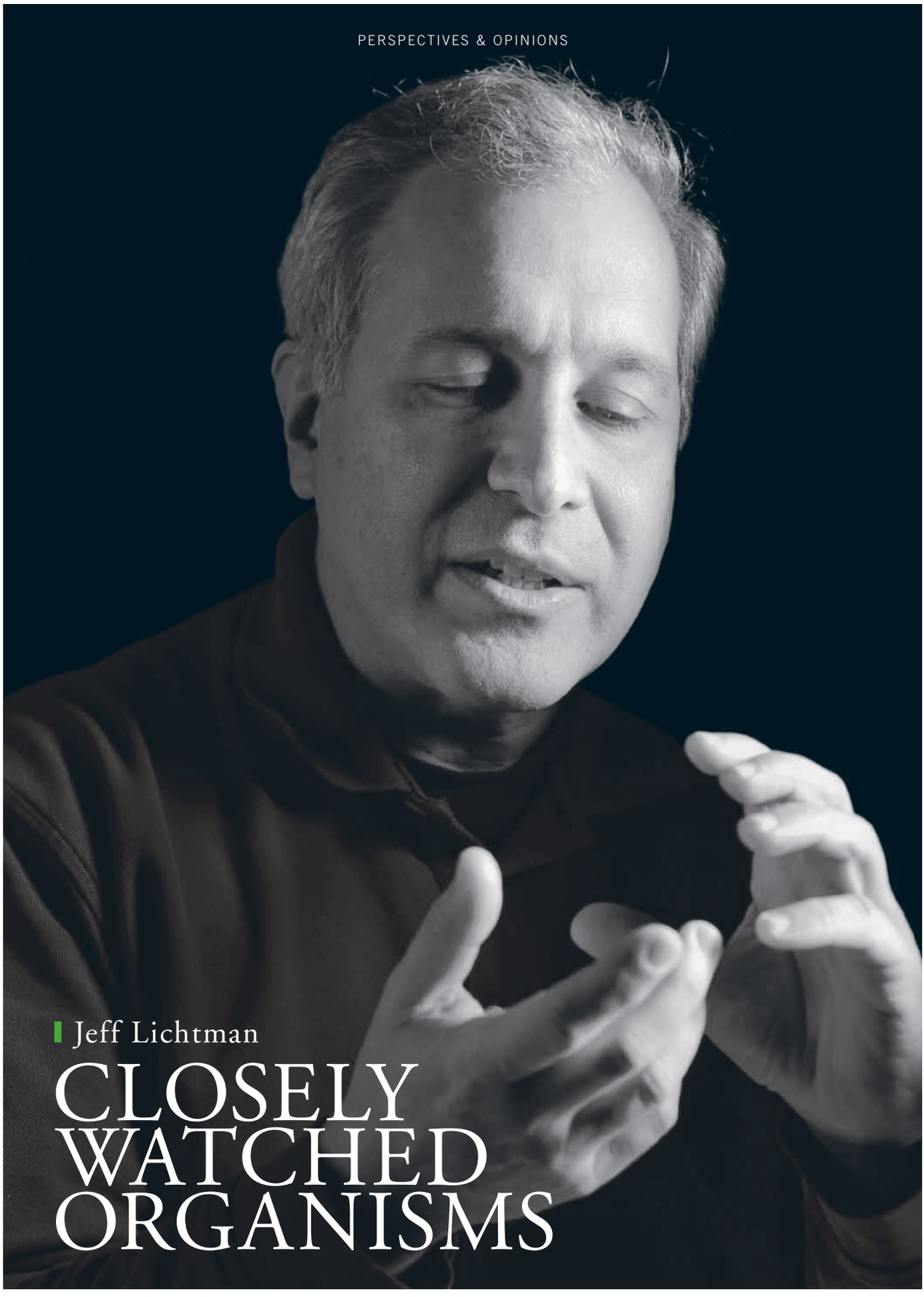
The past few years have seen an explosion of new microscopy methods, which sometimes read like alphabet soup: two-photon fluorescence correlation microscopy (TPFCM), which helped scientists trace drug transport in tumors; three-dimensional live-cell microscopy, which helped identify never-before-seen threadlike transport lines between live cells; and the GRIN lens, a needle-shaped, insertable lens that can create microscopic images several centimeters deep in the brain.

But microscope designers are far from done. At Janelia Farm, group leader Eric Betzig, a physicist, will see if he can build microscopes that use optical tricks to see objects far smaller than previous light microscopes. Betzig is developing what he calls the optical lattice microscope, in which multiple beams of light interfere with each other to create a three-dimensional lattice that will fill a sample with spots of light. The result, according to his theories, would be a microscope that can image objects three times smaller than today's best light microscopes and thousands of times faster. Others in the small field of ultra-high-resolution microscopy are pursuing the same goals using different designs.

"The holy grail is to see dynamically imaged living cells noninvasively, to see at the level of an individual protein molecule, and to see how the molecules interact in a cell," Betzig says. "It's many years away, but you can dream about it." ■

THE CHALLENGES OF IMAGING

TO BRING CELLS INTO SHARPER FOCUS, biologists need a world-class imaging facility, and that's exactly what HHMI plans at Janelia Farm. But building such a facility offers challenges of its own. **FOR STARTERS, DEVELOPING NEW MICROSCOPES** takes a broad range of expertise. For example, Eric Betzig, a physicist and Janelia Farm group leader, has developed a blueprint for a new type of ultra-high-resolution light microscope (see main story) by drawing on mathematics, theoretical and experimental physics, and engineering. To make his blueprint a reality, he'll work with experimental physicists; computer scientists; and electrical, optical, and mechanical engineers to build prototypes and to develop them into reliable instruments. His long-term goal is "to make instruments that are widely used by biologists." **TO DEVELOP BETTER ELECTRON MICROSCOPES,** Chen Xu, a physicist in HHMI investigator and Janelia Farm group leader Nikolaus Grigorieff's lab who will manage Janelia Farm's shared electron microscope facility, will collaborate with microscope manufacturers like FEI and JEOL to obtain the best electron beams, the best phase plates, and the most sensitive CCD detectors—and customize them. Grigorieff's team will develop fast new computer programs that choose particles to analyze, align them, and piece together a three-dimensional structure. **DEVELOPING NEW MICROSCOPES IS** only part of the challenge. "You also need a facility for [biologists] to do high-end microscopy with established techniques," says Winfried Denk, a leading microscopist who directs the department of biomedical optics at the Max Planck Institute in Heidelberg, Germany. At Janelia Farm, HHMI plans to create several core facilities, including Xu's shared electron microscope facility, to provide expert technical support to the biologists who use them. **RESEARCH INSTITUTES LIKE THE MAX PLANCK** Institute and Janelia Farm are great places to develop new technologies, says Denk. "Developing new technology involves taking risks," he says. Academic scientists, with their eye on tenure and their next grant, can't always do that. Research institutes also foster cross-disciplinary collaboration, which is critical for developing new microscopes. At Janelia Farm, physicists will rub elbows with biologists, chemists will talk with computer scientists, and molecular biologists will mingle with mathematicians, says Gerald Rubin, director of Janelia Farm. "We're going to have all those kinds of people working side by side," he says.



Jeff Lichtman

CLOSELY WATCHED ORGANISMS

Sean Kernan

JEFF LICHTMAN LIKES TO invoke baseball legend Yogi Berra's famous line "You can observe a lot by watching" to describe his own work. Lichtman, who is a professor of molecular and cellular biology at Harvard University and has participated in planning for HHMI's Janelia Farm Research Campus, is a self-described "neuronal ethologist." He watches nerve cells in their native habitat—the animal. Powerful new microscopes are facilitating a trend toward observational biology, which Lichtman contends has some key advantages over experimental biology.

For me, an observational approach to biology is very natural, but it's not that common in my field. Most of modern biology, and indeed science education from elementary school on up, is actually rooted in deduction: You start with an idea and use experimental tools to manipulate variables to test that hypothesis.

The aim of the deductive scientific method is to try your hardest to prove the idea wrong. If you keep failing at these attempts, the hypothesis stays alive, even though you haven't actually proved it. But where have you gotten? Given that the idea was already out there, all you've done is maintain the status quo. Refuting a hypothesis, on the other hand, leads to paradigm shifts. But it requires compelling data against the hypothesis, which is difficult to obtain, and when it is negative data, it is even more difficult to publish.

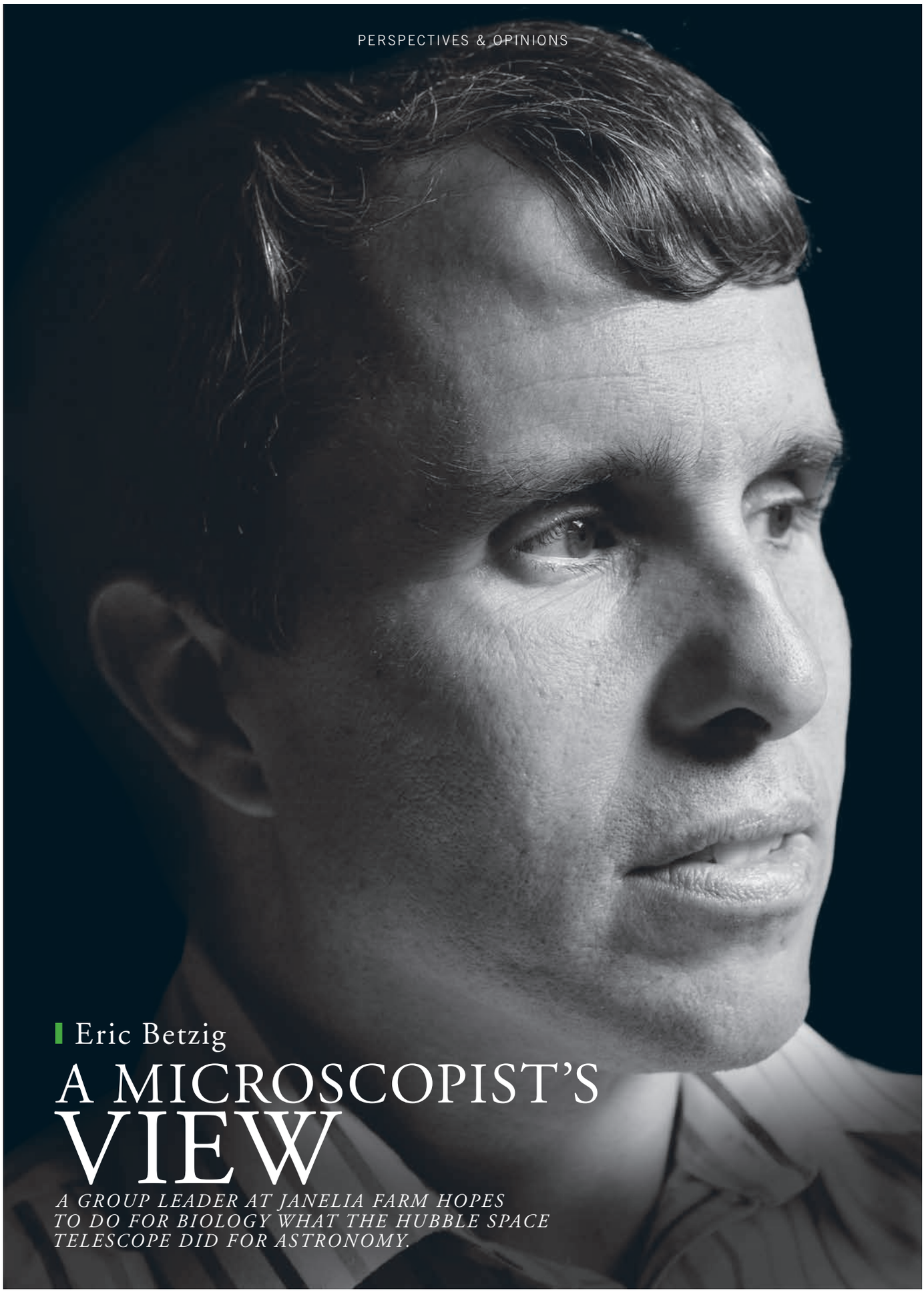
So with deduction, hypotheses tend to become entrenched as the weight of the published record becomes progressively lopsided. You could provide a virtually limitless amount of evidence consistent with the hypothesis that Earth is flat, but that doesn't make it so.

