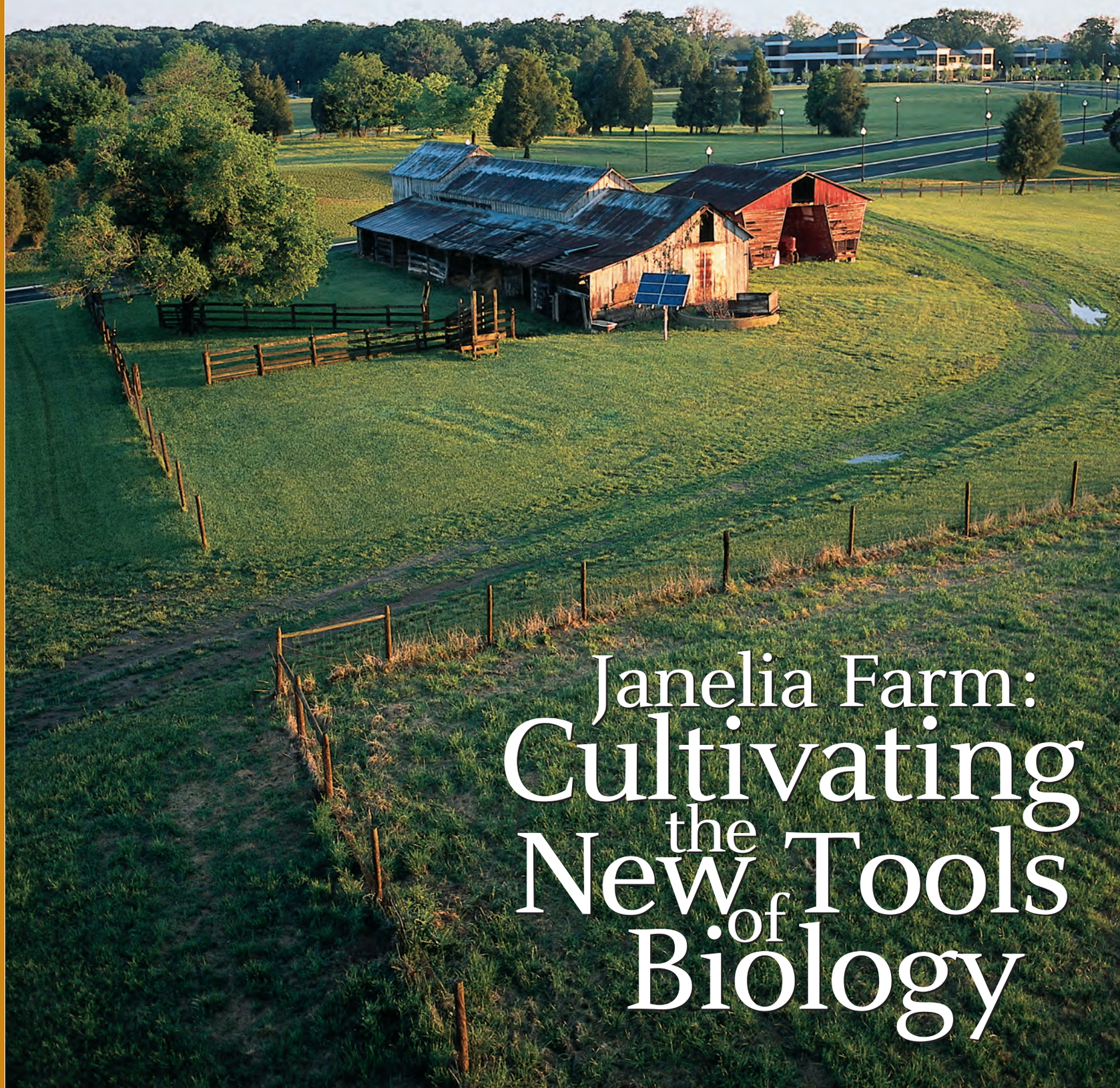
Protein Folding || Brain Disorders || Postdocs || Embryonic Development

JULY 2001

HHMI

Howard Hughes Medical Institute Bulletin



Janelia Farm: Cultivating the New Tools of Biology

FEATURES

10 Janelia Farm

HHMI plans to transform a Virginia farm into a unique laboratory complex where interdisciplinary teams will create advanced tools for biomedical research. The inside story of how a "what if?" idea became a $500 million project.
By M. Mitchell Waldrop

16 When the Brain Fails

Huda Zoghbi unravels the genetics of Rett syndrome and other disorders.
By Nancy Ross-Flanigan

20 Rosetta Tackles the Extreme Origami of Protein Folding

David Baker's model is producing remarkably accurate predictions.
By Dennis Meredith

24 The Invisible Scientists

More pay. Better benefits. Increased mentoring. A report carried out with HHMI support says postdoctoral fellows need all this—and more.
By Karen Young Kreeger

DEPARTMENTS

2 NOTA BENE

3 PRESIDENT'S LETTER
The Chemistry of Creativity at Janelia Farm

UP FRONT

4 *Science as Training for a Lifetime*

6 *Undergraduates Learn About Science and Life in Labs Overseas*

8 *Erin O'Shea: Learning the Secrets of Cell Signaling*

NEWS AND NOTES

28 *Ticks Inspire a Young Scientist*

29 *Discovering a "Sweet-Tooth" Gene*

30 *Kurt Weiss Battles Cancer—This Time, to Help Others*

31 *Science News for the Spanish-Speaking World*

32 *Evolution in a Test Tube*

Tracing Neural Clues to Eating and Willpower

33 *A Teachable Moment in the Waiting Room*

34 CLOSE-UP
The Beautiful Logic of Development

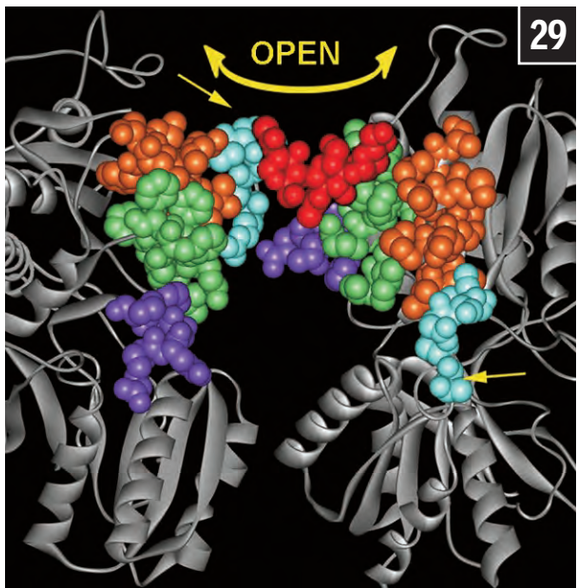
36 OBJECT LESSON
Explaining the Brain

38 FROM THE TOOLBOX
New Technique Reveals Neuronal Calcium Channels in Action

40 INSIDE HHMI
An Investigator's Right-Hand Man in the Laboratory

41 HHMI ONLINE
The Bulletin *Goes Online*

16



29

SCOTT GOLDSMITH (OPPOSITE), PAM FRANCIS (TOP)

On the Cover: A barn stands near the entrance of HHMI's new Janelia Farm site. Photograph by William K. Geiger

The next issue of the *Bulletin* will have a new name on the masthead—and it is a pleasure to introduce Cori Vanchieri, the new editor. Cori was an undergraduate at Cornell University and earned a master's degree in journalism at Stanford University. She has extensive editing and writing experience in science, medicine, and health, and for 10 years was on the communications staff of the National Cancer Institute. We look forward to her leadership in creating a magazine that accurately portrays the work and the spirit of the Howard Hughes Medical Institute—and the incredible pace of discovery and change that is characterizing biomedical research.

—Robert A. Potter, *Director of Communications*

HHMI

Howard Hughes Medical Institute Bulletin

July 2001 ‖ Volume 14 Number 3

HHMI TRUSTEES

James A. Baker, III, Esq.
Senior Partner, Baker & Botts

Alexander G. Bearn, M.D.
Executive Officer, American Philosophical Society
Adjunct Professor, The Rockefeller University
Professor Emeritus of Medicine, Cornell University Medical College

Frank William Gay
Former President and Chief Executive Officer, SUMMA Corporation

James H. Gilliam, Jr., Esq.
Former Executive Vice President and General Counsel,
Beneficial Corporation

Hanna H. Gray, Ph.D., CHAIRMAN
President Emeritus and *Harry Pratt Judson Distinguished Service Professor of History,* The University of Chicago

Garnett L. Keith
Chairman, SeaBridge Investment Advisors, L.L.C.
Former Vice Chairman and *Chief Investment Officer*, The Prudential Insurance Company of America

Jeremy R. Knowles, D.Phil.
Dean of the Faculty of Arts and Sciences and *Amory Houghton Professor of Chemistry and Biochemistry,* Harvard University

William R. Lummis, Esq.
Former Chairman of the Board of Directors and Chief Executive Officer, The Howard Hughes Corporation

Irving S. Shapiro, Esq., CHAIRMAN EMERITUS
Of Counsel, Skadden, Arps, Slate, *Meagher & Flom*
Former Chairman and Chief Executive Officer,
E.I. du Pont de Nemours and Company

Anne M. Tatlock
Chairman and *Chief Executive Officer*
Fiduciary Trust Company International

HHMI OFFICERS

Thomas R. Cech, Ph.D., *President*

Peter J. Bruns, Ph.D., *Vice President for Grants and Special Programs*

David A. Clayton, Ph.D., *Vice President for Science Development*

Stephen M. Cohen, *Vice President and Chief Financial Officer*

Joan S. Leonard, Esq., *Vice President and General Counsel*

Gerald M. Rubin, Ph.D., *Vice President for Biomedical Research*

Nestor V. Santiago, *Vice President and Chief Investment Officer*

HHMI BULLETIN STAFF

David Jarmul, *Issue Editor*

Kimberly Blanchard, *Production Supervisor*

Elizabeth Cowley, *Copy Editor*

Jennifer Donovan, *Education Editor*

Patricia Foster, *Manager of Publishing*

Jim Keeley, *Science Editor*

Dean Trackman, *Copy Editor*

David Herbick Design, *Publication Design*

Telephone (301) 215 8855 ■ Fax (301) 215 8863 ■ www.hhmi.org
The *Bulletin* is published by the HHMI Office of Communications, Robert A. Potter, *Director.*

© 2001 Howard Hughes Medical Institute

Letters to the editor: We invite your comments. Send your letters via e-mail to bulletin@hhmi.org or regular mail to Letters, Office of Communications, Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815-6789. We reserve the right to edit for space and clarity. Please also include your name, address (e-mail or postal) and phone number.

The opinions, beliefs and viewpoints expressed by authors in the HHMI Bulletin do not necessarily reflect the opinions, beliefs and viewpoints or official policies of the Howard Hughes Medical Institute.

NOTA BENE

HHMI investigators **Pamela J. Bjorkman,** California Institute of Technology; **Peter Cresswell,** Yale University; **Pietro V. De Camilli,** Yale University; **John H. Exton,** Vanderbilt University; **Jeffrey M. Friedman,** The Rockefeller University; **Philip P. Green,** University of Washington; **John Kuriyan,** The Rockefeller University; **Richard P. Lifton,** Yale University; and **Ronald D. Vale,** University of California, San Francisco, were elected to membership in the National Academy of Sciences.

Kristi S. Anseth, an HHMI investigator at the University of Colorado at Boulder, won the 2001 Outstanding Young Investigator Award from the Materials Research Society.

Richard Axel, an HHMI investigator at Columbia University College of Physicians and Surgeons, received the New York Academy of Medicine's 2001 Academy Medal for his contributions to biomedical science.

Günter Blobel, an HHMI investigator at The Rockefeller University, received the Grosse Bundesverdienstkreuz mit Stern, the highest civilian honor awarded by Germany.

Mark M. Davis, an HHMI investigator at Stanford University School of Medicine, won the 2000 William B. Coley Award from the Cancer Research Institute for his achievements in the fields of basic and cancer immunology.

Stephen J. Elledge, an HHMI investigator at Baylor College of Medicine, received the 2000 Michael E. DeBakey, M.D., Award for Research Excellence and the 2001 G.H.A. Clowes Memorial Award from the American Association for Cancer Research.

Elaine Fuchs, an HHMI investigator at the University of Chicago, won the 2001 Richard Lounsbery Award from the National Academy of Sciences for achievement in biology and medicine. She also has been elected to a three-year term on the academy's council.

David Ginsburg, an HHMI investigator at the University of Michigan Medical School, received the school's 2000 Distinguished Faculty Achievement Award.

HHMI investigators **Corey S. Goodman,** University of California, Berkeley, and **Thomas M. Jessell,** Columbia University College of Physicians and Surgeons, shared the 2001 March of Dimes Prize in Developmental Biology.

H. Robert Horvitz, an HHMI investigator at the Massachusetts Institute of Technology, shared the 2000 Louisa Gross Horwitz Prize with HHMI investigator **Stanley J. Korsmeyer** of the Dana-Farber Cancer Institute. Columbia University awards the prize to recognize outstanding research in biology or biochemistry.

Robert J. Lefkowitz, an HHMI investigator at Duke University Medical Center, received the 2001 Peter Harris Distinguished Award from the International Society for Heart Research.

Pat Lisoskie, a high school teacher in Turnwater, Washington, won the 2000 Presidential Medal for Excellence in Math and Science Teaching. She was recognized for a unit on bioethics that she wrote as a participant in the HHMI-supported Woodrow Wilson National Fellowship Foundation's Leadership Program for Teachers.

Roderick McInnes, an HHMI international research scholar at Toronto's Hospital for Sick Children, has been appointed scientific director of the Genetics Institute of the Canadian Institutes of Health Research.

Randall R. Reed, an HHMI investigator at The Johns Hopkins University, won the 2000 Frank Allison Linville's R.H. Wright Award in Olfactory Research, given by Simon Fraser University in Canada.

Gerald M. Rubin, HHMI vice president for biomedical research, was a co-recipient of the American Association for the Advancement of Science's Newcomb Cleveland Prize for 2000. Established in 1923, the award recognizes an outstanding paper published in *Science*.

Charles J. Sherr, an HHMI investigator at St. Jude Children's Research Hospital in Memphis, won the 2000 Ernst W. Bertner Award for Cancer Research from the University of Texas M.D. Anderson Cancer Center.

Christine E. Seidman, an HHMI investigator at Brigham and Women's Hospital, Boston, and **Jonathan G. Seidman,** an HHMI investigator at Harvard Medical School, were co-recipients of a 2000 award given by the Gill Heart Institute of the University of Kentucky for their contributions to cardiovascular research.

Jonathan S. Stamler, an HHMI investigator at Duke University Medical Center, received the 2001 Outstanding Investigator Award in Basic Science from the American Federation for Medical Research Foundation.

Susan S. Taylor, an HHMI investigator at the University of California, San Diego, won the 2001 Francis P. Garvan–John M. Olin Medal, awarded by the American Chemical Society to an American woman chemist.

Bert Vogelstein, an HHMI investigator at The Johns Hopkins University School of Medicine, received the 2001 American Association of Pathologists' Award for Excellence in Molecular Diagnostics.

Arthur Weiss, an HHMI investigator at the University of California, San Francisco, won the American Association of Immunologists' 2001 AAI-Huang Foundation Meritorious Career Award.

Owen N. Witte, an HHMI investigator at the University of California, Los Angeles, won the 2000 Warren Alpert Foundation Prize for his research involving chronic myelogenous leukemia. He shared the award with David Baltimore, Nicholas Lydon, Alex Matter and Brian Druker.

PRESIDENT'S LETTER

The Chemistry of Creativity at Janelia Farm

KAY CHERNUSH

I've been thinking a lot about chemistry lately. Not about the science itself but about scientists and the ways they can work best together—perhaps what I mean is the chemistry of creativity. For many years, biomedical research has owed its successes to the meticulous work that innumerable biologists carry out alone or in small groups in individual laboratories. Recently, however, although this approach remains central to the enterprise, more and more discoveries are emerging from teams working collaboratively around the world.

As the landscape of creativity changes, so must the biomedical research enterprise that is built upon it. That's why the Howard Hughes Medical Institute recently unveiled a 10-year, $500 million plan for a biomedical science center that we hope will become a leading source of the advanced technology that science needs to maintain its breathtaking pace of discovery. The center will be home to 24 HHMI investigators and their teams, who will establish ongoing research programs. In addition, there will be extensive laboratory space and living accommodations for visiting scientists from around the world, drawn from the academic community and from government and industry. Researchers interested in both science and technology will be able to interact with colleagues from diverse fields in attacking biological problems that are difficult to address in existing settings.

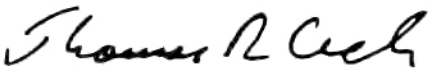
We're building the facility on a 281-acre site, called Janelia Farm, which the Institute recently purchased in Loudoun County, Virginia. The farm is just outside Washington, D.C., convenient to our headquarters and to Dulles International Airport. It's a beautiful place on the banks of the Potomac River, with magnificent old trees and views across a historic landscape. We expect to begin construction in about two years and to move in by the end of 2005.

Even after it's up and running, the new project will account for only about 6 percent of HHMI's investigators and less than 10 percent of its research budget, which totals $509 million this year. The Institute will continue to carry out research primarily with its more than 300 HHMI investigators and their teams at 72 universities and other institutions across the country. We will also continue to support science education, international research and other activities through our grants program, which totals more than $100 million annually. We're pondering how best to integrate Janelia Farm with HHMI's current efforts and those of the federal government, universities, industry and others involved in biomedical research.

Although the Janelia Farm project is small in relative terms, we hope it will have a substantial impact on the Institute and biomedical research generally. We're not interested in activities that are already being done well at most institutions, such as x-ray crystallography or the small-scale bench science that is the bedrock of biology. Rather, we'll look for opportunities to create research tools that have great potential to advance biology and medicine—tools based on new insights from biology, physics, chemistry, computer science, engineering and other fields—and then to disseminate these tools throughout the research community. Several years ago, for instance, we might have helped develop the biochips that have recently become so important to researchers studying gene-expression patterns. Today, we might find opportunities in hot fields like bioinformatics or imaging. Ten or 20 years from now, who knows? Indeed, we deliberately chose a site that contains plenty of extra space where our successors at HHMI can build facilities that cannot even be imagined today.

The new campus will also offer resources that HHMI cannot easily provide at its existing laboratories. For instance, many researchers would welcome access to libraries of chemical compounds and expert guidance on how to screen the compounds for those that inhibit or activate functions of biomolecules. At Janelia, we can provide this, and more.

Recently, I had an opportunity to visit the European Molecular Biology Laboratory in Heidelberg, which has blended biology, technology and other concerns into a vibrant campus with an active visitors' program. My HHMI colleagues and I have also been studying other institutions, and we've benefited from the insights and advice that scientists and others around the world have generously shared. Now, as we embark on this exciting new chapter in the Institute's history, we're determined to build on HHMI's own traditions and the experience of others to create a unique resource that advances science and benefits humanity. We're looking for just the right chemistry to catalyze something wonderful.



Thomas R. Cech
President
Howard Hughes Medical Institute

Up Front

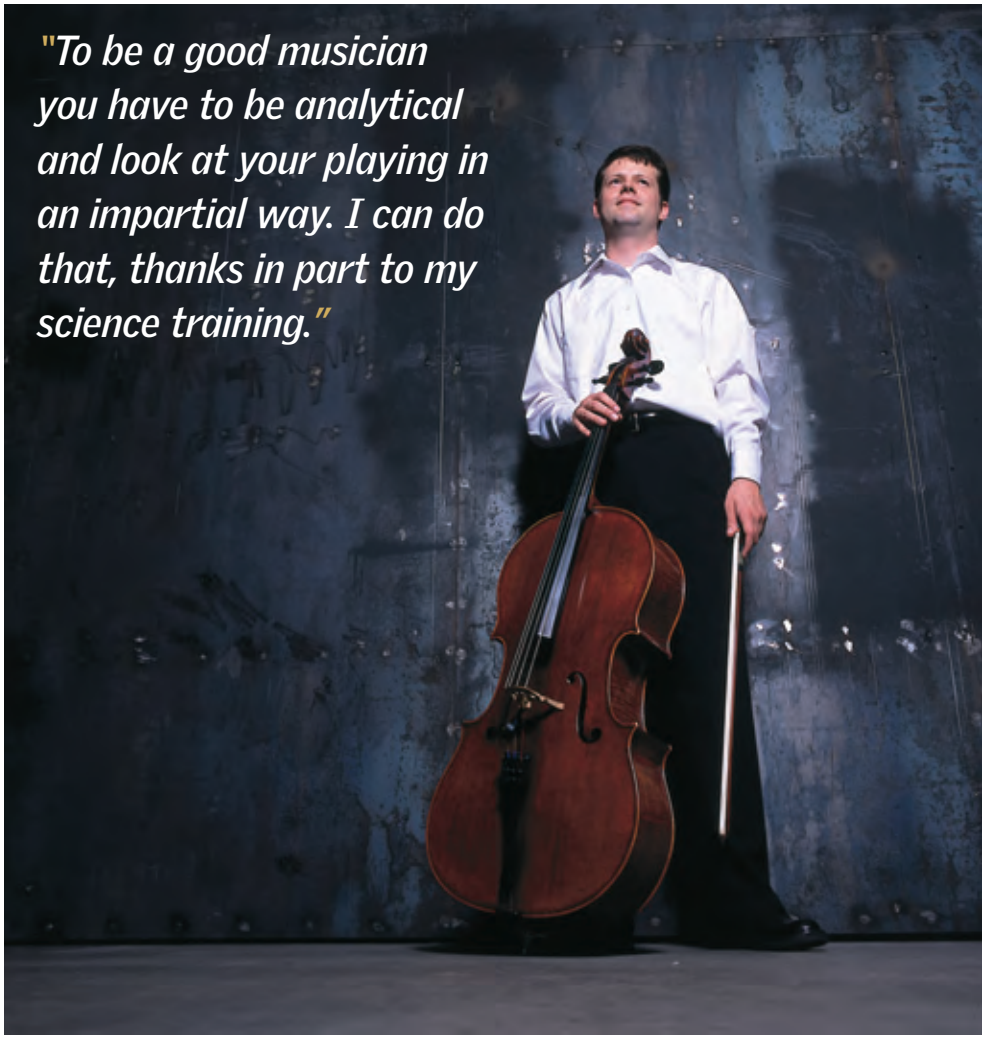
Science as Training for a Lifetime

Like any good scientist, Christopher Stenstrom is both creative and critical. Every day, he throws himself into his work and then coolly steps back to analyze the results. Did his experiments succeed? How well? But Stenstrom is no biologist. His tool is the cello—and his lab is the Nashville Symphony.

At 28, Stenstrom is a musician with a molecular background. As an undergraduate at Ohio's Oberlin College in the early '90s, he majored in both music and molecular biology. Today, he puts the combination to good use. "To be a good musician," Stenstrom says, "you have to be analytical and look at your playing in an impartial way. I can do that, thanks in part to my science training."

Some science students immediately fall in love with the lab, leaving the bench only to grab a soda. But what about the rest? Like Stenstrom, many students discover that science is not for them. These graduates wander into far-flung fields—law, journalism, business, acting, just to cite a few—yet they bring a unique sense of science along, such as problem solving and being creative on a budget. Mainstream science has traditionally viewed these graduates as talent gone astray, but academic instructors say former scientists-in-training are among the best success stories around. "By introducing students to the culture of science, we really do change the face of society," notes Lynne Hunter, a virologist who directs an HHMI-funded undergraduate biology program at the University of Pittsburgh. "We can encourage students to be good scientists for life, whether they're in the lab or just reading the *Wall Street Journal* critically."

All budding scientists dream about that "Eureka!" moment of discovery, but many instead experience the "uh-oh" moment of career uncertainty. For P.J. Gallagher, 25, that sinking feeling arrived one year into graduate school at Emory University in Atlanta, where he studied neuroscience. "I really loved scientific theory more than the day-to-day practice," Gallagher explains. "Actually being there in the trenches wasn't what I wanted to do." During graduate school, Gallagher moonlighted as a Web site designer and, not yet fully aware of his true calling, turned down a handful of Internet-related job offers. But he found his way back. Today, Gallagher is a creative developer at Halo Integrated Solutions Design Group, an Atlanta firm that designs multimedia-ready Web sites. There, he puts the scientific method on the menu every day. "When we're brainstorming for designs and



DAVID MAXWELL

Christopher Stenstrom, a cellist in the Nashville Symphony, majored in both music and molecular biology at Oberlin College.

considering all this programming architecture, I always create hypotheses for what I think will and will not work," he says. "Then I test them vigorously."

The career turning point came earlier for Deborah Yellin, 26. As a biochemistry major at Tufts University near Boston, she had always planned to attend graduate school. During her senior year, however, Yellin took a class on the legal issues surrounding genetic engineering—and was hooked. "I really got into these debates over the ethics of human cloning and animal experiments," she recalls. "It seemed like science and law would make the perfect combination." Yellin recently graduated from the George Washington University Law School in Washington, D.C., and now plans to become a patent attorney. "Scientists can bring a way of thinking to the table—be it more creative or more analytical—that other lawyers may not bring," Yellin says.

That should satisfy any academic science instructor, notes David Hansen, a chemist at Amherst College in Massachusetts. Rather than viewing a student's undergraduate research experience solely as a step toward a scientific career, Hansen says he also evaluates it on its own merits. "I measure the success of the experience at ground zero," Hansen says. "What has the student gained in undertaking his or her research project?" Sometimes the answer is confidence. Students learn they can walk into an unfamiliar environment—like a research lab—and succeed. "That kind of confidence spills over to any endeavor," Hansen says.

It has for Amani Brown, 24, a graduate student at Harvard Divinity School. After earning an undergraduate chemistry degree at Amherst, Brown was doing clinical research on AIDS at Massachusetts General Hospital in Boston when she decided that her heart just wasn't in science. "I had wanted to be a healer so badly," Brown says. "But I realized that my definition of 'healer' was changing. While I was interested in the elegant ways science can approach the body, I felt more drawn to the emotional and spiritual side of the healing process." She jumped into a three-year master's degree program in divinity and hasn't looked back. Today, Brown says her time spent in science is a source of inspiration. "My science major was one of the biggest challenges I've undertaken," she says. "Now I'm confident I can stretch my mind and solve whatever challenging problems come my way."

Similarly, 25-year-old Michael Brennan of Los Angeles relies on his science training to pursue his career choice: acting. Fresh out of Texas Tech University in Lubbock with degrees in biochemistry and theater arts, Brennan set out to start a small film production company. And he succeeded—in part, he says, because he knew how to rein in his ambitions and organize his goals. "Coming from a science background, I knew how to research the field, outline our agenda and get things done," Brennan says. "A lot of actors, too, have ambitions but don't know how to approach them. That's half the battle."

Are there any drawbacks to starting out in science? Brennan's answer is no—and he does get a kick out of telling fellow actors that he began as a biochemistry student. Similarly, Brown says theology students are often surprised to hear she was once a budding chemist. But these might-have-been scientists make the most of their colorful backgrounds, weaving logic and creativity together in new ways. "Science," says Brown, "will serve me well for a lifetime."

—KATHRYN BROWN

JASON GROW

Amani Brown, a former chemistry student now at Harvard Divinity School, was drawn to the spiritual aspects of the healing process.

Undergraduates Learn About Science and Life in Labs Overseas

Until she spent the summer before her junior year of college working in a parasitology lab in Lima, Peru, 23-year-old Rose Q. Do had always lived in Tucson, Arizona. "I couldn't even imagine myself in another country," Do recalls. Then she joined an international research program for undergraduates and spent three months in Peru.

"Living in a Third World country was sobering, but it made me realize that I can do meaningful research with global ramifications," Do says. Now she's finishing her first year of medical school and planning a research career in infectious diseases. Do is one of 94 University of Arizona undergraduates who have conducted laboratory research in 23 foreign countries through a program called BRAVO! (Biomedical Research Abroad: Vistas Open!), supported in part by an HHMI grant.