Observational, or inductive, science proceeds in a different way. The researcher simply observes a biological system, and hypotheses emerge as a consequence of, rather than as the motivation for, the observation. Nobel laureate Sydney Brenner said, "Progress in science depends on new techniques, new discoveries, and new ideas, probably in that order," which is a good way to sum up how inductive science works. This approach, however, has its own pitfalls. First, your hypotheses are only as good as the observational tools at your disposal. Second, your life experience molds your worldview and therefore your observational abilities. You see things that resonate in your mind as real and interesting, and you ignore those things that don't.

The strength of inductive science, however, is that new observational tools often reveal unexpected things that force you to confront the disconnect between the current worldview and the revealed world—especially when you have young colleagues, who bring fewer biases to biological phenomena. Microscopes are particularly valuable tools for this endeavor in neurobiology because they are a direct link between cell-biological phenomena and the visual system, our most sophisticated sensory mechanism.

In the 1990s, neuroscience turned sharply toward molecular genetics. Many remarkable technological advances have resulted, including the ability to make mice that express fluorescent proteins from jellyfish. In our lab and others', these animals have dramatically improved the ability to visualize neurons over time in living animals (so-called intravital microscopy). Automated microscopes and computational tools also allow assessment of the structure of many individual nerve cells in one preparation. Together, these innovations may enable us to describe all the synaptic linkages in neuronal circuits, what I like to call "connectomics." By providing a complete description of the wiring of the brain, these maps may give us a window into the inner workings of the mind and may even yield insights into "connectopathies," where the wiring has gone awry.

Some optimists believed that knowing the complete genome sequences of animals meant it was just a matter of time before we would understand essentially all biological phenomena as molecular cascades. My sense is that this optimistic view now holds less sway. So many concurrent and interacting molecular reactions occur within cells that scientists simply cannot reduce a living cell, much less a multicellular organism, to just so many bags of molecules, hoping to extrapolate the organism's full repertoire of behaviors. Observational approaches of neuronal behavior may provide a much-needed bridge between molecules and brains. Just as nature videographers working in the wild show us a far better view of behavior than we could obtain by observing specimens in a museum case or even a zoo, intravital imaging helps us move out of the culture dish and into the real jungle of the brain. **–Interview by Julie Corliss** ■



Eric Betzig

A MICROSCOPIST'S VIEW

A GROUP LEADER AT JANELIA FARM HOPES TO DO FOR BIOLOGY WHAT THE HUBBLE SPACE TELESCOPE DID FOR ASTRONOMY.

Paul Fetters

PHYSICIST ERIC BETZIG MADE A dramatic contribution to the imaging field in the 1980s and 1990s with his work on the near-field microscope. This technology, which shattered the theoretical "diffraction limit" imposed on spatial resolution by the wavelength of light, imaged small structures at higher resolutions than scientists thought possible. Techniques to peer inside living cells at similar high resolutions are still too slow, however. So when Betzig comes to the Janelia Farm Research Campus, opening this fall, he plans to develop a new method—"optical lattice microscopy"—to rapidly image the constant activity within living cells.

Which fields of biology stand to benefit most from improvements to microscopy?

EB: Basically, the interfaces between cell biology and molecular biology. We understand the genetic sequences by which proteins are made, and we understand, in many cases, the structures of the proteins. What we *don't* understand in sufficient detail is when they're expressed, or not expressed, within the cell; how that relates to environmental factors; what other proteins are present within the cell right then; how the proteins interact with one another; and how those areas of interaction are localized to drive the cell and its function.

Conventional optical microscopy cannot provide high-enough resolution to address these questions. But if the techniques that are at the edge right now pan out, we've only seen the tip of the iceberg. I make an analogy with astronomy: When people at the turn of the last century looked at Mars through telescopes with inadequate resolution to see any detail, the fuzzy lines they saw started them thinking about built canals and Martian civilizations.

Similarly, when you crack open any issue of *Cell* or *Biophysical Journal*, you see tons of interpretive studies based on relatively low-resolution cell images. Oftentimes, the interpretations are necessarily speculative. But as we begin to get higher resolutions, better dynamics, and the ability to access deeper tissue, we're going to get vast improvements in understanding. With factor-of-two or -four increases in each of those areas, we'll be creating this multidimensional space of information that can grow by orders of magnitude. All these systems that we've looked at before very blurrily we will now see in greater detail. It's going to be like the difference between using those old telescopes and using the Hubble Space Telescope.

Which aspects of optical microscopy need to be improved?

EB: The first is new contrast mechanisms: To find out more about the cell optically, you need a wider and better set of labels. Single molecules are basically exquisite reporters of their local environment, and you can optimize them so that the fluorescence of a labeling molecule is sensitive to a parameter of interest. Techniques like fluorescence lifetime imaging give you contrast based on how long it takes for a photon to be emitted from the molecule, which can act, say, as a pH sensor or a viscosity sensor. So this is an ongoing area of interest: both on the chemistry side, in how to create new and better labels, and on the technology side, in how to get the information from the photons.

On a biological level, there are dynamics happening on all time scales, from the femtosecond to the many, many tens of seconds. There's a whole continuum of processes happening, and you want to be able to study as many as you can. So the second goal is to increase the dynamics.

The reason we do optical microscopy (as opposed to electron microscopy)—despite the limited resolution—is that we want to be able to look at living cells without pumping in so much energy that we perturb them. But the more energy you pump into trying to interrogate your sample, the greater the chance of perturbing it. The third goal, then, is to achieve the greatest dynamics possible so that we can look at things in real time, yet do so noninvasively.

Of course, the fourth area would be high resolution: trying to get to the diffraction limit and push beyond it. And finally, deep-tissue imaging: We need to do better than diffraction-limited resolution, which confines us to really thin and idealized samples, and actually push into the brain and other areas. Those are the five main issues that I see people working on in the field of optics.

Is there an ideal goal?

EB: In terms of resolution, once you get to the molecular level, that's pretty much it; there's not much more to do there. But in terms of dynamics, you can always ask for more. In terms of slicing and dicing your signal and getting different contrast, you can always ask for more. In terms of how deep you can go, you can always ask for more. There's loads of room for improvement.

CONTINUED ON PAGE 56

Q&A

What did see you through your first microscope?

Some scientists have been peering through microscopes for decades. Still, they often vividly remember their first glimpse through a lens, squinting at some otherwise imperceptibly tiny thing below. Here, a few HHMI investigators recall that seminal moment. –Edited by Kathryn Brown

DAVID A. AGARD
PROFESSOR OF BIOCHEMISTRY AND BIOPHYSICS AND OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

A:

"I believe I was 7 or 8 when I was delighted to receive a microscope for my birthday. I immediately ran out to the creek to collect some algae—and rather stagnant water—thinking it would be full of bugs. I was right. I also looked at onion cells and cells from my own cheek. What a blast!"

PAMELA J. BJÖRKMAN
MAX DELBRÜCK PROFESSOR OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

A:

"I think it was during a 7th-grade science class that I first looked at a drop of pond water through a microscope and saw an amazing collection of strange creatures—hydra, rotifers, and other tiny organisms. I was fascinated to think that there was a whole world out there we don't usually see. Maybe that inspired me to want to know what else could be revealed by high-resolution imaging."

NIKOLAUS GRIGORIEFF
ASSOCIATE PROFESSOR OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, AND INCOMING JANELIA FARM GROUP LEADER

A:

"When I was about 12, my parents gave me my first microscope. In my excitement to peer through it, I immediately began looking for a suitable substance to study. Eventually, I plucked a hair from my own head."

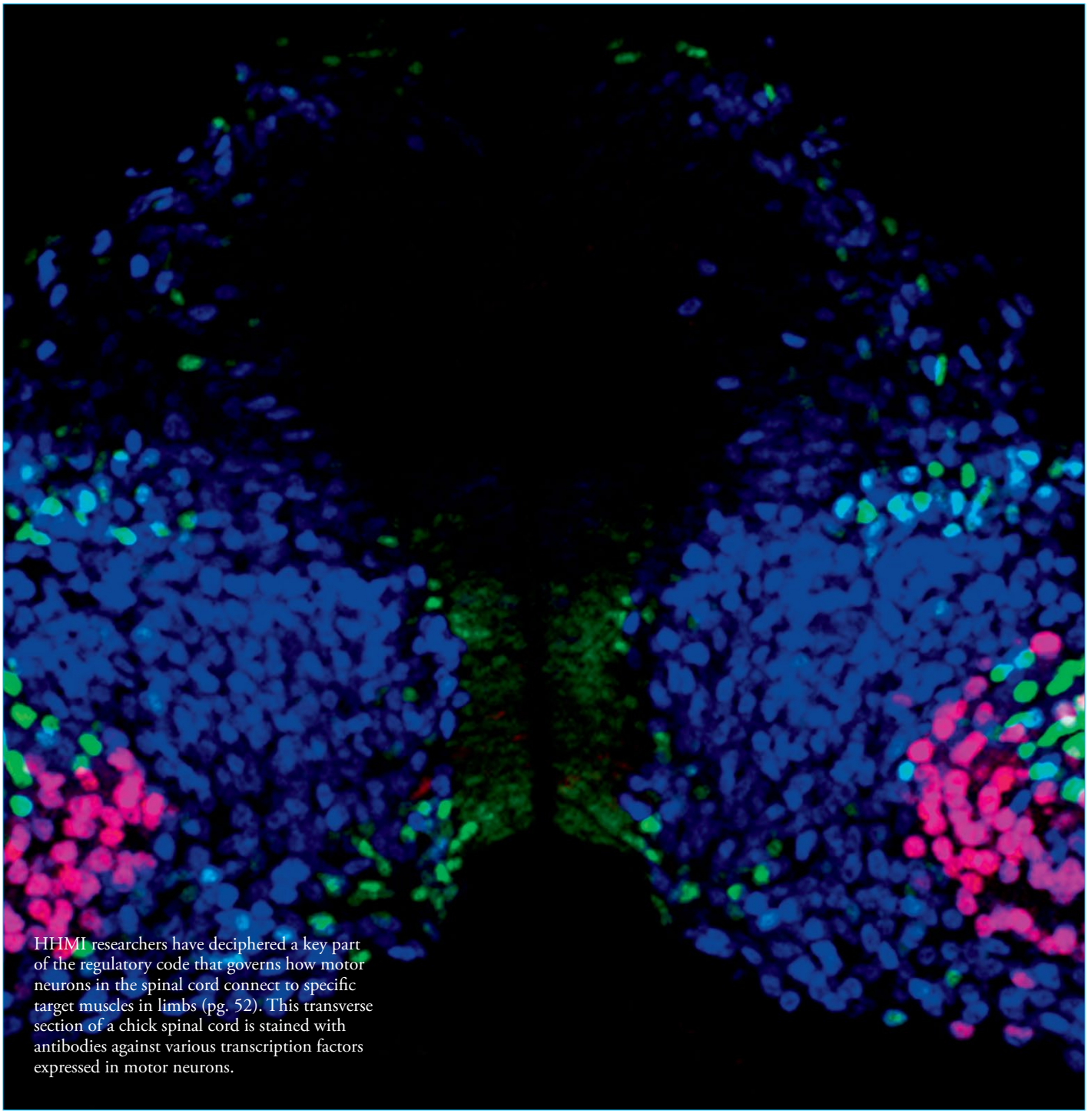
TOM K. KERPPOLA
PROFESSOR OF BIOLOGICAL CHEMISTRY, UNIVERSITY OF MICHIGAN

A:

"When I was growing up in Finland, experimental science was not a big part of school. At home, I once discovered parts of my grandfather's antique microscope. With some tape and cardboard, I managed to fashion these bits into a working scope, producing a blurry image. I was unimpressed. It wasn't until many years later, after finding ways to study molecular events by imaging, that the microscope became one of my favorite tools."

Agard: Mariano Tabios/Agard Lab *Björkman:* Misha Gravenor *Grigorieff:* Paul Fetters *Kerppola:* Paul Fetters

CHRONICLE



HHMI researchers have deciphered a key part of the regulatory code that governs how motor neurons in the spinal cord connect to specific target muscles in limbs (pg. 52). This transverse section of a chick spinal cord is stained with antibodies against various transcription factors expressed in motor neurons.

Improving Cochlear Implants

A young researcher aims to make the technology responsive to pitch and widen deaf people's perceptions of complex sound environments.

Experiencing the limitations of his own cochlear implant inspired Chad Ruffin to better the device.

COCHLEAR IMPLANTS HAVE HELPED BRING PROFOUNDLY DEAF INDIVIDUALS into the hearing world, but one HHMI fellow says the technology needs improvement. Chad Ruffin says users have a tough time understanding speech in noisy environments, largely because today's implants transmit almost no information about pitch—the tone that allows the hearer to distinguish one sound from another, as in picking one voice out of the din. Ruffin knows firsthand; he received an implant 6 years ago.

He hopes to correct the limitations of cochlear implants through his research. An HHMI medical research training fellow at the Virginia Merrill Bloedel Hearing Research Center at the University of Washington in Seattle, Ruffin is studying how cochlear implants can be programmed to transmit more information on frequency, thereby enabling the user to discriminate between the pitch of different voices.

Each implant contains a tiny computer that receives sound information from the environment and then uses speech-processing programs to relay this information—or at least some of it—to the brain. Current speech processors rely almost entirely on the intensity of sounds within a few frequency bands; that is, they are sensitive mainly to the changes in amplitude in a series of spoken syllables or words. Although this feature gives "good speech perception in the quiet," Ruffin says, "if you start to add complex sounds, such as music or noise, the perception goes down dramatically."

For cochlear implants to give users a better level of pitch perception, they must deliver information on the fine structure of the sound—the tiny and rapid changes in frequency. Ruffin is working with Jay Rubinstein, director of the Bloedel Center, to test a speech-processing algorithm designed to do just that. Studies in animals show that this algorithm conveys a sound's fine structure to the brain, Rubinstein says. And some people whose implants were reprogrammed with the new algorithm report improved speech perception. Ruffin is developing psychophysical tests to prove that the algorithm is in fact allowing fine structure information to reach the brain.

For the congenitally deaf, says Rubinstein, the earlier they receive an implant, the better. Older children and adults show a great deal of variability in how well they perform with implants. Ruffin still requires assistive listening devices at the movies and to hear lectures in a classroom environment, but he marvels at the changes he's experienced since his implant. "My social circle expanded exponentially after the implant," and, he says, "Now I can converse in the dark with a friend; I don't have to read lips."

Ruffin began working in the field of implant research in 2004 after completing his first year of medical school at Louisiana State University in Shreveport. His investigations took him to the University of Iowa in Iowa City, where he met Rubinstein. When Rubinstein moved to the University of Washington, Ruffin applied for the HHMI fellowship to join him. Although he plans to become a surgeon, Ruffin would also like to do long-term research. "And I decided that if I was going to do research," he says, "it'd be something that I'm interested in." **–Melissa Lee Phillips** ■

> "I decided that if I was going to do research, it'd be something that I'm interested in."
>
> CHAD RUFFIN

Brian Smale

Never Too Young for Science

A program aimed at preschoolers exploits their natural curiosity and hands-on predispositions.

FOR 4-YEAR-OLDS, WHAT COULD BE more fun than playing with water? And from a science educator's point of view, what could be more powerful than tapping children's fascinations?

In a Head Start classroom at Dominion High School in Loudoun County, Virginia, children dressed in purple smocks are exploring the concept of volume by pouring water from a bucket into another container with a line marked by masking tape. Using beakers and liter containers, they pour water in or take water out, repeatedly trying to judge how much liquid they need to fill the container to the mark. "They are learning to use the tools in ways that were intended—and maybe in ways that were not intended. But they are excited about learning, and that's wonderful," says teacher Kathleen Miller.

In Loudoun County public schools and select schools in two other states, an innovative preschool science program provides such activities to help children begin to think critically and solve problems. The curriculum, steeped in research about how children learn, capitalizes on their natural curiosity and trains teachers and parents to arouse that curiosity.

Teachers can choose from 21 activities in a toolbox that contains experiments, questions, and suggestions. In one activity, students learn to observe and compare by making a fruit salad, discussing the similarities and differences in the pieces of fruit, and counting the number of pieces of each type of fruit they add to the salad. In another activity, children use a pan balance to weigh objects, determine which are heavier or lighter, and sort them by weight.

The activities can be integrated seamlessly into the preschool day, allowing teachers to take advantage of "teachable moments" to reinforce or extend concepts, says Odette Scovel, science supervisor for the Loudoun County schools. To help children develop literacy skills, teachers read them science-related books and encourage them to talk about what they are doing as they work.

Supported by a 2-year, $50,000 grant from HHMI, the program partners the Loudoun County schools and Florida's Fairchild Tropical Botanic Garden, whose staff also work with preschool science teachers at the Miami site of the Children's Home Society of Florida, which serves thousands of children eligible for adoption or foster care. Selected preschool classes in Harrisburg, Pennsylvania, also use the program. The grantees collaborate with the curriculum's developer, Cognitive Learning Systems (CLS), also of Harrisburg.

"So much is going on in children's brains when they are doing something as simple as pouring water," says Joanna Garner, vice president for program development at CLS. She provides teachers and parents with a "science-behaviors checklist" to determine how well children are using

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Students Drawn to Debate on Evolution and Religion

Must evolution and religion be at odds? A theologian, a philosopher, and two scientists help high school students grapple with tough questions.

MANY PEOPLE THINK SCIENCE AND religion make uncomfortable bedfellows. Father James A. Wiseman isn't one of them.

"I am a believing Christian who totally accepts evolutionary theory," the Benedictine monk and theology professor at the Catholic University of America told a group of Washington, D.C.–area high school students. The teenagers gathered at HHMI headquarters for a discussion following the 2005 Holiday Lectures on Science, which focused on evolution.

In addition to Wiseman, the panel included Michael Ruse, a philosophy professor at Florida State University, and the two Holiday Lectures speakers, HHMI investigators Sean B. Carroll of the University of Wisconsin–Madison and David M. Kingsley from the Stanford University School of Medicine.

The high school students peppered the speakers with challenging questions:

(Left to right) Father James Wiseman, philosopher Michael Ruse, and scientists Sean Carroll and David Kingsley

"Should evolution be taught with a disclaimer, presenting it as one theory among many?"

"Could creationism and evolution be different paths to the same answer?"

"Do you believe in God? If so, how do you reconcile that belief with the science that you do?"

Although the speakers brought different viewpoints to the discussion, they agreed that it is possible to be an evolutionist and a Christian. They encouraged the students to use the scientific method to examine the evidence for biological evolution, which Kingsley called "absolutely overwhelming."

"I don't see a fundamental conflict between religion and evolution," he said. "Evolution is a description of how life changes. That's different from addressing where the whole universe came from. Darwin says nothing about why the universe exists. There is still the critically important 'why' question of how the universe came to be that isn't addressed by evolutionary theory."

Ruse observed, "I can't see why one can't be both an ardent Darwinian and a

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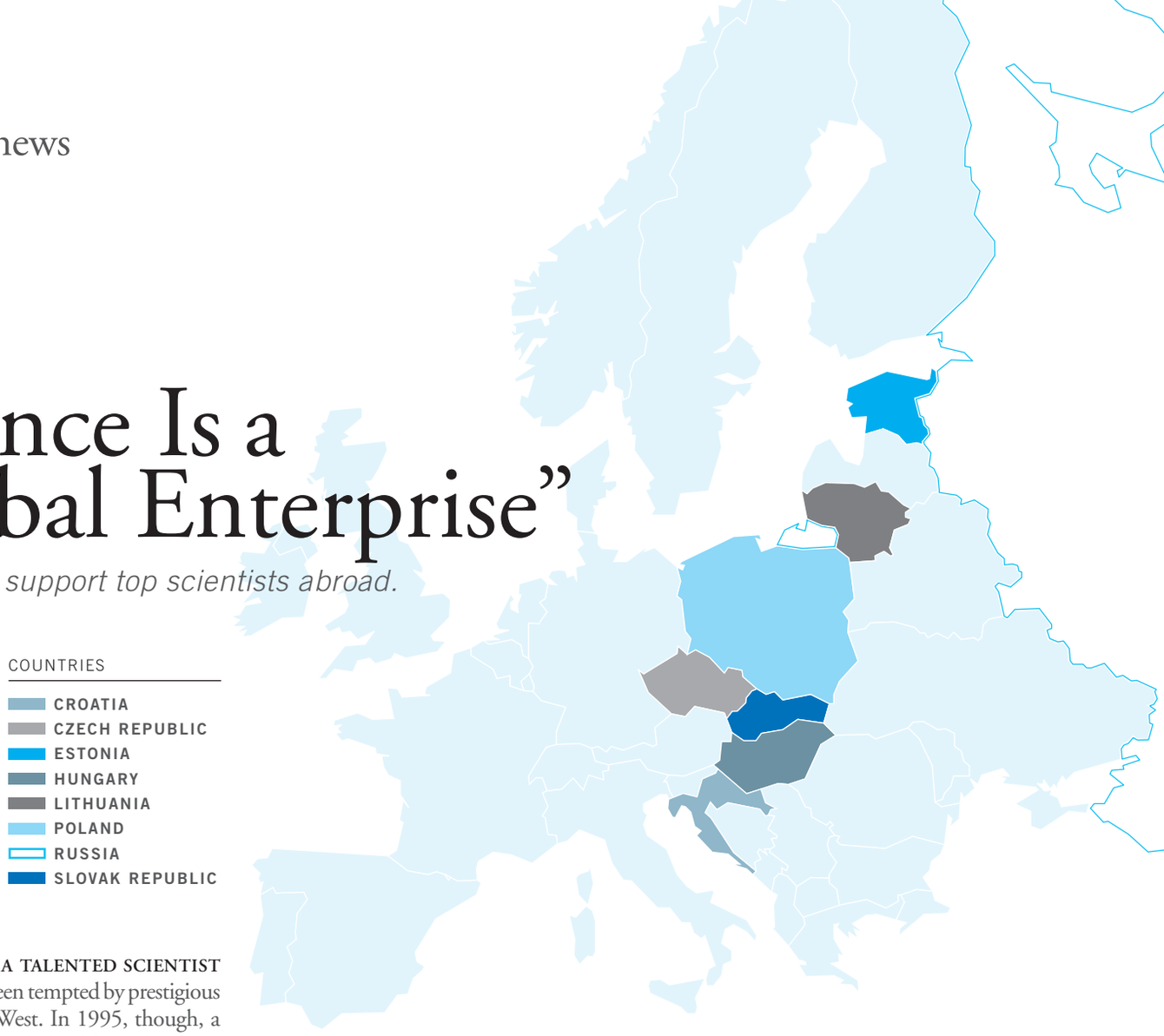
FOR MORE INFORMATION
View the lectures and discussion at
www.hhmi.org/biointeractive/evolution
A free DVD of the Holiday Lectures on evolution and the discussion of evolution and religion will be available in April 2006.

Paul Fetters

"Science Is a Global Enterprise"

New grants support top scientists abroad.



TAMÁS FREUND, A TALENTED SCIENTIST in Hungary, has been tempted by prestigious job offers in the West. In 1995, though, a grant from HHMI helped him decide to stay in his native country. A second grant in 2000 enabled him to build a well-equipped lab in Budapest.

Now, Freund has earned a third grant from HHMI. He is 1 of 28 exceptional scientists in eight countries who were selected in November to receive new international research awards from HHMI.

The new grants support research in Croatia, the Czech Republic, Estonia, Hungary, Lithuania, Poland, Russia, and the Slovak Republic. Each scientist will receive $100,000 a year, and the 28 awards total $14 million over 5 years. Sixteen of the researchers have previously received HHMI international grants.

In addition to supporting scientists in the Baltics, central and eastern Europe, and Russia, HHMI awards competitive grants to researchers in Latin America and Canada and supports infectious disease and parasitology research worldwide. The Institute has awarded a total of $129 million to 353 scientists in 39 countries since 1991.

Freund, now director of the Institute of Experimental Medicine of the Hungarian Academy of Sciences in Budapest, will use his new HHMI grant to explore the role in neuron signaling of a mind-altering compound naturally produced in the human body. He hopes the research will lead to new drugs to treat anxiety and new ways to combat drug dependence.