About one-third of the students traveled to developing countries. Oscar K. Serrano, on the other hand, worked in Italy with neuroscientist Giancarlo Pepeu at the University of Florence, where he was impressed by the close interaction between researchers and students. "European scientists take their role as teacher and guide very seriously," says the University of Arizona senior. "In Florence, it was common to see professors working side by side with their students at the lab bench or computer."

Serrano also learned about anti-Americanism. Born in Arizona but raised in Mexico until he was 11, he often found himself privy to "America bashing" by Europeans who considered him a Mexican. "I never thought of myself as an American nationalist until people started criticizing my form of government, my people and my country," he says.

Living and working in another country is quite different from traveling as a tourist, the students say. They are treated as full-fledged members of their lab families and thereby gain an inside look at a different way of life. Do, for example, accompanied a public health fieldworker up steep, dusty hills to the shantytowns of Lima, where they weighed and measured children and dispensed health advice to parents. "It was amazing to see how public health work is actually done at the local level," she says.

Of course, not every experience is so serious. Dominic Titone, who worked in a lab in Geelong, Australia, remembers tying up traffic while learning to drive on the "wrong" side of the road. And Do recalls how she was nicknamed "chica de los pescados"—fish girl—after she filled a broken garbage bag with fish from a laboratory experiment and left it out in the sun, creating a stench that permeated the neighborhood.

After living their foreign adventures, it's no surprise that students often return home with new goals. Charles A. Hoeffer, Jr., for example, was planning to become a physician when the program took him to the National Institute of Health in Tokyo, Japan, to study apolipoprotein mutations in silkworms. "My experience convinced me not to go to medical school," says Hoeffer, who is now pursuing a doctorate in molecular and cellular biology at the University of Arizona. "I found out how much I like lab research."

Couldn't he and the other students have reached the same conclusions at home? Neurobiologist Leslie Tolbert, a member of the program's advisory board, doesn't think so. "So many of the benefits of this program are intangible," she says, the direct result of being in a new and different environment.

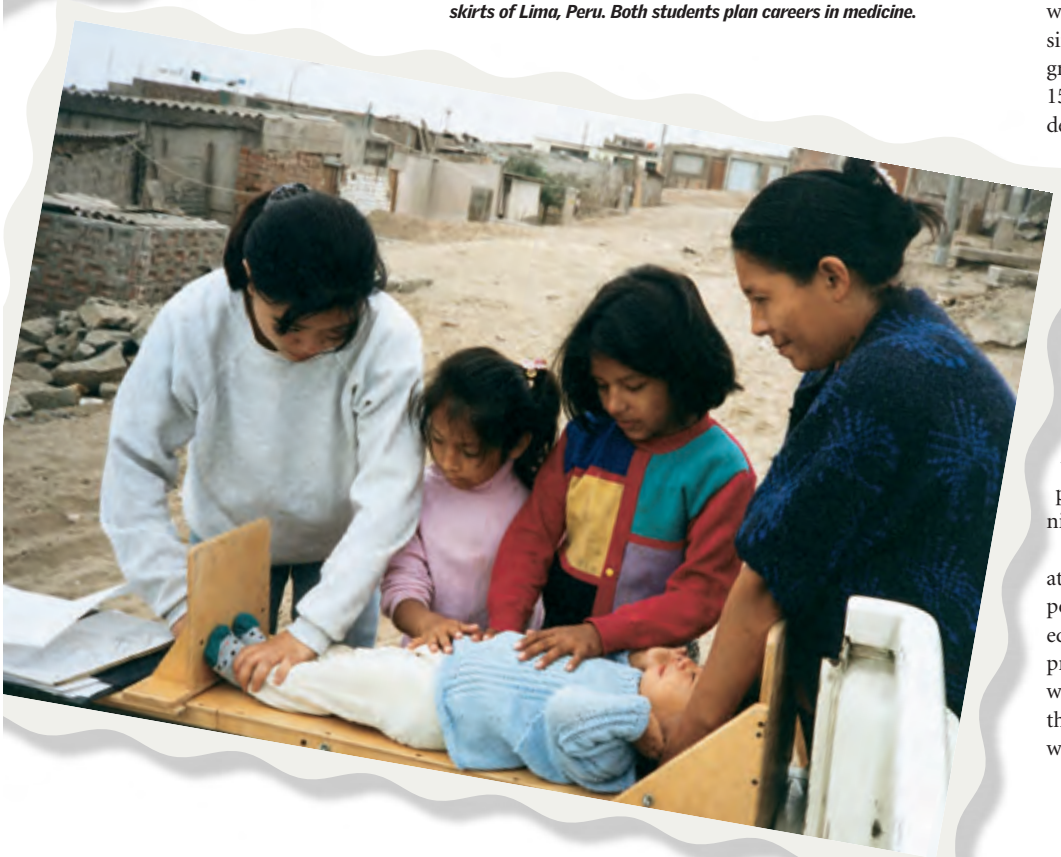
"*In Florence, it was common to see professors working side by side with their students at the lab bench or computer.*"

— Oscar K. Serrano

"*Living in a Third World country was sobering, but it made me realize that I can do meaningful research with global ramifications.*"

— Rose Q. Do

University of Arizona undergraduates get a taste of medical research in foreign countries. Oscar Serrano (above, left) talks with his mentor, Giancarlo Pepeu, at the University of Florence in Italy. Concepción "Nina" Roxas (below, left) studied the growth of children who are infected by parasites in the hillside community of Las Pampas, on the outskirts of Lima, Peru. Both students plan careers in medicine.



"You can't separate the scientific experience from the personal one," Tolbert says, recalling one student whose science was not remarkable but whose foreign mentor "completely changed his outlook on the world and himself. He came back with a self-confidence he'd never had before."

Students also bring home a new appreciation of science as an international enterprise—and of the abundance that Americans take for granted. "When you have to wash and reuse slides and coverslips, you really learn what 'lack of resources' means," says Charles R. Sterling, a University of Arizona professor of veterinary science and microbiology. Do's eye-opening summer was one result of Sterling's connection with a colleague in Peru, with whom he collaborates scientifically and exchanges undergraduates regularly.

Madeleine J. Long, a consultant with the American Association for the Advancement of Science, concurs with Sterling. "What students gain far exceeds knowledge of science and the scientific experience," she says. That reality, however, makes evaluation of the program more difficult—although tangible measures do exist. Of the 94 undergraduates who have participated in overseas research since the program began in 1992, 26 are in graduate school and 3 have received Ph.D.s; 15 are doing research; 17 are medical students; 2 more are applying to medical school and 7 have earned their M.D.s. Two are in M.D./Ph.D. programs; one is a genetic counselor and two are teaching or preparing to teach. BRAVO! participants have co-authored more than 60 journal articles and presented their research at nearly that many scientific meetings.

"These students have developed the ability to communicate their scientific work, and many have come home committed to pursuing careers that will make them part of the international scientific community," program director Carol Bender says.

"When the idea of sending undergraduates overseas to do research was first proposed, some of us were skeptical," acknowledges Sam Ward, a University of Arizona professor of molecular and cellular biology, who heads the HHMI-supported programs there. "The students have shown us how wrong we were."

—JENNIFER BOETH DONOVAN

Erin O'Shea: Learning the Secrets of Cell Signaling

Communication among humans can be an imprecise affair, but deep within our physical selves, precision rules. Cells speak to each other in a crisp, chemical language, their messages conveyed by proteins that rapidly bind and separate. Our immune and hormonal systems are just two of many in which signaling proteins carry unambiguous orders to convert this or release that, stop, change, concentrate or disperse.

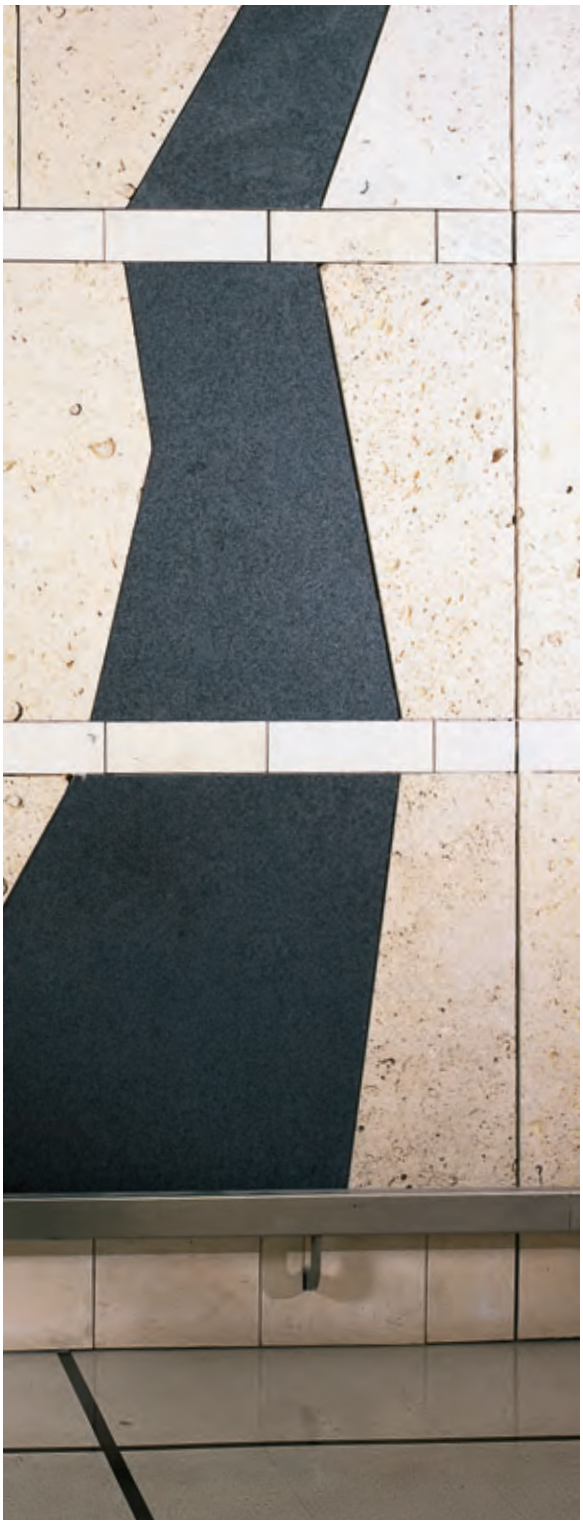
Genes, too, fall into line when signaling proteins convey a message. In a developing embryo, genes write the plan, but signaling proteins call the shots about when genes should go to work.

Although they can cause disease if they misfire, signaling proteins usually follow a specific pathway—much like HHMI investigator Erin O'Shea, who has been tracking, testing and teasing out the secrets of a protein signaling route in yeast for the last eight years. Along with her colleagues at the University of California, San Francisco (UCSF), O'Shea is now probing some of the most important details of this route, such as how it filters out "noise" and whether it uses "switches" to move signals along so precisely.

In humans, this is no mean task, given the vast numbers of proteins we create and the billions of combinations they might form. Yeast, however, has a mere 6,000 proteins and a genome that is easier to manipulate.

A mystery novel enthusiast, O'Shea enjoys searching for clues about how signal pathways operate, which can be hard to find even in yeast. Research on cell signaling is littered with daunting questions: How do protein signals become specific? How do pro-

FRED MERTZ



Erin O'Shea hopes new proteomic techniques may speed drug-development efforts.

teins know when to respond to a certain signal? How does the signal get passed along in a cell to generate a response?

O'Shea is especially interested in learning how kinase enzymes know whether to strike as they come in contact with proteins. When they add phosphate to a protein through the process of phosphorylation, the kinase enzymes cause the protein to change activity. The number of kinase enzymes available for this task, however, is much smaller than the number of proteins that can be changed. "It's a problem of 'specificity,' of the kinase knowing which protein to modify," explains O'Shea.

Since kinases are essential for modifying proteins, O'Shea decided to follow one class of them in detail. She chose a group called Pho85 that she had previously shown plays an important role in sensing phosphate levels outside the cell and in triggering a survival mechanism when these levels dwindle. By learning what happens along the Pho85 signaling pathway, O'Shea hoped to understand more, in general, about the biology of such pathways.

A mystery novel enthusiast, O'Shea enjoys searching for clues about how signal pathways operate.

To accomplish this, her team needed a more refined hunting method. They found it in the new field of proteomics, which embraces a variety of technological approaches for studying all of the proteins from an organism. O'Shea and her research partners at UCSF expressed and purified an existing collection of all 6,000 yeast proteins and screened them to see which were modified by the desired kinases.

Modified, however, does not necessarily mean flipping the switch in a signaling pathway. "It makes sense that in some cases there has to be a switching system that says 'all' or 'none,'" explains O'Shea. Without such a system, she reasoned, even random fluctuations in a signal could trigger a response, which might be catastrophic in the case of, say, a developing embryo.

"This yeast library shows us which proteins might be modified by which kinases," O'Shea explains. Finding this offers tantalizing clues—but only clues—about how kinases control events in cells. Together with her colleagues, O'Shea has now begun using computer modeling systems to identify the most promising avenues of experimentation. Her team members attach a green fluorescent protein to an area of the yeast genome that controls gene transcription. Then they measure the amount of fluorescence emitted by the cells, using a fluorescence-activated cell sorter that provides a measure of the signaling pathway's activity. By varying the stimulus outside the cell and then measuring the output, O'Shea has found that she can determine if the pathway she is studying is indeed "switch-like."

O'Shea thinks the evidence is now persuasive that a switch does exist in the pathway she studies, but she is quick to add that switches are just one part of the larger picture of signal processing. Determining what components give rise to the switch itself still remains on her "to do" list. Despite the list's length, O'Shea is confident her approach will bear fruit before long. "Just as in electrical engineering," she says, "if you can understand the devices and properties, you can understand and predict behavior."

She also hopes new insights into signaling pathways and new tools in proteomics will speed efforts to develop drugs for a variety of diseases. That her work might one day contribute to a new class of medicines gratifies O'Shea, of course, but it provokes irony as well. "I've always had an interest in human biology and was accepted to the M.D./Ph.D. program at Yale," she recalls. "I changed my mind at the last minute because I didn't want to see patients."