> "For science to flourish, fresh ideas often come from fresh new scientists."
>
> PETER BRUNS

Commenting about the awards, HHMI President Thomas R. Cech said, "It is vital to invest in the scientific capacity of economically less advantaged countries because science is a global enterprise."

In the same spirit of global science, HHMI and the European Molecular Biology Organization (EMBO) have singled out six outstanding central European scientists to receive EMBO/HHMI Startup Grants—awards to help the scientists establish their first independent laboratories in the Czech Republic, Estonia, and Hungary.

Each scientist will receive U.S. $75,000 a year for 3 years. HHMI will contribute $50,000 per scientist, and EMBO and participating member countries will provide the additional $25,000.

"HHMI already has an ongoing program in support of established science in central Europe, but we recognize that for science to flourish, fresh new ideas often come from fresh new scientists," said Peter J. Bruns, HHMI vice president for grants and special programs. "This new program aims to help promising new scientists get established with resources, space, and time in the early years of their independent careers." ■

FOR MORE INFORMATION
For details about the new grants, including the names of the grantees, go to www.hhmi.org/news/122205.html and www.hhmi.org/news/120605.html

Craig Alexander Named as HHMI's General Counsel

THE TRUSTEES OF THE HOWARD HUGHES Medical Institute have elected Craig A. Alexander as the Institute's vice president and general counsel. He assumed his new role in January.

Alexander, 46, had served as HHMI's deputy general counsel since 1994. He succeeds Joan Leonard, who retired as general counsel after 11 years in that post. She is now senior counsel to the Institute's president, Thomas R. Cech.

"Over the past decade, Craig has been Joan Leonard's active collaborator and partner in managing the Office of the General Counsel. His broad legal expertise and deep familiarity with HHMI make him uniquely qualified for this new assignment," said Cech.

As deputy general counsel, Alexander played a major role in formulating and implementing HHMI's policies on intellectual property, in providing legal advice concerning the investment of the endowment, and in guiding the development of the Janelia Farm Research Campus in Ashburn, Virginia.

Alexander joined HHMI as an associate general counsel in 1992 from the Indianapolis law firm of Sommer & Barnard, P.C. Before that, he handled numerous matters involving HHMI while an associate in the Washington, D.C., office of Paul, Weiss, Rifkind, Wharton & Garrison.

A magna cum laude graduate of the Georgetown University Law Center, where he was editor of the law journal, Alexander received a bachelor's degree

in accounting from Butler University in Indianapolis. He is also a certified public accountant. Alexander is a member of the tax, science, and technology sections of the American Bar Association, the National Association of College and University Attorneys, and a variety of other organizations. ■

> "His broad legal expertise and deep familiarity with HHMI make him uniquely qualified for this new assignment."
>
> THOMAS CECH

Spotlight | Janelia Farm Dinner

A MODEL EVENING: Members of the Virginia House of Delegates visited the Janelia Farm Research Campus last November to learn firsthand about HHMI's investment in Northern Virginia. The dinner and reception were part of the annual retreat for members of the Appropriations and Finance committees.

A FEW POINTERS: GERALD RUBIN, director of Janelia Farm, uses a scale model to explain the campus layout to Delegate VINCENT J. CALLAHAN (R-34th District), who is dean of the Northern Virginia delegation and chair of the Appropriations Committee.

INFORMAL BRIEFING: Loudoun County Supervisor LORI WATERS (right), whose district includes the Janelia Farm campus, joined other local officials for the evening. GERALD RUBIN and KEVIN MOSES (center), associate director for science and training at Janelia Farm, provided an update about recruitment and the Janelia Farm graduate program.

Photos by Paul Ferters

FOR MORE INFORMATION
To learn more about Janelia Farm, visit www.hhmi.org/janelia

New Agreements on Mice

HHMI aims to help manage the logistics and allay the cost of the ever more refined yet ubiquitous laboratory mouse.

WITH THE USE OF LABORATORY MICE skyrocketing, it's no surprise that scientists across the country face a murine housing crunch—and that's just for starters. Not only do the mice represent a significant investment of time and laboratory resources but requirements by organizations like HHMI and the National Institutes of Health (NIH) mean that researchers also have to find cost-effective ways to share mouse stocks with their colleagues and preserve them for future experiments.

"Because most institutions' facilities are bursting at the seams, any way that you can more effectively maintain your mouse colonies will be a big benefit to the community," says Nathaniel Heintz, an HHMI investigator at the Rockefeller University. He ought to know: Heintz has created hundreds of transgenic mice in studying the development of the mammalian brain and deposited many of them in an NIH-supported facility that makes them available at nominal cost.

More than one-third of HHMI's 321 investigators now use laboratory mice in their research, and that percentage is likely to increase. With spending on animal breeding and maintenance hitting an estimated $47 million between 2002 and 2004, an Institute-wide initiative was required.

Enter Philip Perlman, one of HHMI's senior scientific officers, whose own research focused on mitochondrial genes in yeast—a more manageable model organism. Perlman has spent the past 18 months identifying approaches to improve the management of mouse colonies in HHMI laboratories so that resources could be freed for more research.

After consulting with HHMI investigators, several NIH scientists, and experts in mouse genetics and animal care, the Institute has taken two steps. First, it has entered into a new agreement with the Jackson Laboratory (TJL), the leading independent center for mouse genetics in the United States, as well as one of three NIH-supported mouse repositories and home to two HHMI investigators. The agreement focuses on improving ways to archive and distribute valuable strains of mice and develop better tools for managing mouse colonies. Second, HHMI is running a short-term trial program with Transnetyx, a Memphis-based company, to outsource genotyping, the important task of determining the genetics of a mouse.

"This gives us an opportunity to invigorate our historic relationship with the Jackson Lab by funding projects that are relevant to the aims of both TJL and HHMI and are also cutting-edge," says David Clayton, HHMI's chief scientific officer, noting that HHMI has collaborated with TJL since the 1980s.

About 75 percent of the nearly 3,000 mouse strains at TJL are stored as frozen embryos or sperm. Cryopreservation is efficient, but recovering live mice requires techniques not widely used in HHMI labs. So part of the initiative focuses on training lab staff and developing better cryopreservation approaches. Other goals include creating more effective ways to tag individual mice and enhancing mouse colony management software that was largely developed by HHMI investigator Simon W.M. John.

Perlman says the Institute is planning another project to help defray the costs of archiving mutant mice developed in HHMI labs. With HHMI partially covering the expenses, Perlman expects the labs to break even in the first year. "We're always looking for ways we can improve how research is being done," he notes.

Because many mutant mice do not look any different from wild-type mice or from other mutant mice, DNA genotyping of every mouse born in a mouse facility is a necessary part of mouse research. This genotyping determines which individual mice have certain mutant and wild-type genes. It is a time-consuming and costly activity, which is why HHMI is funding the pilot program with Transnetyx. More than 70 HHMI investigators are now trying out the company's automated approach to mouse genotyping.

"We're always looking for ways we can improve how research is being done."

PHILIP PERLMAN

"This is the kind of work that is repetitious and not very interesting for a researcher to do, but we have to know the answer," says Richard A. Flavell, an HHMI investigator at Yale University School of Medicine and one of the scientists participating in the program.

Each of these initiatives can make mouse research in HHMI laboratories a little more efficient and can potentially reduce the cost of mouse breeding for research. Notes Perlman, "Whichever projects prove to be effective for HHMI researchers will also be useful for the wider community of mouse researchers." ■

Courtesy of the University of Texas Southwestern Medical Center at Dallas

HHMI and *Science* Partner to Improve Science Education

HHMI AND THE JOURNAL *SCIENCE* have begun a collaboration to showcase innovative approaches to teaching science. A new monthly section of the journal will engage research scientists in thinking about ways to improve education at all levels by providing a forum for sharing ideas and sparking discussion.

The new education section will be produced by *Science*'s editorial staff. It will feature peer-reviewed research as well as scholarly literature reviews, essays, and other original writing on science education. The section will focus on undergraduate and graduate level education but will also showcase innovations in K–12 science education.

"Why *Science*?" asked Peter J. Bruns, HHMI vice president for grants and special programs. "Because that's where the scientists are. *Science* is read by scientists, and scientists are an important key to great science education. Good research and good teaching can go hand in hand to the mutual benefit of both."

In an editorial in the December 16, 2005, issue of *Science*, HHMI President Thomas R. Cech and *Science* Editor-in-Chief Donald Kennedy argued that research scientists should care about the strength of science education in view of the "pipeline issue"—where we will get the next generation of research leaders—and in view of the policymaking threat posed by voters who do not understand science or the process of scientific thinking.

"If the electorate distrusts science and doesn't understand how scientists explore and interrogate the natural world," the authors asked, "how will they vote on issues ranging from stem cell research and global climate change to the teaching of intelligent design in our schools?"

The new section in *Science* debuts this year. ■

Courtesy of Rockefeller University

Sir Paul Nurse Elected as HHMI Trustee

SIR PAUL NURSE, PRESIDENT OF THE Rockefeller University, has been elected a Trustee of the Howard Hughes Medical Institute. He is one of 11 Trustees of the Institute.

Nurse, 56, is a distinguished scientist who shared the 2001 Nobel Prize in Physiology or Medicine with Leland H. Hartwell and R. Timothy Hunt for fundamental discoveries concerning control of the cell cycle. A geneticist who uses fission yeast as a model system, he continues an active research program that focuses on the cell cycle and how the cell organizes its internal structures to prepare for cell division.

A native of England, Nurse became Rockefeller's ninth president in 2003. He had been chief executive of Cancer Research UK, the world's largest cancer research organization outside the United States.

Nurse graduated from the University of Birmingham in 1970 and received his Ph.D. from the University of East Anglia in 1973. He headed laboratories at the University of Sussex, the Imperial Cancer Research Fund (ICRF), and Oxford University before rejoining the ICRF in 1996 as its director general. He presided over its merger with the Cancer Research Council.

Nurse's work has been recognized around the world. He is a fellow of the British Royal Society and, in 1995, became a foreign associate of the U.S. National Academy of Sciences. He has received the Gairdner Foundation International Award (1992), the Alfred P. Sloan Jr. Prize from the General Motors Cancer Research Foundation (1997), and the Albert Lasker Award for Basic Medical Research (1998). ■

Awards Smooth Path to Research Career

THE TRANSITION FROM ADVANCED training to operating a self-sufficient lab is difficult for any scientist. Physician-scientists, who are expected to handle a clinical caseload while they are trying to establish a research career, can find it especially challenging.

To address the need for support during that transition, HHMI is establishing a program of early career awards. Former HHMI medical student fellows and HHMI-NIH research scholars who are just finishing their advanced training or are in the first 2 years of their first independent positions are eligible to compete for the awards.

The Institute will award 13 grants annually. Each award totals $150,000 over 3 years. Institutions employing the awardees must agree to let them spend 70 percent of their time on research. The funds may be used for research expenses, such as supplies, technical support, and small equipment.

HHMI will make its first early career awards in June 2006. ■

Cancers Use "Cellular Bookmarks" to Target Favorite Sites of Metastasis

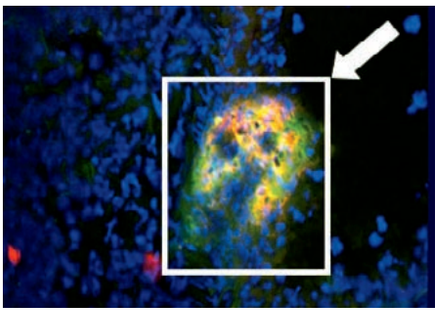
HHMI RESEARCHERS AND THEIR COLLEAGUES HAVE discovered that nonmalignant bone marrow cells establish "cellular bookmarks" in target organs that guide the spread of cancer cells to their predetermined destinations.

The researchers say their findings could have a major impact on how oncologists assess the likeliness of metastasis to specific organs. Their discovery may also help identify subsets of high-risk cancer patients prone to distant metastases who would likely benefit from a more aggressive therapy to prevent cancer relapse.

Ultimately, understanding how cellular bookmarking works at the molecular level could lead to information that may help thwart metastasis, a major cause of death among cancer patients, says one of the study's senior authors, Shahin Rafii, an HHMI investigator at Weill Medical College of Cornell University.