Future patients may be glad she made that choice, and colleagues already are recognizing her potential. Earlier this year, the National Academy of Sciences selected O'Shea to receive its annual award for "a recent notable discovery in molecular biology by a young scientist." It's an honor presented previously to some of biology's most celebrated researchers. **—JEFF MILLER**

Janelia Farm

HHMI plans to transform a Virginia farm into a unique laboratory complex where interdisciplinary teams will create advanced tools for biomedical research. The inside story of how a "what if?" idea became a $500 million project || *By M. Mitchell Waldrop*

PHOTOGRAPHY BY WILLIAM K. GEIGER

A Normandy-style farmhouse is the focal point of the bucolic 281-acre site.

Not too many years from now and not too far from Washington, D.C., HHMI plans to open what some are calling "the Bell Labs of biology."

Officially announced on February 1, 2001, the initiative is intended to create a free-ranging environment in which chemists, physicists, computer scientists and other specialists can collaborate with biologists. In a state-of-the-art facility equipped with ultra-advanced research tools, they will be able—even encouraged—to attack much more than one class of diseases or set of research problems. "We're trying to do something that will benefit the scientific enterprise as a whole," says Institute President Thomas R. Cech.

Thomas R. Cech

KAY CHERNUSH

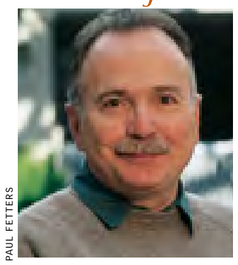
The new laboratory complex will occupy a 281-acre site in Virginia, called Janelia Farm, located some eight miles north of Dulles International Airport. HHMI expects to spend at least $500 million over the next decade to construct and operate the facility.

"We're trying to do something that will benefit the scientific enterprise as a whole."

Clearly a major, perhaps defining, project for his administration, Cech reasons that HHMI's uniqueness in the biomedical research world enables it, with financial resources second only to NIH itself and the flexibility that comes from being a private organization, to undertake projects that can transform the biomedical sciences, not just move them along a little faster.

David A. Clayton, a developmental biologist, began thinking along these lines shortly after he joined HHMI as a senior scientific officer in 1996, coming from Stanford University. "I had begun to realize how challenging a task it is to deploy state-of-the-art technologies to researchers in the field," recalls Clayton, who is now HHMI's vice president for science development. The initial cost is actually the least of it, he explains—although electron microscopes, tandem mass spectrometers and the like certainly aren't cheap. "First, you have to find the space to put the machine—and there isn't any academic institution I know of where space is not at a premium. Then, you have to provide a protected environment for it, with air conditioning, seismic isolation and so forth—which is very hard if you have an old building, as most university buildings are. You soon find that the environment is costing you several times the cost of the instrument itself.

"I had begun to realize how challenging a task it is to deploy state-of-the-art technologies to researchers in the field."



PAUL FETTERS

David A. Clayton

"Finally," says Clayton, "you have to find the technicians and engineers who know how to operate the machine and keep it in repair. That's the real challenge because it's expensive and time-consuming to train these people—and then if they're good, the pharmaceutical or biotechnology companies come knocking at their doors, offering them commercial-sector salaries that the academics can't hope to match."

Given that reality, he says, one obvious solution was consolidation: Use HHMI's considerable resources to establish a central campus equipped with an excellent staff and the best instrumentation—and then bring the scientists to the machines instead of the machines to the scientists.

Cech was taken with Clayton's idea as soon as he heard it; establishing an HHMI research campus seemed to offer exactly the new direction he was looking for. "So David and I talked about it before I started as president," says Cech, "and then the idea matured as we continued to discuss it with lots of people."

An active participant in those discussions was geneticist Gerald M. Rubin, an HHMI investigator at the University of California, Berkeley whom Cech was in the process of recruiting as the Institute's vice president for biomedical research. "I liked the concept of doing something outside the usual process," says Rubin. "It made the job seem more intellectually challenging."

Rubin was especially intrigued by the evolving strategy for staffing the new campus. In addition to a resident staff of about 240 scientists, there would be an active program for visiting scientists. Some

To Dream the Plausible Dream

To understand why HHMI is building its own research campus for the first time, says Institute President Thomas R. Cech, you just have to look at where the biomedical sciences are headed in the next decade or so: To turn these dreams into reality, you have to deploy the most advanced tools in the right kind of environment.

Take imaging tools, for example. "That's a broad term that means trying to locate the molecular components of living cells in as much detail as possible," says Cech, and "then seeing how those molecules move within the cell, how they change partners within the cell and how they change position within the cell—say, from the cytoplasm to the nucleus."

Present-day examples include the use of rapid freezing to lock cellular structures in place so that they can be mapped by a high-voltage electron microscope. "But there's no reason to restrict it to the cellular level," Cech adds. "People are also interested in imaging how whole tissues and organs respond to various stimuli," for instance, by using nuclear magnetic resonance techniques to map the changes taking place in the brain as experience becomes fixed into memory.

Then there are the computational tools. "Computers have finally come into biology in a big way," says Cech. "Many of our investigators and their students are spending considerable amounts of time mining the databases of genomic sequence information that are becoming available at such a rapid rate."

Today, thanks to the Human Genome Project, virtually the entire three billion-letter sequence of *Homo sapiens*' DNA is available to anyone with access to the Internet. But soon, Cech says, "you can imagine a Web site where you could scoot around inside a cell as if you were in a video game, and look at any molecule you want." In effect, he adds, such a site would function as an electronic atlas that mapped the location of every type of molecule in every type of human cell at every stage of the cell cycle—or at every stage of turning cancerous. "It will take very large computers to store that kind of information. But our techniques are getting to the point where this is a plausible dream, rather than a wild flight of imagination."

Researchers are also "modeling macromolecular structures or, in the neurosciences, using computational approaches to map out neural connections and then model them with neural networks," says Cech.

The new HHMI campus, he notes, will push hard to advance these tools and many more, with heavy emphasis on cutting across traditional disciplines.

Location, Location, Location

HHMI's new research campus doesn't yet have an official name, says Institute President Thomas R. Cech. For internal planning purposes, it is being referred to simply as HCAT, the Hughes Collaboration in Advanced Technologies. In the end, however, the site may very well keep the name it already has—Janelia Farm—following in the tradition of Cold Spring Harbor and Woods Hole. "That has the advantage that it doesn't mean anything scientifically," Cech says. "As new generations of programs evolve there, the name of the place doesn't go out of date."

The property, along Virginia Route 7, is eight miles north of Dulles International Airport.

Besides, it still looks like a farm. Located along the south bank of the Potomac River about 30 miles upstream from Washington, D.C., the 281-acre site is mostly given over to woods, pasture and two ponds. Its centerpiece is a Normandy-style farmhouse that was built in 1936; both it and the adjoining carriage house are listed on the National Historic Register. Janelia Farm is not entirely rustic, however; it contains three nearly completed office buildings—the remnants of a technology park planned by the farm's previous owner, the Vanderburg Group, a subsidiary of the Dutch software firm Baan Companies.

In terms of location, at least, the site appeared to have virtually everything HHMI was looking for. Janelia Farm lies on a major highway—Virginia Route 7—just eight miles north of Dulles International Airport, which will mean easy access for out-of-town visitors. It is likewise very close to the Internet companies that have lately been springing up around Dulles, which will mean easy access to fiber-optic lines and other cutting-edge information technologies—not to mention top-flight computer talent. Being located in fast-growing Loudoun County, Virginia, provides access to housing and good schools. Most important, Cech says, the farm offers a pleasant setting in which to work. So, when it turned out to be available, HHMI snapped it up.

The details of the site plan remain to be determined, though it is already clear that the existing office buildings are not suitable for biomedical laboratories; one of them will likely be used for campus administration and bioinformatics, while the other two will be rented out. The farmhouse will probably be renovated, perhaps as a reception center. The main campus, which will include laboratory space for 24 investigators and some 300 staff members, along with conference facilities, visitor housing, recreation facilities and the like, will be constructed from scratch on the 183 acres of the site that remain open for development.

The designs will be as environmentally sensitive as possible, says HHMI's vice president for science development, David A. Clayton, who is in charge of the planning. For example, HHMI will remove only as many trees as it has to for the actual construction of the buildings. The landscaping will do everything possible to enhance the feeling of peace and quiet—and the portion of the site lying in the Potomac River floodplain, which is protected by state law, will not be disturbed. "For something so close to the city," Clayton says, "Janelia Farm is an amazingly bucolic site." **—MMW**

"What's needed is a place for people to come together—not just to have a meeting but to work together collaboratively over an extended period of time."

PAUL FETTERS

Gerald M. Rubin

visitors would come for just a week or so to attend a workshop or to brush up on a new software technique. Other visitors would come for about a month and bring specimens and other materials for testing. Still others might come for a whole sabbatical year or longer. No one on the campus, however, would have anything like tenure; the expectation is that most would eventually move on.

Perpetual turnover would, presumably, help ensure the intellectual vitality of the campus, since new visitors would constantly bring fresh ideas with them. It would likewise ensure the rapid dissemination of any technologies developed there, since visitors would be taking ideas home with them.

Rubin saw another opportunity. "It's an experience we've all had," he explains. "You're at a meeting, maybe sitting around with some colleagues in a bar, and you get an idea for a great research project you could do together—but you can't just go out and do the project. First you've got to scrape up funding from government agencies—and then you've got to struggle to coordinate the efforts of team members who are scattered among home institutions all over the country, if not the world.

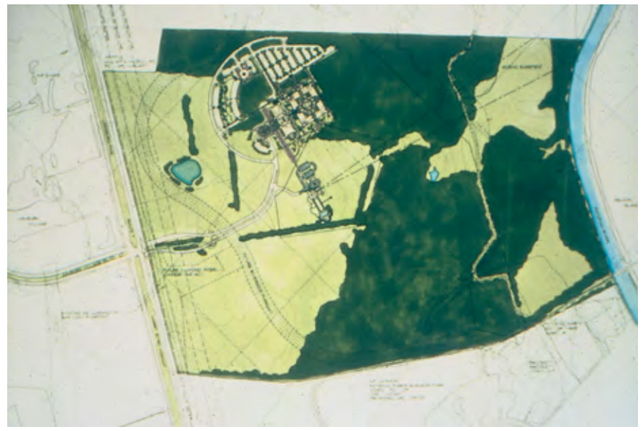
"So what's needed is a place for people to come together—not just to have a meeting but to work together collaboratively over an extended period of time," says Rubin. The new HHMI campus can offer exactly that opportunity. By building enough capacity in the beginning, Rubin points out, HHMI can operate the new campus partially as a "research hotel," providing space and resources for scientists to come for several years and tackle a hot new idea as a group. With people rotating through instead of spending their entire careers there, the place would always have lab spaces opening up.

Cech took office as HHMI's president on January 1, 2000, the same day that Clayton and Rubin assumed their current posts. In the months that followed, says Cech, the HHMI Trustees also proved to be enthusiastic about the new program and a formal proposal was developed, followed by the site selection. Then, in December 2000, HHMI purchased Janelia Farm from the Dutch software maker Baan Companies for $53.7 million.

Plans for the new campus are continuing to evolve. Take the obvious issue of commercialization, for example: Will visitors who want to commercialize technologies they've developed at the campus be allowed or even encouraged by HHMI to form start-up companies? "We haven't gotten far enough along to answer that," says Clayton, "although obviously we'll have to work it through to make sure we're staying true to HHMI's mission and to the rules for not-for-profit organizations."

Some principles are settled, however. One is that the new campus will not be restricted to the existing cadre of HHMI investigators; instead, it will be open to researchers from all over the world. Another is that cooperative research and cross-disciplinary thinking really will be given the highest priority. "We'll be making every effort to mix engineers, biologists and so on," says Cech. "We'll even be working with the architects to make sure the physical layouts of the buildings are such that people can't pull away into isolated groups. . . . But we will also have to find people who genuinely buy into the cooperative model. It may not be for everyone; some researchers work best as loners. But if everyone on the campus is a loner, then this experiment will fail.

"At a typical university," Cech adds, "if someone's publications are all collaborations, he or she will often have trouble getting promoted. But at Janelia Farm, if we see that someone's papers are all collaborations, we can say, 'This is wonderful!'" H



Much of the property lies in the Potomac River floodplain, and will be left undisturbed.

WHEN THE BRAIN FAILS

Huda Zoghbi unravels the genetics of Rett syndrome and other disorders

BY NANCY ROSS-FLANIGAN

Gowned and gloved, huda zoghbi holds a squirming mouse in her hand and peers through a plastic hood. “Did you see that?” Zoghbi asks as the mouse flails. “He just had a seizure.”

The wriggling laboratory mouse could have major implications for human health. “Through studies of this animal model,” Zoghbi says, “we hope to gain insight into autism and into one of the most common causes of mental retardation in females, namely Rett syndrome.”

With the mouse safely back in its cage, Zoghbi, an HHMI investigator at Baylor College of Medicine in Houston, turns her attention to another one. Its crooked spine and unsteady gait are tip-offs to its condition, a mouse version of the human neurodegenerative disorder called spinocerebellar ataxia type 1 (SCA1). The mouse and its mates are helping to reveal the chain of glitches that ultimately cause specific groups of neurons to degenerate in people with SCA1.

In recent years, Zoghbi and her collaborators have racked up an impressive list of accomplishments in these and related areas of research: discovering the gene for Rett syndrome (*RTT*); identifying the mutation involved in SCA1 and beginning to understand how it wreaks havoc on neurons; and advancing knowledge of the sensory components of coordination. Their work could provide insights into disorders such as autism and Huntington’s disease.

A DISEASE’S DEVASTATION

It all started when Zoghbi saw her first patient with Rett syndrome; nearly 18 years later, the memory remains fresh. Beautiful, brown-eyed Ashley, who began life like any healthy baby and made her parents proud as she learned to crawl, walk, babble and sing “ee-i-ee-i-oh,” had suddenly changed at around 18 months of age.

Instead of gaining skills, Ashley seemed to be losing them, becoming uncoordinated and uncommunicative. She stopped playing with her toys, coloring with crayons and running to the door to greet her daddy when he came home from work. Instead, she just rocked back and forth, staring vacantly and grinding her teeth. When Zoghbi saw her at age three and a half, she was struck by the heartbreaking contrast between Ashley’s beauty and the bizarre behaviors that characterized her Rett syndrome.