The researchers, led by David Lyden and Rafii, published their findings in the December 8, 2005, issue of the journal *Nature*. Lyden is at Memorial Sloan-Kettering Cancer Center and Weill Medical College.

Rafii and Lyden's group had established that a specific subset of bone marrow–derived cells (BMDCs)—which are composed of hematopoietic progenitor cells capable of dividing and forming colonies—are recruited by tumors and aid in the growth of new blood vessels. The generation of new blood vessels occurs through a process called angiogenesis. In previous studies, the researchers had shown that co-recruitment of hematopoietic BMDCs expressing an angiogenic factor receptor, VEGFR1, along with the vascular cells accelerated the assembly of newly formed blood vessels and tumor growth.



CLUSTERS OF BONE MARROW-DERIVED CELLS (GREEN) AND ANGIOGENIC FACTORS (RED) MAY MARK AREAS FOR METASTASIS

"In the current paper, we set forth another novel concept by demonstrating that a nonmalignant cluster of VEGFR1-positive hematopoietic BMDCs were recruited to a premetastatic niche, thereby establishing a permissive docking site prior to the arrival of the circulating tumor cells," says Rafii. A "premetastatic niche" is a cellular microenvironment that is specialized for the development of metastatic tumor cells.

In experiments with mice that had been implanted with highly metastatic lung cancers or melanoma cells, the scientists discovered that BMDCs arrived at the premetastatic sites before the cancer cells did. They also found that such clusters appeared before metastases developed in mice genetically predisposed to developing tumors—a system that closely mimics how cancers develop. **–Dennis Meredith** ■

IN BRIEF

BREAST CANCER DRUGS MAY SLOW GROWTH OF LUNG CANCER

A few years ago researchers discovered that, much like breast tumors, some lung tumors also thrive on estrogen. Now a medical student conducting research on an HHMI fellowship and colleagues have managed to stop the growth of human lung cancer cells in mice with a class of breast cancer drugs called aromatase inhibitors.

"It was a natural progression of the work that had already been done linking estrogen and lung cancer," said **Olga Weinberg**, who delayed her fourth year at Vanderbilt University School of Medicine to work on the project. The findings suggest a new way to treat lung cancer in women—a group whose death rate from the disease is surging.

"More women are dying now from lung cancer than from breast cancer," said senior author Richard Pietras, Weinberg's research mentor at the University of California at Los Angeles. "We followed one of the clues as to why this is happening, namely that estrogen drives the growth of certain types of lung cancer in women."

To see if they could block this growth, the team started with the enzyme aromatase. It was a natural target because it converts testosterone into estradiol, a potent form of estrogen also used in hormone replacement therapy. In addition, drugs that inhibit aromatase have already made it to market as treatments for breast cancer. "The production of estrogen takes several steps, and aromatase is the key to the process," said Weinberg. "Without aromatase, you don't get estrogen."

The studies were reported in the December 15, 2005, issue of the journal *Cancer Research*.

BIOTECHNOLOGY'S NEW CHEMICAL TOOL

Researchers have developed a new technique that allows them to modify specific sequences within a DNA molecule. The approach not only will reveal the impact of biochemical alterations to DNA but also could have far-reaching implications for DNA-based medical diagnosis and nanobiotechnology.

Combining chemistry with biotechnology, **Saulius Klimašauskas**, an HHMI international research scholar at the Institute of Biotechnology in Vilnius, Lithuania, and chemists at the Institute of Organic Chemistry in Aachen, Germany, harnessed a group of essential enzymes to add various chemical groups to DNA, thereby altering its function.

The enzymes at the heart of the study, known as DNA methyltransferases, are one of the tools cells use to turn genes on and off. In this study, the scientists demonstrated that methyltransferases can be used to transfer sizable chemical groups to large DNA molecules in a sequence-specific manner.

Earlier studies had suggested that transferring chemical groups larger than a methyl group was not feasible. "No one has really thought about possible applications [of this] before because no one thought it was possible," said Klimašauskas. He predicts that DNA methyltransferases will become a standard laboratory tool like restriction endonucleases.

The work was published in the January 2006 issue of *Nature Chemical Biology*.

Protein-Pairing Method May Yield New Drug Targets

PLASMODIUM FALCIPARUM, THE PROTOZOAN THAT causes the most severe form of malaria, is one of the deadliest of human pathogens, killing up to 2.7 million people each year. But the parasite is particularly difficult to study with standard genetic methods, thus hampering the search for drugs or vaccines, says Stanley Fields, an HHMI investigator at the University of Washington. In the hope of bypassing this bottleneck, researchers from his laboratory and Prolexys Pharmaceuticals recently adapted a method that Fields invented, called the yeast two-hybrid assay, to map protein-protein interactions in *P. falciparum*.

The test, performed by expressing pairs of randomly chosen *P. falciparum* proteins in yeast cells, tells researchers whether the two proteins can physically touch one another, or "interact," within the cell. "The utility of the assay is based on the principle of guilt by association," Fields explains. Proteins that interact are likely to participate in common cellular processes. If you know the function of one of the proteins, you can reason that the proteins it interacts with may be involved in the same process.

Using robots and other high-throughput technologies, the researchers screened more than 32,000 protein combinations, identifying 2,846 unique pairwise interactions in their study. Even so, says Fields, "We've only scratched the surface of what's out there." He and his colleagues published their results in the November 3, 2005, issue of *Nature*.

An illuminating finding from the analysis is that some groups of proteins with seemingly disparate functions may nevertheless interact. One large cluster includes proteins implicated in chromosome modification, gene regulation, and protein and mRNA stability. Another cluster includes proteins important in host-cell invasion. "This shared function implies that in the second cluster, some uncharacterized proteins might also be involved in infectivity," Fields says, and this offers hope that they could become targets for future antimalarial drugs.

The *P. falciparum* protein-interaction network developed by the researchers has also yielded information about evolution. In a letter in the same issue of *Nature*, HHMI medical student fellow Taylor Sittler and colleagues at the University of California, San Diego, compared *P. falciparum* network clusters with those in flies, yeast, worms, and bacteria. *P. falciparum* is already recognized as an evolutionary "oddball"—only about 40 percent of its proteins have similar counterparts in other organisms. Sittler's analysis found that the parasite's protein networks are as unconventional as the proteins themselves. Only three *P. falciparum* networks had counterparts in yeast, and the protozoan had no networks in common with the other organisms. Because some *P. falciparum* proteins have counterparts in other species, the research suggests that similar proteins might serve distinct roles in different species by making unique network connections. **–Paul Muhlrad** ■

IN BRIEF

TECHNIQUE CAPTURES NEW INFORMATION ABOUT PROTEIN SYNTHESIS MACHINERY

HHMI researchers have deduced the structure of a molecule that orchestrates the early stages of protein synthesis. They used the newly solved structure to better understand protein synthesis and to learn how the hepatitis C virus may hijack the protein-synthesizing machinery in human cells.

The new view of the protein complex, known as eIF3, reveals a five-lobed structure, with the lobes arranged much like a head, arms, and legs. The scientists' findings help explain how eIF3 uses these appendage-like lobes to maneuver components of a cell's protein-making factory, allowing the conversion of RNA to protein to begin. Their findings also reveal how hepatitis C virus (HCV) interacts with eIF3, a finding that could yield new drug targets in treating HCV, they said.

The research involved a collaboration between the laboratories of HHMI investigators **Eva Nogales** and **Jennifer A. Doudna**, both at the University of California, Berkeley. Bunpote Siridechadilok and Christopher S. Fraser were co-lead authors of the research paper, which was published in an early online article in *Science Express* on December 1, 2005. Another coauthor, Richard Hall, is at Lawrence Berkeley National Laboratory.

RESEARCHERS IDENTIFY FRUIT FLY INTESTINAL STEM CELLS

HHMI researchers have identified stem cells in the gut of the fruit fly—a finding that may lead to new insights into digestive diseases, intestinal cancers, and the infection strategies used by insect-borne parasites. The discovery puts to rest a scientific debate over whether invertebrates have gut stem cells.

The discovery of immature cells that differentiate into multiple types of gut cells suggests that the digestive tract of the fruit fly is likely to be a more similar, albeit simpler, version of that found in humans. Scientists now envision using the fruit fly to explore normal and pathogenic regeneration of the digestive tract in ways that were not available before.

The two HHMI research teams reported their independent findings on December 7, 2005, in two online research articles published in the journal *Nature*. The two groups were led by Craig Micchelli and HHMI investigator **Norbert Perrimon**, both at Harvard Medical School, and Benjamin Ohlstein and HHMI investigator **Allan C. Spradling**, both at the Carnegie Institution of Washington.

REGENERATING WORMS HELP ELUCIDATE STEM CELL BIOLOGY

Using a flatworm known for its ability to regenerate lost tissue, researchers have identified a gene that controls the ability of stem cells to differentiate into specialized cells. The gene encodes a protein that is similar to the protein PIWI, an important regulator of stem cells in organisms ranging from plants to humans.

The replacement of tissue lost to injury or shed during the body's normal activities is essential for the survival of most organisms. The study, published in the November 25, 2005, issue of *Science*, helps scientists understand how stem cells make this process possible. The research, performed at the University of Utah School of Medicine, was led by HHMI investigator **Alejandro Sánchez Alvarado**.

The Immune System: Imaged at Last

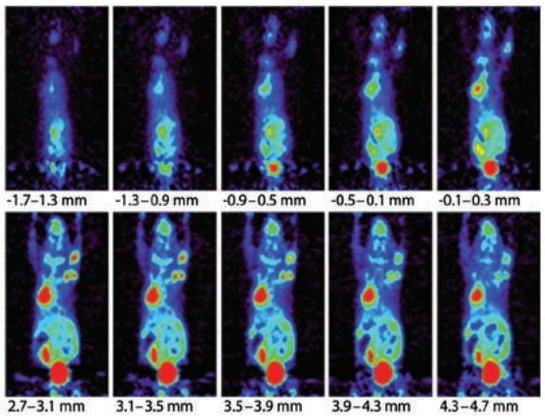
IF THE THOUGHT OF INVASIVE MEDICAL PROCEDURES makes you queasy, HHMI investigator Owen N. Witte points out that "there's a noninvasive trend in medical diagnosis—to measure things inside the body without having to stick tubes in a patient or do an operation." Witte, a researcher at the University of California, Los Angeles, has recently furthered this trend, leading a team from three medical institutions to develop a noninvasive technique based on positron emission tomography (PET). The scientists captured three-dimensional views of one body component never before seen from the outside—the immune system.

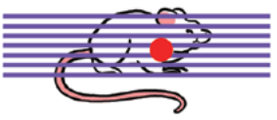
The immune system is particularly challenging to image, Witte says, because "it's everywhere in the body." Thus, the researchers targeted one contiguous aspect of the immune system—the primary immune response to localized tumors, which they induced experimentally in laboratory mice. They focused their PET scanning method on the increased metabolic activity of activated lymph nodes. Lymph nodes are relatively inactive in healthy mammals, but when they face foreign antigens, such as from a growing tumor, "it's like cranking up a rheostat," Witte says. "All the same metabolic processes go on, but at a rate that's 10 to 100 times higher."

The team injected trace amounts of a radioactive compound called [^{18}F]FDG into mice with tumors. The chemical, an analog of glucose, concentrates in metabolically active tissues, allowing the PET apparatus to image its radioactive emissions. Tumors themselves, composed of cells grown out of control, are also high-energy tissues, and hospitals have used FDG for many years to monitor tumor progression. "While we expected to see the cancer with FDG, it was a bit unexpected as to how well we saw the immune system at the same time," Witte says.

The team reported on its work in the November 29, 2005, issue of *Proceedings of the National Academy of Sciences*.

Besides its utility in evaluating the immune systems of cancer patients, the technique may also help monitor other diseases, Witte says. "We've had contacts from a number of people who want to try this technology in animal models of various autoimmune disorders, and eventually in people." **–Paul Muhlrad** ■





WHOLE-BODY SECTIONS OF A MOUSE WITH CANCER SHOW A RADIOACTIVE ANALOG OF GLUCOSE CONCENTRATED IN HIGHLY METABOLIC TISSUES, INCLUDING ACTIVATED IMMUNE CELLS FIGHTING THE TUMOR.