“She was constantly wringing her hands, having trouble with balance and staring past all of us,”

PAM FRANCIS

Huda Zoghbi (right) and her team, including HHMI postdoc Kei Watase, are studying the genetics of brain disorders.

Zoghbi recalls. “She would alternate rapid breathing with holding her breath.” Zoghbi, then a pediatric neurology fellow, felt compelled to devote herself to research.

“Many basic scientists go into research because of curiosity about certain biologic problems, but I was driven into science because it was so devastating to see patients with these neurogenetic diseases,” Zoghbi recalls. “Most of the time we could do very little for the kids, and that was very hard on me.”

Having children of her own—Roula, now 16, and Anthony, 14—also fueled Zoghbi’s fervor. “Once I became a parent,” she says, “I realized what pain the children’s parents must be going through, and I thought, ‘I just can’t sit there and watch; I have to do something.’ ”

Little did she know that “to do something” about Rett syndrome would entail such a long and tedious process. Finding the gene responsible for a disease is tricky enough when the disorder runs in families and researchers can study large groups of affected kin. With this condition, however, fewer than one percent of cases are inherited; most are sporadic, meaning that the mutation can show up anywhere in the population.

Because Rett syndrome occurs mainly in girls, who generally have two X chromosomes instead of an X and a Y, the X chromosome seemed a logical place to start looking for the gene. Still, it took 14 years to pinpoint the gene’s exact location—on the far end of the chromosome’s longest arm—and then to begin deciphering its role. Finally, in 1999, Zoghbi and Uta Francke, then an HHMI investigator at Stanford University School of Medicine, reported the discovery of several mutations in a particular gene known as *MECP2* in patients with Rett syndrome.

Normally, *MECP2* produces a protein that “silences” other genes, preventing them from being expressed. Such inhibition can be critical during development, when various genes must crank out their protein products for precise periods, then stop and remain inactive. Though it’s not yet clear exactly how mutations in *MECP2* cause Rett syndrome, Zoghbi and Francke speculate that the defect allows genes to stay busy when they should be still, leading to overproduction of certain still-unidentified proteins, which somehow disrupt neural development.

PAM FRANCIS

Invigorating synergy: Having kids helped Zoghbi become a better mentor, and having graduate students helped her become a better parent.

Discovery of *MECP2* led to a diagnostic test for Rett syndrome, and once researchers began testing patients, it became obvious that the gene’s importance extended beyond this one disease. Several *MECP2* mutations have been found in female patients with autism or with mental retardation alone, and even in some boys with severe mental retardation and seizures, or with features of autism. “So we see now,” says Zoghbi, “that the spectrum of phenotypes that results from mutations in this gene is quite broad, way beyond Rett syndrome.”

The next step is to learn which genes are normally silenced by *MECP2* and which proteins those genes produce. To that end, researchers in Zoghbi’s lab will use the mouse model of Rett syndrome they’ve developed to home in on the genes.

THE JUNE FLOOD

Tropical storm Allison dumped roughly three feet of rain on Houston in early June, killing 20 people and causing property damage estimated at $1 billion. Huda Zoghbi and other HHMI investigators at Baylor College of Medicine did not escape the storm’s wrath.

More than 30,000 of Baylor’s research animals died when basement and sub-basement animal facilities flooded. “We lost about 40 percent of our mice,” Zoghbi says. The manager of HHMI’s administrative office in Houston, Randal Condit, estimates that other investigators who work with mice experienced similar losses. Power failures destroyed many cell cultures, but investigators and their teams used more than 1,500 pounds of dry ice a day to save what they could. “We preserved the things that were irreplaceable, such as patient cell lines and donated tissue,” Zoghbi says, adding that “this was hardest on the graduate students and fellows. A few months’ or a year’s setback is enormous for them.”

Although it is on the first floor of a building near a bayou, HHMI’s administrative office at Baylor sustained no permanent damage. The lab of Richard Gomer, an HHMI investigator at Houston’s Rice University, was untouched. Only a block from Baylor, Rice is on slightly higher ground, and all of its research animals and labs are above ground level.

A GENETIC STUTTER

Zoghbi and her colleagues are also studying SCA1, a disorder that, unlike Rett syndrome, shows no early neurological symptoms. Patients with SCA1 are usually asymptomatic until adulthood, when they begin to stagger and stumble. Eventually, muscle control degenerates to the point that patients cannot talk, swallow or, finally, even breathe.

In 1993, Zoghbi and collaborator Harry Orr of the University of Minnesota cloned the *SCA1* gene and found that the disease-causing mutation is a kind of genetic stutter. In the normal gene, the nucleotide sequence cytosine-adenine-guanine (CAG, the DNA code for the amino acid glutamine) is repeated about 30 times. But in patients with SCA1, CAG is reiterated 40 to 100 times. These extra repeats cause the gene to produce an abnormally long polyglutamine tract in the resultant protein, ataxin-1.

Somehow, these polyglutamine proteins do devastating damage to neurons, and not just any neurons. In patients with SCA1, the mutant proteins prey mainly on the Purkinje cells of the cerebellum and certain brainstem neurons. Just how does the damage occur? And why are some neurons singled out? These are questions that Zoghbi and colleagues are continuing to explore.

They’ve learned that the number of repeats affects the disease outcome—the more there are, the earlier in life the disease strikes, and the more severe the symptoms. And they’ve found that the abnormal protein forms clumps in the nuclei of affected neurons, perhaps because the cellular machinery that normally breaks down and clears out excess protein doesn’t work efficiently on the polyglutamine chains.

“If it is not cleared sufficiently from the cell,” says Zoghbi, “the protein is free to do things in a neuron that it shouldn’t be doing,” such as interfering with the expression of important genes. To explore these possibilities, the researchers are studying proteins called chaperones, which help other proteins fold properly or hold them in configurations that can be degraded. In work with the mouse model, published in the February 2000, issue of *Nature Neuroscience*, they also found evidence that ataxin-1 interferes with normal gene expression very early in the disease process.

“Early on, before you see aggregations of protein, before you see signs of the disease, we found that the protein alters the expression of genes that are essential for the normal function of the neuron,” says Zoghbi. Several of those genes are involved in regulating calcium, which plays a critical role in normal neuron function.

This picture of how the SCA1 mutation leads to the loss of neuron function is supported by results from a related line of Zoghbi’s research. In that work, she and Baylor colleague Juan Botas are using a fruit fly model of SCA1.

“The power of the model in flies is that we can now screen thousands of genes to see which ones, if they are lost or overexpressed, will make the disease better or worse,” Zoghbi explains. In work recently published in the November 2, 2000, issue of *Nature*, the researchers identified a number of genes that suppress or enhance the neurodegenerative activity of SCA1.

As expected, those genes that impair the protein-degrading pathway make the disease worse. “But we also identified a new molecule that we had not suspected before to be a key player,” says Zoghbi. “Now we will take what we’ve discovered in the fly and try to discover in the mouse model and in human cells which of these components are directly or indirectly working with ataxin-1, so we can understand how they are contributing to neuronal dysfunction and death.”

The eventual goal, of course, is to figure out how to manipulate the process—by enhancing the clearance of ataxin-1 from the cell, for example—to slow the progression of SCA1 or prevent it altogether. To families plagued by SCA1, that would be a great payoff, but the benefits could be even broader. SCA1 is just one of eight neurodegenerative diseases caused by mutant polyglutamine proteins; Huntington’s disease is another. Though different populations of cells are affected in different polyglutamine disorders, the underlying mechanisms seem to be the same, so pinning down the molecular basis of one should shed light on the others.

STRIKING A BALANCE

As Zoghbi teases apart problems of genetic expression, she struggles with expression issues of an entirely different sort, such as how to nurture students while still insisting that they do their best. “It’s a fine line to walk,” she says. “You want to demand excellence, but you don’t want to destroy someone in the process.”

When students get discouraged, Zoghbi sits them down and confides that she spent three years working on the wrong region of the *SCA1* chromosome. Then she reminds them: “That’s why we call them experiments—if they’re all going to work, we don’t need to do them.… When things don’t work out, we pick up the pieces, we think of an alternative way, we ask another question and we go on.”

Zoghbi tries to strike a balance, too, between family life and professional responsibilities, and she finds an invigorating synergy in the combination. “Having kids helped me be more compassionate and understanding with graduate students and their ups and downs—thinking of their needs and listening to them,” says Zoghbi. “And having graduate students helped me become a better parent, because I learned that they leave you and fly off on their own. You want to see them go into that independent world, and you adjust, and you expect it and you’re happy for it.”

There’s one more important area where Zoghbi reminds herself of the need to accept frustration and stay steady. Like many researchers, she must regularly balance her desire to find cures with the knowledge that the work often proceeds slowly. “In science,” she says, “you have to set some really great goals and realize that you’re going to have to take minuscule steps toward achieving them.”

Another aid to maintaining balance is the firm belief that her research, even when it occasionally comes to a dead end, contributes to the overall efforts and their ultimate products. “Whether it’s our lab or someone else who will discover therapeutics based on some of the work that we do,” says Zoghbi, “it *will* happen.” H

THE SENSORY SIDE OF COORDINATION

While its SCA1 studies are providing insights into the motor control involved in balance, Zoghbi’s group is also exploring the sensory side of coordination. “When you walk down a hallway, you don’t have to look at your feet; you know exactly where they are,” says Zoghbi. “Even if you close your eyes, you know where your hands and feet are, because there is continuous sensory feedback to your brain about the position of your limbs.” Sensory receptors in the muscles, tendons, joints and inner ear detect motion and position, and they signal the brain through a route known as the proprioceptive pathway.

To better understand that pathway, Zoghbi followed up on a lead from geneticist Hugo Bellen, another HHMI investigator at Baylor, who called her attention to a line of fruit flies that are uncoordinated because they lack a gene called *atonal*. This gene controls the development of the chordotonal organs, major sensory organs in the peripheral nervous system of the fly. Collaborating with Bellen, Zoghbi’s group found the mouse version of *atonal*, which is named “Mouse atonal homolog 1” (*Math1*), and began studying its function.

“What we have found is that *Math1* is essential for many components of this pathway, controlling multiple neurons,” says Zoghbi. “These neurons are quite different and diverse in their functions, but they’re all components of a pathway that does the same thing as the chordotonal organs in the fly.” —NRF

Rosetta Tackles the

Extreme Origami of Protein Folding

David Baker's model is producing remarkably accurate predictions.

BY DENNIS MEREDITH

protein folding has been called one of the great unsolved mysteries of molecular biology, a process too complex and elusive to predict with accuracy. Recently, however, a team led by HHMI investigator David Baker at the University of Washington School of Medicine has begun making predictions that one admiring expert compares to a string of home runs.

Baker has developed a computational technique, called Rosetta, that predicts the ways in which proteins, which start out as the string-like amino acid sequences that emerge from the protein-synthesis machinery,

REX RYSTEDT

undergo a folding process that might be dubbed "extreme origami."

Unlike the intricate Japanese art of paper folding, the result of protein folding is not just an elegant shape; it is also a functional one, akin to folding sheets of metal to create a working gasoline engine. The strings of amino acids collapse into the globular three-dimensional structures of enzymes and other life-sustaining cellular components.

Late last year, Rosetta proved its worth during the fourth "Critical Assessment of Techniques for Protein Structure Prediction" (CASP4). In this biennial series of experiments begun in 1994, researchers are given the amino acid sequence of target proteins and then asked to develop three-dimensional models of the final folded versions. Their predictions are compared with the actual protein structures, which have been solved experimentally by x-ray crystallography or NMR spectroscopy, but not yet published.

In the CASP4 experiment, which began in April 2000, more than 100 research groups generated three-dimensional structures for 40 candidate proteins. They presented and discussed their results at a conference in Asilomar, California, in early December (*prediction center.llnl.gov/casp4*).

"The CASP experiments have been among the most important influences in advancing this field," says Baker. "One of the problems with structure prediction is that it is all too easy to produce a program that correctly predicts the structure of a protein if you know the correct structure in advance. By challenging researchers to produce models before knowing the right answer, the CASP experiments have provided an invaluable boost to the field."

RISING TO THE CHALLENGE

Protein-structure prediction methods fall into three basic classes, explains Baker. For proteins whose sequences closely resemble those of other proteins with known three-dimensional structures, the structure can be modeled using the known protein as a template—a method known as comparative modeling. A second class of methods, called fold recognition, attempts to identify a known protein structure that is a good match for an amino acid sequence. Researchers use these methods when there is relatively little "sequence similarity" to a protein of known structure. The methods can succeed when a protein under study has a structure that is similar to one already known, but will fail if its structure is very different from those that have been determined previously.

The third class of methods is *ab initio* structure prediction, which attempts to model proteins by starting from an extended chain and folding up the sequence on the computer. These methods have the advantage that they do not depend on the existence of an already determined structure to serve as a template. Until recently, however, success in *ab initio* prediction was considered highly unlikely, says Baker.

The most exciting progress at CASP4 was in this area of *ab initio* structure prediction. As participant Peter Kollman, an expert in computational molecular modeling at the University of California, San Francisco, explained shortly before his death in late May, "The evaluators of the structures for the *ab initio* predictions gave two points for a structure which was 'among the very best,' one point for a structure that was 'pretty good' and zero if the structure was reasonably far from the correct one."

Rosetta did quite well under these ground rules. "The amazing thing is that David Baker's group had 31 points and the next-best group had 8 points," said Kollman, who compared the results to a season when Babe Ruth hit four times more home runs than any other player.

HOW ROSETTA WORKS

In research that received the Nobel Prize in 1972, Christian Anfinsen showed that a completely unfolded protein could fold spontaneously to its biologically active state, which means that a sequence of amino acids contains all of the information needed to specify its three-dimensional protein structure. In the years that have followed, scientists have verified that a large number of proteins fold spontaneously to their biologically active states. They've accounted for these results with the hypothesis that a sequence of amino acids folds naturally into a protein structure that requires the lowest amount of energy, and the folding process is essentially a search for this structure.

Since most proteins do fold spontaneously to this correct native structure, why have researchers found it so difficult to mimic the process with a computer? There have been two main problems. The first is the sheer bulk of the calculations; the number of possible conformations that a polypeptide chain can adopt is too vast to analyze with anything less than a very powerful computer. Second, it is difficult to calculate with accuracy the energy of a protein chain in the watery environment of a living cell. Rosetta solves both problems by restricting the number of conformations that it considers for each short segment of the protein chain. It only considers conformations that the segment has actually adopted in proteins whose structures have been solved. It then searches through the possible local conformations to find the combinations that produce the most favorable "low-energy interactions" throughout the protein.

By greatly restricting the universe of options, Rosetta can search for low-energy conformations more quickly and eliminate numerous conformations that might be selected incorrectly with imperfect formulas. It's a procedure that mimics the refolding of real proteins, with segments of the protein chain "flickering" among different possible conformations until an overall conformation is found in which favorable interactions exist throughout the protein.

Recently, Baker's team improved the program by incorporating an insight from experimental data on protein-folding rates. "We noted a very strong correlation between how fast proteins folded and how close together along the amino sequence were the amino acid residues that touched in three dimensions in the structure," Baker explains. "It's actually very intuitive. In a protein in which most of the contacts in the three-dimensional structure are between residues that are close to each other along the sequence, the structure can assemble much more readily than if most of the contacts are between residues that are far apart. Proteins whose contacts are primarily local, or close along the sequence, fold much more rapidly than those whose contacts are primarily 'nonlocal.'

"Because the search for low-energy conformations in Rosetta is quite short," Baker continues, "we thought it might produce structures with primarily local contacts. This indeed turned out to be the case. One of our most important advances with the CASP4 predictions was to correct this bias towards proteins with all local contacts."

DRAWING INFERENCES

Where Rosetta can be improved, says Baker, is in predicting the folding of proteins with many nonlocal interactions among amino acids and by generally increasing the accuracy of the predicted structures. Still, he concludes, Rosetta's results in the CASP4 experiments demonstrate that enormous progress has been made in *ab initio* structure prediction. "Analysis of the predicted structures showed that for the majority of proteins with no sequence similarity to proteins of known structure, we had produced reasonable low-resolution models for large fragments of up to about 90 amino acids." By contrast, he notes, at the CASP2 meeting four years ago, there were few reasonable *ab initio* structure predictions.

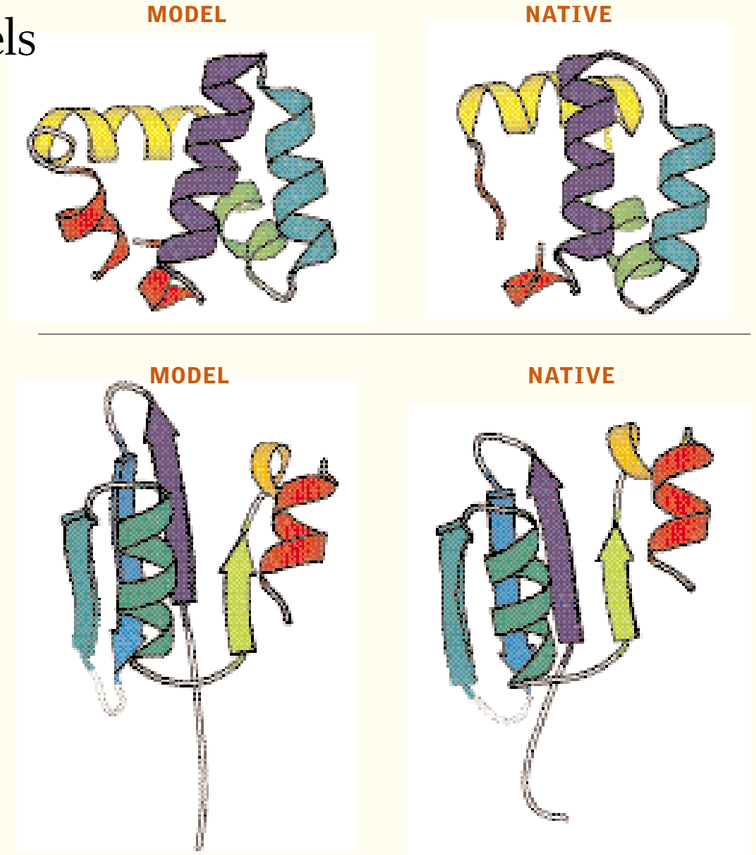
"One of the exciting things about the results of [CASP4] is that it has become very clear that incorporating insights from experiments into our computational methods really helped a lot," says Baker. "For a long time there's been a hope that experimental study would contribute to structure-prediction methods, but it's been only very recently that such insights have actually contributed to making protein-structure prediction better." Other researchers share this excitement. "Nonetheless," Kollman cautioned, "there is still some way to go in predicting these structures to experimental accuracy."

Baker acknowledges that "these three-dimensional structures are not detailed enough, for example, for structure-based drug design." Still, "they can yield invaluable insights into the function of unknown proteins," he says, "so our aim is to use our *ab initio* structure-prediction method to produce three-dimensional models for proteins of unknown function on the genome scale. A number of our CASP4 predicted structures provided insights into protein function that were not evident from the linear amino acid sequence, and we are optimistic that, using Rosetta, we can provide some insights into the functions of the significant fraction of proteins in recently sequenced genomes whose functions are not currently understood."

Baker's group is currently generating models for all large protein families whose three-dimensional structure is unknown. He and other computational structural biologists are also seeking to identify particularly interesting proteins whose structures are unknown. They're asking biologists to submit their top choices for a "10 Most Wanted" list of important proteins whose structures have not yet been solved (*predictioncenter.llnl.gov*).

How the Models Compare with Reality

Two examples of how the protein structures predicted by Rosetta compare with the proteins' actual shapes. In both examples, the *ab initio* structure predicted by Rosetta is on the left, and the experimentally determined x-ray crystal structure is on the right. For clarity, the amino acid side chains are not shown and the protein backbone is colored to show the beginning and end of the chain. In both cases, the overall fold of the predicted structure is very similar to that of the native structure but has some details incorrect. The predictions provide valuable insights that are not evident from the proteins' amino acid sequences alone. When the protein predicted in the first example was compared with known protein structures in a database, for example, it closely resembled the structure of a protein that plays a role in killing bacteria. Sure enough, the predicted protein turned out to play a similar role, even though it has an unrelated amino acid sequence. The second example shows one domain of a large 811-residue protein that was found to resemble proteins with related functions but unrelated sequences.



The Invisible Scientists

More pay. Better benefits. Increased mentoring. A report carried out with HHMI support says postdoctoral fellows need all this—and more.

BY KAREN YOUNG KREEGER

An entire community of scientists has been overlooked and underserved, according to a recent study of a joint committee of the National Academy of Sciences, the National Academy of Engineering and the Institute of Medicine. The study*, issued last fall, recommends that postdoctoral fellows should receive better pay, benefits and mentoring, as well as enjoy a more clearly defined status—not just for humanitarian reasons but for the health of the science enterprise in the United States.

The National Academies' Committee on Science, Engineering and Public Policy (COSEPUP), which conducted the study, estimates that there are currently some 52,000 postdocs in this country, constituting an integral part of the scientific workforce. Three-quarters of them are in the life sciences and more than half are from foreign countries. "This group of people had just fallen into a big crack," says Maxine Singer, COSEPUP chair and president of the Carnegie Institution in Washington, D.C.

COSEPUP arrived at this bleak conclusion after conducting workshops and focus groups in which it gathered information from 39 groups of postdocs and advisers at 11 universities, seven national laboratories, and five private research institutes or companies. HHMI and other organizations provided support for the project.

The study, Enhancing the Postdoctoral Experience for Scientists and Engineers, reports that most postdocs with whom the committee spoke are garnering valuable research experiences and laboratory skills. It also observes, however, that many of them are not gaining the lab-management and grant-writing experience that they need. It highlights such chronic problems as low levels of professional recognition and inadequate research and career guidance from postdoctoral advisers.

The myriad of postdoc designations at different institutions is also a problem; many universities don't even know how many postdocs are on their campuses. The situation is exacerbated, especially in the life sciences, by the length of a postdoc position, which in biology is generally three and a half years or more. The main issue, however, as Singer points out, is "terribly low stipends."

TOO LITTLE TO LIVE ON

"One of the concerns that we recognized is that many postdocs feel very underpaid," said COSEPUP member Brigid Hogan, an HHMI investigator at Vanderbilt University. The committee found that in 1997–1998 (the last year for which data were available) the median annual salaries for postdocs during the first six years after receiving the Ph.D. were $28,000 in academia, $36,000 in industry and $37,000 in government.

Such salaries may be adequate in certain parts of the country but COSEPUP noted that no allowance is made at most institutions for geo-

FRED MERTZ

* *Enhancing the Postdoctoral Experience for Scientists and Engineers: A Guide for Postdoctoral Scholars, Advisers, Institutions, Funding Organizations, and Disciplinary Societies* (Washington, D.C.: National Academy Press, 2000). A Web site that links to the report's text and related resources is at *www.nationalacademies.org/pd/postdoc.nsf.*

"Most postdocs I know would like to put away for retirement and save, but it's just not an option."

graphical differences in the cost of living. How do postdocs in urban areas—the land of four-digit rents—make ends meet? "For myself and some others, we have spouses who aren't postdocs and [who] do get paid well," says Mary O'Riordan, a second-year postdoc at the University of California, Berkeley.

Having a well-paid wife or husband is not a universal situation, however, and many postdocs must resign themselves to several years of poverty and even go into debt for such basic items as childcare and rent. "It's unfortunate," says O'Riordan, who is president of the Berkeley Postdoctoral Association. "Most postdocs I know would like to put away for retirement and save, but it's just not an option."

Some institutions in high-cost regions do make allowances. The Gladstone Institute at the University of California, San Francisco, adds a 10 percent living allowance to its postdoc stipends, which are already more than 15 percent above the government's level. Farther up the coast, the Fred Hutchinson Cancer Research Center in Seattle provides subsidized childcare. These examples are not the norm, however, nor does tweaking the stipends in high-cost regions fundamentally address the modest salaries that postdocs receive, regardless of their locations.

The National Institutes of Health recently increased stipend levels for its National Research Service Awards (NRSA) by 5 percent. For 2001, the NRSA annual salary is $28,260 for entry-level postdocs, $29,832 for researchers with one year of experience and jumps to $35,196 for those with two years of experience. In response to the COSEPUP report, NIH pledged to raise the NRSA levels by 10–12 percent a year to a target of $45,000 for new postdocs, and to institute annual cost-of-living increases.

"It's clear that NIH's NRSA stipends are the de facto standard for many stipends in the life sciences," says O'Riordan. What's more, she adds, "NIH is trying to be more responsive in raising stipends." Walter Schaffer, an NIH research training officer, says the agency is supportive of future increases, although obliged to proceed carefully. NIH addressed the COSEPUP report at length in a special Web site at *grants.nih.gov/training/nas_report/NIHResponse.htm*. Although NRSA stipends may set the standard, there is no guarantee that other institutions will meet it. Last fall, for instance, in a survey of postdocs at one research university, 13 of 25 respondents reported annual salaries at least $1,000 lower—and sometimes much lower—than the 1999 NRSA guidelines.

ADVICE TO STAKEHOLDERS

To remedy the general situation, the COSEPUP report spells out responsibilities for each group integral to the postdoc experience: the postdocs themselves, postdoc advisers, administrators and institutions, professional societies and funding organizations. "Each one needs to do different things," says Singer. "The societies can do a lot to foster attention by mentors. On the other hand, a lot rests on the particular mentors themselves, but also on the postdocs. Certainly the funding agencies could catalyze change by providing more money for stipends. There's something for everyone to do."

The committee's main recommendations address the complexity of this issue:

- **Postdocs** should define their objectives, contribute their best efforts, take equal responsibility with advisers for communicating their

A Supportive Boss

Postdocs can be both productive and happy. The report from the National Academies (*Enhancing the Postdoctoral Experience for Scientists and Engineers*) highlights the problems that many postdocs face, but also acknowledges that many postdocs have stimulating and well-supervised research experiences. Amelie Gubitz and Westley Friesen, two postdocs at the University of Pennsylvania School of Medicine, are among those who recount positive and even glowing experiences with their mentors and laboratories.

Both young scientists are members of HHMI investigator Gideon Dreyfuss's team that is studying the biology of spinal muscular atrophy (SMA), a degenerative disease of motor neurons in the spinal cord and the leading genetic cause of infant death. By most standards, the Dreyfuss lab is large: one research assistant professor, ten postdocs, five graduate students, three technicians, two undergraduate students and one administrative assistant. Large, however, need not mean inattentive. "Because making the lab an enjoyable place to work has always been a priority for me, it's important that I like the people I work with and that they get along with each other," says Dreyfuss of his efforts to create an atmosphere of cohesiveness within his research group.

This attitude strikes a responsive chord in his postdocs. "[Dreyfuss] is a supportive boss and always encouraging, even if you have a negative result," says Gubitz, who joined the Dreyfuss lab in the summer of 2000 to work on the cellular biology of SMA. "He's very supportive in finding alternative strategies so that you can try out different approaches in parallel and not get stuck on one idea or model."

This receptivity applies as well to collaborations among postdocs. "Some of our papers are 'single-author,' but many are joint," says Dreyfuss. He attributes such teamwork to the lab's common scientific interests, the interaction he encourages at its weekly meetings and his own interest and accessibility. "His office is at one end of the lab and you can always find him there and the door is usually open," says Gubitz, who has a mild form of SMA herself.

Friesen, who studies the molecular function of the SMN protein (the product of the SMA gene), knew pretty much what to expect prior to setting foot in the Dreyfuss lab—the result of his thoroughness in looking for a position. "I interviewed [Dreyfuss's] postdocs and students before coming here to find out how he works," recalls Friesen, now in the third year of his postdoc in the lab. He says his experience confirms what he was told, namely that Dreyfuss "has an unassuming management style where he lets you have some freedom." Friesen concludes that he was attracted to the lab overall because of Dreyfuss's "exuberance for discovery, which infects the entire lab." **—KYK**

Amelie Gubitz and Westley Friesen have had a positive experience in the laboratory of Gideon Dreyfuss, an accessible and encouraging mentor.

needs and bear primary responsibility for their own success.