IN BRIEF

GRABBING ADDICTION BY THE TAIL

Canadian scientists have developed some clever molecular trickery that is helping reduce the drug cravings of addicted rats. One problem in addiction is that neurons in some parts of the brain lose glutamate receptors from the cell surface, and those receptors are important for communication between neurons. The researchers sidestepped this problem by crafting a peptide that mimics a portion of the tail of the glutamate receptor and, once inside a neuron, serves as a decoy to prevent the loss of glutamate receptors.

Yu Tian Wang, an HHMI international research scholar, and colleagues at the University of British Columbia in Vancouver reported their findings in the November 25, 2005, issue of *Science*.

In addicted rats, cell-to-cell communication is compromised as a result of certain long-term changes at the level of individual neurons. The group's research produced a targeted drug that tricks brain cells into preventing those changes. "We think this is a good candidate for a drug against addiction that has very few side effects," said Wang, a neuroscientist. Although the initial studies are promising, Wang cautioned that the drug is in the early stages of development and is years away from testing in humans.

RADICAL APPROACH TO CARDIAC RESYNCHRONIZATION

Correcting the timing of heart contractions through cardiac resynchronization therapy can be a lifesaver for people with advanced heart failure. But the procedure, as it is done today, fails in about 15 percent of patients.

Steven Mickelsen, a third-year medical student at the University of New Mexico School of Medicine, developed a minimally invasive technique that—at least in pigs—overcomes the procedure's main shortcomings. Mickelsen conducted the research during a year away from medical school as a Howard Hughes Medical Institute–National Institutes of Health research scholar at the National Heart, Lung, and Blood Institute.

A critical step in cardiac resynchronization therapy is the placing of leads—thin wires that resynchronize the beating of the left and right ventricles—onto the heart. The standard approach to lead placement on the left ventricle is through a blood vessel on the heart's surface, a technical challenge in some patients that limits lead placement to where blood vessels are. A substantial number of cardiac leads fail to work because they are positioned poorly, or they become dislodged. When lead placement fails, the most common next step is open chest surgery to place the lead—an invasive procedure that requires a surgeon and general anesthesia.

Mickelsen searched for a better way to position the critical leads, testing his technique in small pigs whose hearts are about the size of a human's. Using a catheter inserted through the pig's jugular vein, he implanted pacing leads by puncturing the upper chamber of the heart to reach the pericardium, the fluid-filled sac around the heart.

The work was reported in the October 2005 issue of the journal *Pacing and Clinical Electrophysiology*.

Image adapted from *PNAS* 102(48): 17412-7. ©2005.

Lawrence C. Katz
1956–2005

An HHMI investigator at Duke University Medical Center, he died of melanoma on November 26, 2005, at his home. He was 48 years old.

LARRY KATZ WAS A HIGHLY ESTEEMED NEUROBIOLOGIST whose research on the development and function of the mammalian cortex was recognized internationally. The early part of Katz's research career focused on identifying cellular events and cues used by the developing brain to form, maintain, and modify local neuronal circuits in the primary visual cortex.

He pioneered the use of a variety of techniques, including fluorescent tracers, optical imaging in brain slices and in vivo, and combinations of optical and electrophysiological methods to help define the rules by which specific circuits in the cortex emerge and function as neuronal assemblies.

In recent years, Katz continued to investigate the organization of the visual system, and his group had begun to study the olfactory system. In particular, his lab used the mouse as a model to examine how olfactory signals important for basic, built-in behaviors are encoded by the main olfactory system, which detects airborne odors, and the vomeronasal system, which detects species-specific chemical signals called pheromones. Katz's long-term goal was to understand how the neuronal circuits activated by the olfactory and vomeronasal systems elicit species-specific behaviors.

Katz and his colleagues used brain-imaging techniques to visualize the representations of individual odorants and mixtures in space and time in the living brain. By applying advanced microscopy techniques, they were able to visualize the microstructure of neuronal circuits in living mice and to follow changes in those circuits as animals learned new olfactory tasks.

Katz was named an HHMI investigator in 1996. Recently, he was one of the Institute's advisers in the planning of its Janelia Farm Research Campus. "Larry made several important contributions to the planning and development of Janelia Farm," says HHMI President Thomas Cech. "The good advice he gave us will form at least a small part of his lasting legacy."

At Duke University Medical Center, Katz was the James B. Duke Professor of Neurobiology. He published more than 50 original scientific articles and received numerous professional awards for his research. Katz was also an avid and skilled fly fisherman.

Before moving to Duke, Katz did postdoctoral work with Torsten Wiesel at the Rockefeller University. "My first encounter with Larry was when he was a graduate student in Masakazu 'Mark' Konishi's laboratory at Caltech," Wiesel recently observed, "and I remember being struck by his enthusiasm for rather daring experiments. It was no surprise to me that, at graduation, his thesis was selected by the university as the best that particular year. It was a sheer pleasure intellectually and experimentally to have Larry in my laboratory, first as a postdoc and later as a junior faculty member. Larry had an unbounded energy and imagination; not one day would go by without lively discussions and arguments about research questions and how a problem should be approached experimentally."

Another colleague, HHMI investigator Rafael Yuste at Columbia University, remembers Katz as both a mentor and a friend. "He supervised my Ph.D. thesis and, even more importantly, first introduced me to my wife on a blind date," Yuste says. "So he 'fixed' my professional and personal life. From the first time I met him, I admired his unique combination of intelligence and creativity."

Katz is survived by his wife, Doris Iarovici, of Durham, North Carolina, and two children, Ariel and Justin.

Courtesy of Duke University

Scientists Crack Code for Motor Neuron Wiring

Understanding how a developing chick embryo assigns different functions to nerve cells in the spinal cord may yield clinical payoffs for humans.

AS YOU TURN THE PAGES OF THIS *BULLETIN*, MOTOR NEURONS that project from your spinal cord are coordinating the precise actions of more than 50 muscles in each of your arms. Each muscle is individually controlled by its own motor neuron cluster, which has a distinct identity and pattern of connectivity. "Motor neurons represent an extreme example of neuronal diversification," says HHMI investigator Thomas M. Jessell, whose research group at Columbia University Medical Center is seeking to understand how a developing embryo delegates specialized functions to different nerve cells.

"Its first task is to make motor neurons, as a class, different from all the other classes of neurons," says Jessell. "And once the embryo has solved that problem it has to generate distinct columns of motor neurons in the spinal cord, with each column controlling a particular body region, such as a limb. Then, within each column, the embryo has to generate motor neuron pools, each of which activates one particular muscle." Motor axons must then grow from the spinal cord into the limbs and elsewhere and target the right muscle.

Jessell's team recently deciphered the code that assigns unique identities to the motor neuron pools. This code, as the researchers explained in the November 4, 2005, issue of *Cell*, is written in the language of Hox proteins—a family of transcription factors (proteins that activate specific sets of genes) found in virtually all organisms.

Scientists have long recognized that Hox proteins, by orchestrating a cascade of gene expression in the early embryo, ensure animals' overall body plan. They place the head at the top, the feet below, and the correct arrangement of ribs in between.

Four years ago, the Columbia group discovered that Hox proteins also influence the arrangement of the motor neuron columns within the spinal cord. That finding prompted the recent study, in which Jeremy S. Dasen, an HHMI research associate in Jessell's lab, painted chick embryos with a palette of fluorescently tagged antibodies directed against many of the 39 Hox proteins. The experiments were painstaking and meticulous, says Jessell. Merely generating the antibodies was a 5-year effort by Bonnie C. Tice and Susan Brenner-Morton, Dasen's labmates and coauthors of the *Cell* paper.

The hard work paid off. What has emerged from these experiments is a detailed motor neuron atlas that shows the locations of relevant Hox proteins in the chick embryo at different times during development. The appearance and disappearance of the different protein types in distinct motor neuron pools revealed the molecular logic at work to Dasen and his colleagues. "Different Hox proteins have specific tasks that progressively determine motor neuron identity," Jessell explains.

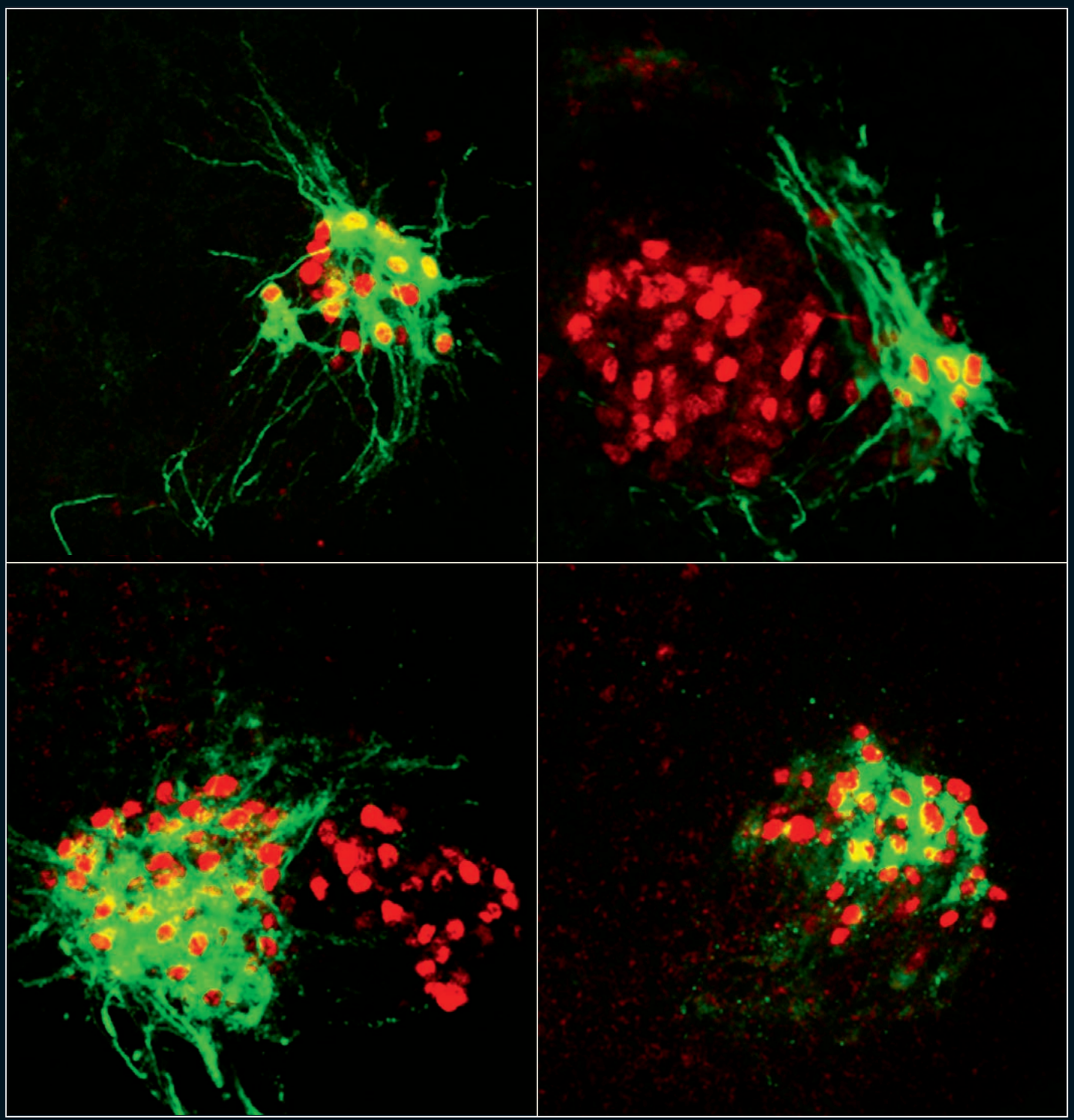
What's more, it became clear from the pictures that certain pairs of Hox proteins exclude each other from an individual neuron, whereas other combinations of Hox proteins can coexist. In effect, the Hox proteins wage a battle for dominance within the cells of each pool. "Hox protein A may win out in one neuron, and Hox protein B may prevail in another neuron," says Jessell. "And since the emergence of that final Hox pattern determines identity, the nature of the interactive circuit between Hox proteins is in itself driving the diversification of neurons."

These findings may have broad implications. "We already know that the basic organization of the chick motor system is conserved within higher vertebrates, including humans," Jessell says. "If you look at chick locomotor behavior, it's strikingly similar to humans walking." It's possible, then, that understanding the Hox code may one day help guide progress in restoring motor neuron function in people whose spinal cords have been damaged by trauma or disease. —**Paul Muhlrad** ■

"Different Hox proteins have specific tasks that progressively determine motor neuron identity."

THOMAS JESSELL

Jessell: Christopher Denney *Facing page:* Images by Jeremy Dasen/Jessell Lab



Outfitting Motor Pools

Researchers in Thomas Jessell's lab have created an atlas of images that shows the location of various Hox-related proteins in the chick embryo at different developmental times.

For this set of images, the researchers injected different muscles in the chick forelimb with a green tracer, which then spread to the corresponding motor neuron pools in the spinal cord.

Each panel shows cell nuclei stained with red-tagged antibodies that bind to different transcription factors. The transcription factors, shown clockwise from top left, are Runx1, Pea3, Scip, and Pea3.

The images demonstrate that different combinations of transcription factors are present in different motor neuron pools.

SPOTLIGHT

Whitehead Institute Taps Page as Leader

HHMI investigator **David C. Page** has been elected the fourth director of the Whitehead Institute for Biomedical Research. Page, who came to the nonprofit institute in 1984 as one of its first fellows and became a faculty member 2 years later, began his new post in December 2005, after a year's appointment as interim director.

"To my mind, the Whitehead Institute is an artist colony extraordinaire," said Page in a press release. "My vision is that in the years ahead we will continue to attract the best young minds and provide them a place to realize dreams."

Page, also a professor of biology at the Massachusetts Institute of Technology, has spent his career examining the Y chromosome: tracing its evolution, determining its nucleotide sequence, and unraveling the mechanism that allows the compact chromosome to maintain its genes. His work has led to an appreciation of the complexity of the Y chromosome and to a better understanding of male infertility.

The Irvington Institute for Immunological Research created a special award in honor of **Frederick W. Alt**, an HHMI investigator at Children's Hospital, Boston, and chairman of Irvington's Scientific Advisory Board. The Frederick W. Alt Award for New Discoveries in Immunology will be presented to former Irvington Institute fellows who have shown outstanding success in academia or industry. The first recipient will be announced in October 2006.

Bonnie Bassler, an HHMI investigator at Princeton University, won the 2006 Eli Lilly and Company Research Award, given to recognize fundamental research of unusual merit in microbiology or immunology by an individual on the threshold of his or her career.

Two HHMI investigators received awards for outstanding scientific achievement from the National Academy of Sciences (NAS). **Ronald R. Breaker**, Yale University, shared the 2006 NAS Award in Molecular Biology with Tina M. Henkin, Ohio State University, for "establishing a new mode of regulation of gene expression." **Frederick M. Rieke**, University of Washington School of Medicine, received a 2006 Troland Research Award for "experimental and theoretical analyses of information coding in the central nervous system and its relation to perception."

Scientific American magazine named **Patrick O. Brown**, an HHMI investigator at Stanford University School of Medicine, one of the 2005 *Scientific American* 50, a designation recognizing people, teams, and organizations whose recent accomplishments demonstrate leadership in shaping both established and emerging technologies. Brown received the honor with Michael B. Eisen for founding the Public Library of Science and for their continuing efforts to make scientific publications accessible to scientists and the public.

Guy Caldwell, an associate professor of biological sciences at the University of Alabama, was named Alabama's 2005 Professor of the Year by the Carnegie Foundation for the Advancement of Teaching and the Council for Advancement and Support of Education. Caldwell was supported in his first 3 years at the university by an HHMI undergraduate biological sciences education grant, beginning in 1999, and he continues to participate in HHMI grant activities there.

SPOTLIGHT



LEFT TO RIGHT: TYLER JACKS, SCOTT W. LOWE, AND JEFF L. WRANA

Three Scientists Win Cancer Research Prize

Two HHMI investigators and one international research scholar have been honored with the 2005 Paul Marks Prize for Cancer Research. The prize, named after Paul A. Marks, president emeritus of the Memorial Sloan-Kettering Cancer Center, recognizes significant contributions to the basic understanding and treatment of cancer by scientists no more than 45 years of age.

The winners are **Tyler Jacks**, of the Massachusetts Institute of Technology, for advancing our understanding of the pathogenesis of cancer; **Scott W. Lowe**, of Cold Spring Harbor Laboratory, who studies how genes influence a patient's response to chemotherapy; and **Jeff L. Wrana**, of the University of Toronto and the Samuel Lunenfeld Research Institute, for his work analyzing the impact of cell-cell communication on tumor development.

Page: Stanley Rowin *Jacks:* Paul Fetters *Lowe:* Zack Seckler/AP. © HHMI. *Wrana:* Courtesy of Jeff Wrana

Kevin P. Campbell, an HHMI investigator at the University of Iowa Roy J. and Lucille A. Carver College of Medicine, was elected in 2005 as a member of the National Arthritis and Musculoskeletal and Skin Diseases Advisory Council.

Three students supported by HHMI's undergraduate grants program are in the 2006 class of Rhodes Scholars. They are **Adam D. Chandler** and **Rahul Satija**, both students at Duke University, and **Elizabeth W. Mayne**, who is enrolled at Stanford University. Each of the three conducted research projects supported by HHMI.

Two researchers were honored recently by the American Association of Immunologists (AAI). **Max D. Cooper**, an HHMI investigator at the University of Alabama, Birmingham, received the 2006 AAI-Dana Foundation Award in Human Immunology Research for his "record of significant achievement and sustained accomplishment in immunology research." **Ruslan Medzhitov**, an HHMI investigator at Yale University School of Medicine, won the 2006 AAI-BD Biosciences Investigator Award for his "outstanding, early-career research contributions to the field of immunology."

Cristoph G.F. Dehio, an HHMI international research scholar at the University of Basel, in Switzerland, shared the Pfizer Research Prize 2006 in infectious disease with his coworker Ralf Schülein. The prize is awarded annually to young, outstanding scientists for basic or clinical research done in Swiss research centers or hospitals.

Gregory J. Hannon, an HHMI investigator at Cold Spring Harbor Laboratory, received the 2005 American Association for Cancer Research Award for Outstanding Achievement in Cancer Research. The award honors an accomplished investigator in the field who is no more than 40 years old at the time the award is conferred.

Stephen C. Harrison, an HHMI investigator at Harvard Medical School, was awarded the 2005 Bristol-Myers Squibb Company Freedom to Discover Distinguished Achievement Award in infectious diseases research for pioneering virus x-ray crystallography.

Lahn: Courtesy of the University of Chicago *Hannon:* Zack Seckler/AP. © HHMI.
DeRisi: George Nitkin/AP. © HHMI.

SPOTLIGHT



LEFT TO RIGHT: BRUCE T. LAHN, GREGORY J. HANNON, AND JOSEPH DERISI

Researchers Shine in *Esquire*

Three HHMI investigators were listed among *Esquire* magazine's "Best & Brightest" of 2005. The popular magazine's picks appeared in the December 2005 "Genius Issue."

Gregory J. Hannon, an HHMI investigator at Cold Spring Harbor Laboratory, was cited for his harnessing of RNA interference to silence genes, which *Esquire* named the "Breakthrough of the Decade." **Bruce T. Lahn**, an HHMI investigator at the University of Chicago, was hailed for his research on the factors driving evolution of the brain, which the magazine called the "Impolitic Idea of the Year."

HHMI investigator **Joseph DeRisi** was singled out for his work at the University of California, San Francisco, studying the molecular biology of infectious diseases, such as malaria, that disproportionately affect developing countries. The magazine dubbed DeRisi a "brilliant do-gooder" for his work and for making his research protocols and published articles freely accessible to other scientists and to the public, placing him among the "vanguard of scientists dedicated to open-source medical research."

Wayne A. Hendrickson, an HHMI investigator at Columbia University College of Physicians and Surgeons, and **Joan Massagué**, an HHMI investigator at Memorial Sloan-Kettering Cancer Center, won 2005 Mayor's Awards for Excellence in Science and Technology in the category of biological and medical sciences. The awards are administered by the New York Academy of Sciences.

Arthur L. Horwich, an HHMI investigator at Yale University School of Medicine, received the 2006 Stein and Moore Award from the Protein Society. The award will be given jointly to Horwich and F. Ulrich Hartl, director of the Max Planck Institute for Biochemistry in Munich, for their discovery of and groundbreaking work on chaperone-assisted protein folding.

Eric R. Kandel, an HHMI investigator at Columbia University College of Physicians and Surgeons, won the 2005 Austrian Medal of Honor for Science and Art.

David R. Liu, an HHMI investigator at Harvard University, received the 2006 American Chemical Society Award in Pure Chemistry.

Craig C. Mello, an HHMI investigator at the University of Massachusetts Medical School, was awarded the 2005 Massry Prize with colleague Andrew Z. Fire at Stanford University School of Medicine for their discovery of RNA interference (RNAi). Mello and Fire share the award with David C. Baulcombe of the Sainsbury Laboratory, John Innes Centre, in Norwich, England, whose research in plants contributed to the discovery of RNAi.

Sergei A. Nedospasov, an HHMI international research scholar at the Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, in Moscow, won the 2005 Helmholtz Humboldt Research Award from the Humboldt Foundation and the Helmholtz Association in Germany in recognition of his accomplishments in research and teaching.

Michael K. Rosen, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas, was one of three researchers to receive an inaugural Edith and Peter O'Donnell Award from the Academy of Medicine, Engineering, and Science of Texas honoring the work of outstanding up-and-coming researchers.

CONTINUED FROM PAGE 23
(JOAN STEITZ)
somewhere else," says Steitz. If this turns out to be the case, the RNA portion of a snRNP would be considered a ribozyme.

By this time, Steitz had already advanced up the Yale ladder to become a full professor. Her lab subsequently discovered a second spliceosome that eliminates a rare class of "black sheep" introns that have atypical sequences at their splice sites.

And with her discovery of another kind of snRNP particle, small nucleolar RNPs (snoRNPs), she proved that the term junk DNA was a misnomer. Introns, the so-called noncoding regions of DNA, sometimes code for the small nucleolar RNA found in a snoRNP. These molecules (pronounced snow-RNPs by Steitz) chemically modify ribosomal RNA and are essential to its function.

Currently, Steitz is exploring viral snRNPs as well as the welter of effects splicing has on the downstream life of an RNA message. "For instance," she says, "we know that in the process of splicing proteins are put on RNA that are important for getting the RNA out of the nucleus to the cytoplasm."

While her work is on the bench side of science, others are translating her findings in the clinic in ways Steitz finds "absolutely amazing." A recent paper in *Science* details a way to use aberrant splicing to prevent the ravages of muscular dystrophy in dog models. "Basically, they designed a snRNP to undo the drastic consequences of a mutation," Steitz marvels. "I think that is just extremely cool!"

THE PLEASURE OF HER COMPANY

Apart from the official kudos Steitz has received, including the National Medal of Science, postdocs and graduate students in her lab say it's a genuine pleasure to be there. They rave, for example, about her "really great parties." At their most recent Halloween bash, Joan was the Statue of Liberty and Tom was Uncle Sam. And Doudna recalls delightful afternoons spent sailing with Steitz and her husband, drinking wine, discussing science—or the wind. "Working as a postdoc under Joan was such a fantastic experience," says Baserga, "that I spent the first several years on my own wishing I were still there."

While science itself is clearly Steitz's first priority, education is her second. "I adore teaching undergraduates and consider it a privilege to interact with the fabulous students at Yale," she says. Her recent participation in a committee that wrote the National Academy of Sciences report titled "Bio 2010" inspired her to completely revamp a course for advanced undergraduates that teaches them, by group participation, how to read the literature. "Almost every time I lecture at another university, someone comes up to me and says, 'I took your biochemistry course back in 19xx, and it was terrific.' What more can one wish for?"

Another passion is a desire for women scientists to be appreciated as men's equals. Steitz stands firmly by her 2001 comment in *The New York Times* that a woman scientist needs to be twice as good for half the pay, although, Thomas Cech points out, she doesn't picket for change but rather leads by example. Steitz spends time on oversight issues to remedy remaining inequality problems—time she would far rather devote to her science.