- **Advisers** should provide an educational experience that helps advance the postdoc's career, clearly communicate expectations, act as mentors in research and for related skills and issues, see that postdocs meet other potential mentors, evaluate progress at least once a year, provide career counseling and assist in job placement.
- **Institutions** should establish explicit policies and minimum salaries, provide access to health insurance, maintain a postdoc office or officer, offer a standard employment contract, ensure career guidance, ask advisers each year for written evaluations of their postdocs and support a postdoc association.
- **Professional societies** should play a larger role in career guidance, support job hunting, develop norms for postdocs, collect and analyze postdoc employment data and organize workshops that help postdocs gain useful career skills.
- **Funding organizations** should craft a definition of postdocs that recognizes their unique status, establish terms and conditions that include appropriate salaries, benefits and reviews and play a larger role in encouraging best practices.

The report also had a few suggestions exclusively for the National Institutes of Health and the National Science Foundation: establish a central postdoc office, develop criteria for postdoc pay scales, meet regularly with postdoc organizations and, for the NIH only, permit institutions and principal investigators to combine funding for traineeships and research grants, allowing the needed flexibility to increase stipends.

DAVID GRAHAM

BECOMING BOLDER

Though the COSEPUP report was seen as an important step, postdocs agree that the momentum for improving their situation must be maintained. Thus some 300 people, mostly postdocs themselves, convened in March at the National Academy of Sciences (NAS) in Washington, D.C., for a follow-up meeting. There, in the auditorium off the Great Hall where Foucault's Pendulum keeps tabs on Earth's rotation, they shared their impressions of the report and of what their institutions planned to do about the committee's many recommendations.

Melanie Leitner, co-chair of the steering committee for the Neuroscience Postdoctoral Fellows Association at Washington University, for example, pointed out that the report has led her institution to begin creating a postdoc office. In a similar spirit, Michigan State University has hosted a workshop on conflict resolution exclusively for postdocs, and the University of Chicago has formed a postdoc welcoming committee, postdoc e-mail list and Web page devoted to postdoc-related issues. What's more, postdocs themselves are organizing. "If you look at postdoc organizations that are more than five years old, there are a handful," said Leitner, "but if you count the ones that are two years old and younger, there's a big bump up."

Those attending the NAS meeting observed that it will be challenging to coordinate postdocs on a national level, given the disparities in everything from where postdocs work to what they're called. The American Association for the Advancement of Science has provided some help: last November, Science magazine's NextWave Web site (nextwave.sciencemag.org) created the Postdoc Network to serve as a national forum for exchanging ideas on issues faced by postdocs. Every week, the site posts discussions on subjects such as salary, benefits, career needs and the evolution of postdoc associations. It also links to more than 50 postdoc associations at institutions in the United States, and it provides data on salaries and policies for most of them.

The sense of the NAS meeting, however, was that the real work has yet to begin, especially when it comes to increasing salaries. "I'm glad that all the parties got together," says Leitner, who was also active in graduate-student leadership when she was an HHMI predoctoral student at Washington University. "It was very valuable. But as a postdoc I was struck that no group picked up the charge and said: 'This is terrible. We need to do everything we can to fix it.' "

The March convocation was "very encouraging," agrees Berkeley's O'Riordan. "I felt there was a real willingness from all parties to consider the [COSEPUP] report's guidelines. But one of my impressions was that there was a real lack of clear solutions for implementation. It remains unclear who will take responsibility for what."

Still, the consensus was that legions of postdocs out there are thankful for the new threshold set by the report. "I know that postdocs themselves have become bolder because they can point to this," remarks Singer. "I believe that this report has really catalyzed something."

SCOTT GOLDSMITH

Aspiring-physician Joshua Courtney and his professor, Richard Dryden, wear protective clothing when they go out to gather deer ticks.

Ticks Inspire a Young Scientist

Undergraduate tracks how a pathogen spreads

Joshua Courtney had two goals when he was a freshman: to finish school as soon as possible and become a chiropractor—but fate, and deer ticks, got in the way. His four-year undergraduate journey at Washington & Jefferson College, a liberal arts school in western Pennsylvania, took a dramatic turn in 1999 when he enrolled in a microbiology class taught by Richard Dryden. There, Courtney got his first taste of research and before long began working closely with the Centers for Disease Control and Prevention (CDC) to track a public health threat.

Today, the tall, soft-spoken 21-year-old has abandoned all chiropractic goals in favor of epidemiology. He's spent much of the past two years identifying the location and "spread patterns" of pathogens transmitted by deer ticks in Pennsylvania. After donning a special suit to gather the freckle-size arthropods, he extracts their DNA and sends it to the CDC in Atlanta for genetic sequencing.

Deer ticks can carry Lyme disease and a newly identified illness called human granulocytic ehrlichiosis (HGE), which produces flu-like symptoms and can be fatal. Thus, the ticks are of high interest to the CDC, which has formed a collaboration with the college under the direction of Dryden, Courtney's faculty advisor.

"A lot of amazing things have happened in the year and a half since I took that course," Courtney says. He interned at CDC last summer and, having just graduated, plans to help other W&J students extend the research beyond Pennsylvania into neighboring Maryland. "Not in a million years did I think I'd be doing this," says Courtney, who now hopes to become a physician specializing in emerging infectious diseases.

Dryden began crafting W&J's connection with the CDC when he studied tick-borne pathogens there during a three-month sabbatical in 1998. "My goal," he says, "was to learn enough to provide research opportunities to our undergraduates." Dryden was also looking for a new focus for that research. "I saw a couple of articles on tick-borne diseases in various parts of the United States, including Pennsylvania," he recalls, "but when I called the state Department of Public Health, they told me they didn't have the money or manpower to study tick-borne diseases." Now it's state officials who frequently turn to Dryden and Courtney, mailing them tick samples from all over Pennsylvania to analyze.

Dryden speculates that the collaboration with the CDC, which also involves the agency's bacterial research branch in Fort Collins, Colorado, will continue for "quite some time" because of the rapid spread of Lyme disease and the emergence of HGE, which was first detected in 1994. The CDC's involvement—and the opportunity for hands-on undergraduate research at W&J—means a lot to the school, says Alice Lee, associate professor of biology and director of an HHMI-funded program at the college that supported many of Dryden's and Courtney's activities. "It's scientifically exciting and will attract many more students and faculty members, enriching the lives of students for a long time. Hopefully, some will choose to go into research."

The undergraduate research program has meant a lot to Courtney, who grew up in one of the gritty steel towns that dot the region. His tick research earned him the Pennsylvania Outstanding Vector Control Researcher Award for 2000—an honor usually reserved for graduate students. He won a $1,000 Allied Health Scholarship and coauthored a paper that will be published in the *Journal of Clinical Microbiology*.

Robert F. Massung, a CDC researcher in viral and rickettsial diseases, praises his young collaborator, saying that "people like Josh bring enthusiasm that you just don't see from a grizzled veteran researcher. We've had student interns before, but for an undergraduate to come in with his own project—that's unusual."

—MELODY SIMMONS

Discovering a "Sweet-Tooth" Gene

Three research groups have independently identified a human gene, expressed by the tongue's taste cells, that encodes a likely receptor for sweet compounds.

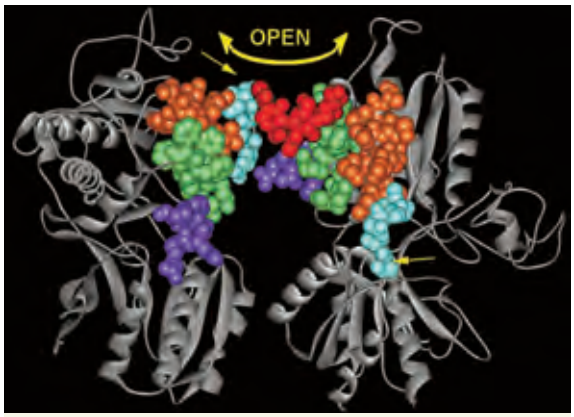
Discovery of the candidate sweet receptor gene, called *T1r3*, was reported in the May 2001 issue of *Nature Neuroscience* by HHMI's Linda Buck and colleagues at Harvard Medical School and in the May 2001 issue of *Nature Genetics* by HHMI's Robert F. Margolskee and colleagues at the Mount Sinai School of Medicine at New York University. Susan L. Sullivan and her team, working in the Laboratory of Molecular Biology at the National Institute on Deafness and Other Communication Disorders at the National Institutes of Health, published similar findings in the April 27, 2001, *Journal of Neurochemistry*.

The starting point for all three groups was the *Sac* locus in the mouse genome, a region of mouse chromosome 4 known to govern detection of sweet-tasting substances. From there they identified an analogous region in the human genome. Only one of the region's genes coded for a protein that fit their working assumption that the Sac gene product could be a G protein-coupled receptor that detects sweet compounds. Importantly, all three groups confirmed that the gene was selectively expressed in a proportion of taste-bud cells. Each laboratory is now exploring the molecular function of the candidate sweet receptor.

Buck and Margolskee envision this work leading to the development of a better sweetener. "The artificial sweeteners now used in an attempt to control weight just don't do a very good job of mimicking the taste of the natural sweetener," says Margolskee. "If we understood the sweet receptor and its binding mechanism, we could design a sweetener molecule that would fit perfectly and be a million times more potent than sugar, yet have the same sweetness as a natural sugar sweetener."

Margolskee says he also hopes this research will lead to treatments to enhance the activity of taste receptors in elderly people. The sense of taste is often lost in the elderly, leading to disinterest in food and to malnutrition.

Buck adds that identifying the sweet receptor offers an important pathway for exploring how the brain processes taste information. "If you have genes that code for receptors that recognize particular tastes such as sweet versus bitter, you can use those genes as tools to actually visualize what's happening inside the brain," she says. "For example, is there a sweet spot in the brain, a bitter spot or a sour spot?" Researchers might also be able to explore whether the olfactory and taste senses—long known to function in concert—share neural circuitry at some point in the brain.



The predicted structure of the ligand-binding portion of the mouse T1r3 taste receptor. In mice that are relatively indifferent to the taste of sweetness ("nontasters"), T1r3 is predicted to have the chain of sugars shown in red. These added bulky molecules keep apart the two units of the T1r3 pair. Studies are under way to determine if the ligand-binding properties of T1r3 change when the bulky sugars separate the two T1r3 units. The straight arrows indicate amino acid residues of T1r3 that differ in taster vs. nontaster strains of mice.

REPRINTED WITH PERMISSION FROM *NATURE GENETICS*, 28:62, FIG. 6C. 2001 © Nature PUBLISHING GROUP.

Kurt Weiss Battles Cancer—This Time, to Help Others

As a teenager, Kurt Weiss battled and beat osteosarcoma, an aggressive bone cancer that spread to his lungs and took his leg. Now a medical student, Weiss is on a year's leave from Jefferson Medical College in Philadelphia, exploring life as a physician-scientist as he studies how osteosarcoma metastasizes and why it always migrates to the lungs.

As a member of the Research Scholars Program sponsored by HHMI and the National Institutes of Health, Weiss is conducting basic research at NIH, in the laboratory of Pediatric Oncology Branch Chief Lee Helman. He is living with his wife, Laura, and infant son, Connor, in the program's Cloister building on the NIH campus.

Using osteosarcoma cells that he has coerced into producing a green fluorescent protein, Weiss is trying to establish the route and measure the travel time of injected cells as they move from the hind legs of mice to the lungs. In addition, he is looking to see if these glowing cells journey through the lymph nodes along the way. By and large, osteosarcoma metastases don't show up in lymph nodes, but Weiss thinks the cells probably do travel through the lymph nodes on their way to the lungs, where they often begin proliferating and may lead to death.

"Only very rarely do patients present with osteosarcoma cells in their lymph nodes," he says. "It's likely that the cancer cells do go first to the lymph nodes but are cleared by cells in the immune system. If I can establish that the osteosarcoma cells spread to nodal tissue as part of their natural biology, then I can start searching for a mechanism by which the immune system eradicates the metastasizing disease."

Weiss is interested in this as-yet-unknown mechanism because he believes it could be the basis for a new therapy aimed at halting the spreading cancer before it reaches the lungs—a major improvement on surgery and standard chemotherapy. He knows through his own experience how such research can lead to improved treatment; he is alive today as the result of an experimental immunotherapy. At age 15, when he was a lineman on his high school football team, Weiss started feeling a dull ache in his right shin. At his mother's insistence, he finally went to the doctor and learned that he had advanced osteosarcoma, which had already spread to his lungs.

A surgeon in nearby Pittsburgh removed the primary tumor and performed an allograft, transplanting a donor's bone tissue in place of some bone that also had to be removed. After Weiss started chemotherapy, however, more lung metastases appeared. "You never want the cancer to return at all," he says, "but you especially don't want it to come back when you're on chemotherapy, because that's a pretty good indication that the chemotherapy just isn't working."

It was then that his older sister, Gretchen, sent the family a small article she discovered in *The Allentown Morning Call* about the work of Eugenie Kleinerman, a researcher at the M.D. Anderson Cancer Center in Houston. Kleinerman was attempting to use an immunostimulatory molecule called MTP-PE to enhance the ability of the macrophages in the lung to fight cancer. Weiss leaped at the chance to participate in her study. "I was very, very sick," he says. "If I was going to die, I was going to make damn sure somebody was going to learn something from it."

The treatment worked, and this past March, Weiss celebrated 11 years of being cancer-free. The intervening years weren't without setbacks—his allograft never took, for example, and ultimately he developed a severe bone infection that required amputation of a leg. "I have been through everything with this disease," Weiss says, "except death—my own death, that is. A number of friends have died from osteosarcoma. I think I can bring a unique understanding to my patients who are suffering from this disease."

His experience also has given Weiss some insight into the economics and difficulties of developing new drugs. The drug that saved his life still hasn't made it to the marketplace, in part because relatively few people develop the disease. One drug developer decided the drug wasn't economically feasible and abandoned its development. The drug now does have new financial backing but has yet to be proved effective enough for FDA approval. "This isn't an epidemiologically high-impact disease," Weiss says. "I see it as sort of a lifelong duty of mine to raise awareness of this disease and get people fired up to do the research and to fund it."

In the meantime, in conjunction with the Make-A-Wish Foundation, he has started the Kurt R. Weiss Undergraduate Scholarship—raising money to enable kids who've survived life-threatening illnesses to attend college even though their families have been burdened with high medical bills. "After fighting so hard to stay alive," Weiss says, "these young men and women deserve the opportunity to chase down their dreams."