She bristles when asked about Harvard President Larry Summers' recent suggestion that women have less innate scientific ability. But she's certainly circumspect in her reply: "What he said, and the sequelae at Harvard and throughout the nation, is the best thing to happen for women in science since the MIT report." She is referring to the report out of MIT in the late 1990s that found women scientists at that institution suffered significant discrimination in terms of pay and stature. After that report was made public, remedial changes were initiated at many universities. Steitz says she is optimistic that Summers' comments will again prompt positive change for women in science. Regarding his continuation as Harvard's president, post-gaffe: "That's something I find very interesting," replies Steitz without expression.

It's not hard to imagine how she would respond to Dr. Famous today if he questioned her place in and dedication to science. She might show him her weighty CV and invite him sailing with her beloved son and husband to remind him that a career in science does not exclude a happy family life—even without the station wagon. ■

CONTINUED FROM PAGE 39
(ERIC BETZIG)

Do you have thoughts on how to speed up progress?

EB: Well, that gets into the wider philosophical issues of how research is done, which Janelia will try to address in some ways. In particular, I'm hopeful that the innovative engineering group within Janelia will help, at least for stuff we start to develop internally.

The problem with the near-field microscope—a device I was using fairly successfully—was that there was no mechanism for turning it into a good turnkey instrument. And it's still too embryonic for most biologists to consider using. There are hundreds, if not thousands, of examples in science and technology of good ideas that just languish because of the gulf that exists between the conception/demonstration of an idea and something that's economically viable.

My hope is that Janelia will be a step in the right direction, because mechanisms will be in place there to take ideas that have been shown to work from a proof-of-principle standpoint to the point where they might be broadly applied. Right now, that's pretty damn rare. **–Interview by Jennifer Michalowski** ■

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(NEVER TOO YOUNG FOR SCIENCE)
tools, observing, drawing conclusions, and making predictions.

Evaluations show that the children's vocabulary for the names and functions of science tools increased significantly over a 5-month period during the 2005 program and that most were able to select the appropriate tool to solve a new problem. The results "tell us that children not only know how to use the tools but are also more likely to transfer that knowledge into a new situation," says Garner.

"When we looked at outcomes," adds CLS president Keith Verner, a former HHMI grantee at the Penn State College of Medicine, "we saw increases that were not dependent on a particular teacher or a particular class. We believe it was the program itself that made the difference."

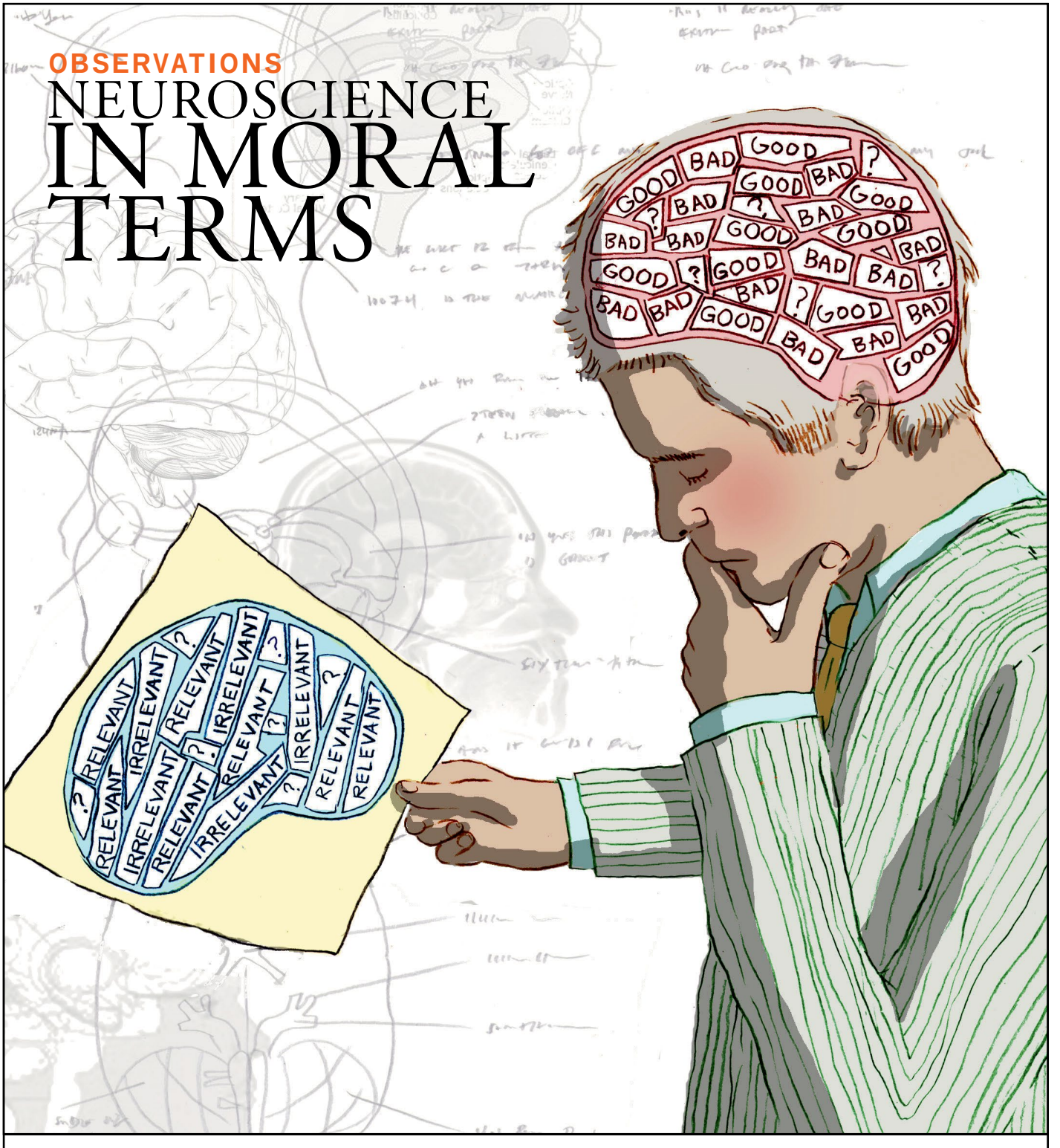
Loudoun County's Scovel agrees, and notes, "We don't want to repeat what children will learn in kindergarten, but we want to build skills they can use in kindergarten and beyond." **–Judith B. Saks** ■

CONTINUED FROM PAGE 43
(EVOLUTION/RELIGION DEBATE)
nonbeliever like myself or a believer like Father Wiseman. It seems to me that [science and religion] are two separate things." He added, "The Bible is not a work of science."

"I find it beyond ironic that society depends on DNA evidence for questions of life and death," Carroll remarked, "yet we're not willing to contemplate the DNA record of natural history and evolution." **–Jennifer Boeth Donovan** ■

OBSERVATIONS

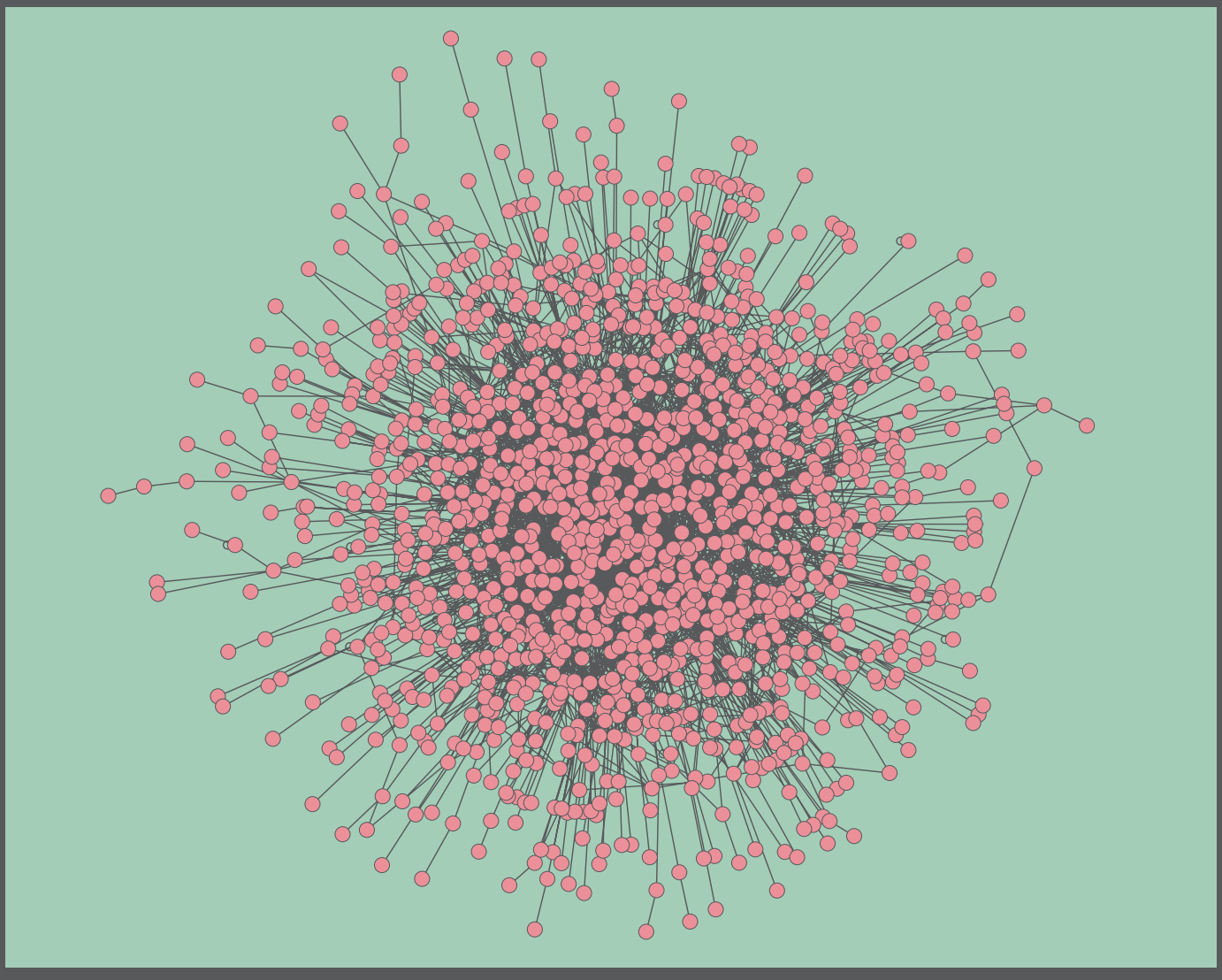
NEUROSCIENCE IN MORAL TERMS



Leif Parsons

Ethical problems seem never to be wholly new; there are always precursors and therefore analogies to be drawn and prior conceptual schemes to be considered and revised or reformed. To the extent that there is an appearance of novelty as ethical issues come to widespread awareness, it is mainly because of peculiar aspects of a particular case that oblige a novel analytic approach. In the early days of bioethics, many issues attracted attention because of new technological capabilities such as the implications of life-extending modalities for the definition of clinical death. With its access to improving technologies, especially functional imaging, work now proceeding in the neurosciences provides rich ground for such cases. Many of those engaged in these efforts will find themselves the subjects of the sort of public attention previously experienced by their colleagues in nuclear physics and genetics. Neuroscientists will increasingly be challenged to explain the significance of their work in moral as well as scientific terms.

A member of HHMI's Bioethics Advisory Board, Jonathan Moreno is the Kornfield Professor and director of the Center for Biomedical Ethics at the University of Virginia and a fellow at the Center for American Progress.



Courtesy of Fields Lab

Mapping How Parasite Proteins Relate

THE THOUSANDS OF INTERACTIONS THAT TAKE PLACE BETWEEN PROTEINS OF THE MALARIA-CAUSING PARASITE PLASMODIUM FALCIPARUM ARE BEING TRACKED AND MAPPED IN THE UNIVERSITY OF WASHINGTON LABORATORY OF HHMI INVESTIGATOR STANLEY FIELDS. IN THIS DEPICTION, INDIVIDUAL PROTEINS ARE INDICATED BY PINK CIRCLES AND THE INTERACTIONS BY THE LINES THAT CONNECT THEM. UNDERSTANDING HOW THE PROTEINS RELATE TO EACH OTHER MAY ILLUMINATE VULNERABILITIES IN THE PARASITE'S DEFENSES (SEE PG. 49).

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