Donations to the scholarship fund can be made to Make-A-Wish Foundation of Western Pennsylvania, Westin William Penn Hotel, Suite 417, Pittsburgh, PA 15219.

—LISA CHIU

WILLIAM K. GEIGER

Kurt Weiss is taking time off from medical school to study osteosarcoma, a cancer that nearly claimed his own life.

ASIA KEPKA

Postdoc Alejandro Aballay translates biomedical research news for Spanish-speaking readers.

Science News for the Spanish-Speaking World

Like most postdoctoral fellows, Alejandro Aballay has to balance research, publishing and presentations at scientific meetings. (He has already landed a research job, or he'd be looking for work, too.) The Argentinian researcher at Boston's Massachusetts General Hospital also does something quite separate from his efforts to learn how organisms respond genetically to infection from food-borne bacteria: he explains scientific advances to the Spanish-speaking world.

Aballay is the translator for HHMI's biomedical news service, a part-time position in which he translates—on deadline—reports about research discoveries. HHMI distributes the free news service via e-mail to Spanish-speaking readers worldwide. (The articles are available at *www.hhmi.org/news/research-esp.html.*) Aballay, who never translated professionally before, has found the work rewarding. "We're really doing something to help people understand current biomedical research," he says.

Aballay enjoys the linguistic challenge of translating news about subjects and procedures for which Spanish words may not yet exist. No word for "screening," for example? No problem. Aballay combs the literature and consults with his wife, Graciela Vidal, a language instructor and trained translator. "It's a team effort," he says, and together they've compiled a glossary of terms (*see box*). In some cases, they've had to create phrases such as *examen genético*, for "screening." Vidal first worked with scientific language while helping Aballay edit his doctoral dissertation, which was written in both English and Spanish. "She has always been involved in my writing," he says.

Aballay says translating is "especially gratifying" because of inadequate communication between his homeland and the worldwide community of English-speaking scientists. "In Latin America, not many people study English, even in science," he says. His work helps researchers in Argentina and elsewhere keep up with the latest news and also serves lay readers who usually see little in the way of science reporting. Watching his stories make their way into local publications has been satisfying for Aballay, who says "we've been telling the whole Spanish-speaking world what cutting-edge science is."

He and Vidal will be going back to Argentina in the next year or so, where Aballay will become an investigator at the Consejo Nacional de Investigaciones Científicas y Técnicas, or CONICET, in Mendoza. He's using the remaining time in his current position to build a base for his independent research.

Aballay will be happy to return to friends, family and soccer; like most Argentinians, he says, "I miss *asado* [grilled meats] and *dulce de leche* [milk-based caramel]." No matter what the future brings, Aballay says he hopes to continue working as a scientific translator so that he—and other Spanish speakers—can keep tabs on the latest science news.

—CAMILLE MOJICA REY

¿Cómo se dice?

Alejandro Aballay has created more than 100 words and phrases while translating HHMI's research news into Spanish. Here's a sample:

English	Spanish
brain-cell-clogging	placas cerebrales obstructivas
down regulation	regulación por disminución
expressed sequence tag	secuencias de expresión marcadoras
gel-mobility shift assay	análisis de rastro en gel
gene shuffling	intercambio de genes
genetic linkage analysis	análisis de enlace genético
genome-wide scale	escala de la amplitud del genoma
nonsense-mediated decay	degradación mediada por el antisentido
prion proteins	proteínas priónicas
scattering studies	estudios de dispersión de rayos X
wiring	patrón de conexiones nerviosa

Evolution in a Test Tube

Assuming that nature's first functional proteins evolved from random protein sequences, a group of researchers set out to learn just how easy or hard it is to create a functional protein from random DNA sequences. They devised a way to subject six trillion proteins to a kind of natural selection process in a test tube.

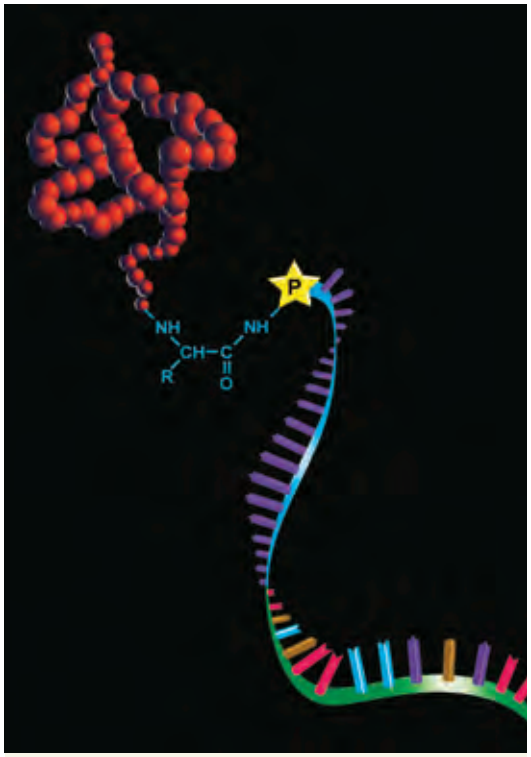
In an article published in the April 5, 2001, issue of *Nature*, HHMI investigator Jack W. Szostak and his colleague Anthony Keefe at Massachusetts General Hospital reported that they started with a massive library of 400 trillion random DNA sequences, from which they generated six trillion different proteins. They subjected the proteins to in vitro selection and tested the resulting proteins' ability to fold into a shape that could be functional—in this case, able to bind the common biological substrate ATP.

"With this experiment, we wanted to try to see whether evolving new proteins is something that could happen easily and frequently, or whether it was an incredibly rare event," says Szostak. Those trillions of proteins yielded four functional proteins—roughly one new protein for every 100 billion random-sequence proteins. "Whether these kinds of odds make protein synthesis appear easy or difficult depends on your point of view and how many starting sequences you're willing to look at," states Szostak.

The new proteins were a bit of a surprise. "They don't really look much like anything we've seen in nature," says Szostak. He cautions, however, that when he and his colleagues determine the three-dimensional structures of their artificially evolved proteins, they might indeed see some structures reminiscent of natural enzymes.

It will be "intriguing," says Szostak, if future experiments show that biology uses just a small subset of possibilities, which may have been the first to evolve by chance. Alternatively, he notes, "there may be many choices, and the ones that survive might do so because they can be optimized for other functions in addition to ATP binding." For example, the living cell might select for protein structures that have certain qualities of stability or a capacity to interact with other proteins.

The research was supported in part by grants from the National Institutes of Health and the NASA Astrobiology Institute.



Amino acids (orange beads) have folded into the protein shown at the top. Attached to the protein and serving as a "tag" is the lengthy messenger RNA (mRNA) that translated a DNA sequence into the protein's genetic code. Puromycin, shown by the letter "P," links the protein and mRNA by a covalent bond. Keefe and Szostak use the mRNA to recover the genetic information coding for each functional protein.

REPRINTED WITH PERMISSION FROM *JOURNAL OF MOLECULAR BIOLOGY* 297:309-319, COVER IMAGE, 2000 © ACADEMIC PRESS, LONDON.

Tracing Neural Clues to Eating and Willpower

"Why can't I resist that second piece of pie?" The answer to that guilt-ridden question may not be far off, now that researchers have taken a first step toward understanding the neural connections that affect a mammal's decisions about whether or not to eat. They've developed a genetically altered virus that for the first time allows them to trace circuits in the mouse brain that control food intake.

HHMI investigator Jeffrey M. Friedman and colleagues at The Rockefeller University, and at Princeton University and the University of California, San Diego (UCSD), used pseudorabies virus to create an elaborate biological tracer that becomes activated only in neurons of their choosing. For this study they targeted neurons that express either the leptin receptor, which is activated by the hormone leptin to reduce appetite, or neuropeptide Y, an appetite-stimulating substance. The virus traveled upstream from the site of infection, jumping from neuron to neuron. The scientists were able to trace the virus as it moved through the brain by engineering it to carry a gene for green fluorescent protein.

When they examined slices of mouse brain treated with the virus, the scientists could see which regions of the brain make connections with the leptin receptor or neuropeptide Y neurons in areas known to regulate feeding behavior. "We could see inputs from a number of other regions to the hypothalamus, which is where basic drives for feeding are controlled," Friedman says. "Specific inputs from brain centers that control emotion and from others that receive olfactory information were seen. We also saw inputs from centers in the mouse that are the equivalent of centers that control higher cortical or cognitive functions in humans."

The researchers published their findings in the March 30, 2001, issue of the journal *Science*. The lead author of the research article was Jeff DeFalco, a member

of Friedman's laboratory, and the coauthors included Lynn Enquist and Mark Tomishima at Princeton and Jamey D. Marth, an HHMI investigator at UCSD.

"While our study is only a beginning and doesn't address behavioral issues, it's pretty clear that people differ in how much willpower they have," Friedman says. "Willpower is not metaphysical; it's a complex network of neural connections and neural circuits. It's not inconceivable to me that individuals who have greater conscious ability to consume less food might have slightly different neural circuitry or more powerful neural connections that might ultimately be visualized through mapping studies.

"To address these sorts of issues we need to learn how this neural system is organized," Friedman continues. "Then we can begin to think about what is different about this system in obesity versus leanness and how the higher circuitry interacts with the circuitry that responds to basic physiological drives."

Friedman and his colleagues are beginning studies using other pseudorabies virus strains that can trace connections that begin in the hypothalamus, as well as combinations of viruses engineered with different markers to trace multiple pathways simultaneously. They also plan to explore the hierarchy of the circuitry by using advanced microscopy and computer systems to generate three-dimensional reconstructions of the labeled cells. H

A Teachable Moment in the Waiting Room

Three-year-old Carlito intently examines the image of a mouse bone magnified on a computer monitor in the pediatric waiting room of Boston's Whittier Street Neighborhood Health Center. "Es un pollo" ("It's a chicken"), the Salvadoran boy concludes as he compares the tiny bone with its enlarged image and recalls a familiar meal of *arroz con pollo*. Although he did not recognize the mouse bone, the interactive game has captured his attention, turning a boring wait into what the activity's designer, M.I.T. artist-in-residence Diane Willow, calls "a teachable moment."

ASIA KEPKA

Jessica Gomez and her brother Eric learn about their bodies and health while waiting to see the doctor at a health center in Boston.

The magnified mouse bone belongs to an activity named *Looking Large!*, part of a program called *Interactive Check-Up*, which takes science education out of the classroom and into community health centers in disadvantaged neighborhoods. At places such as the Whittier Center in the Roxbury section of Boston and South Cove Community Health Center in the city's Asian community, children are waiting with family members and already thinking about their bodies and health; they can explore and learn without the pressures they may feel in school.

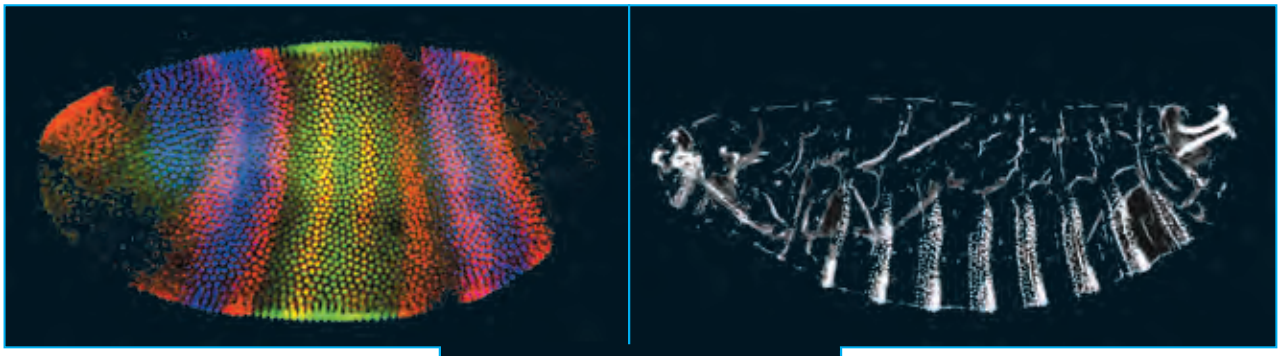
Interactive Check-Up was developed by Willow, formerly a science educator and director of an experimental media studio at Children's Museum in Boston, with support from an HHMI grant. It is designed to appeal visually to its target audience of minority children under the age of 11 and their families. *Looking Large!*, for instance, features a screensaver with pictures of children from the two minority communities and text in English, Spanish and Chinese.

Science educators say inquiry-based activities such as *Interactive Checkup* are among the best ways to stimulate children's interest in science. In a community setting that encourages family participation, *Looking Large!* seems to be doing just that. Carlito is engrossed by magnified images of fur, skin and gauze bandage. The video magnifier also has captivated Emily, a 5-year-old from the Dominican Republic who would like to take her turn with it. Willow finally distracts Emily by describing a new *Interactive Checkup* activity that she is designing: *Parts of a Whole*, a life-sized, illuminated body puzzle made from x-rays and other scans of bones, arteries and organs. "You can play with it next time you come to the clinic," the artist promises.

Willow is developing *Parts of a Whole* in collaboration with Diego Jaramillo, a pediatric radiologist at Massachusetts General Hospital. Another planned interactive activity is *Sensational Me!*, focusing on the senses. Children will use computers and a variety of sensing devices to compose portraits of their sensory organs and explore their own auditory, visual, olfactory and tactile sensations. **—DELIA K. CABE**

CLOSE-UP

The Beautiful Logic of Development



A 3-hour-old fruit fly embryo (left) develops into a larva, or maggot (right, shown at 24 hours old). Soon after fertilization, gene products (shown labeled with colored dyes) are already establishing the embryo's basic orientation. Within 24 hours, a wave of protein activity has built the structure of the maggot's 14 equal segments. The gene product "hairy" appears in red, Kruppel in green and "giant" in blue.

A fundamental mystery of biology—how one simple cell becomes hundreds of specialized cell types in an adult animal—is coming into clearer focus. By combining molecular biology and visual art, HHMI investigator Sean Carroll and his colleagues have learned to make images that show how cells distinguish themselves from each other, enabling an organism to organize itself correctly.

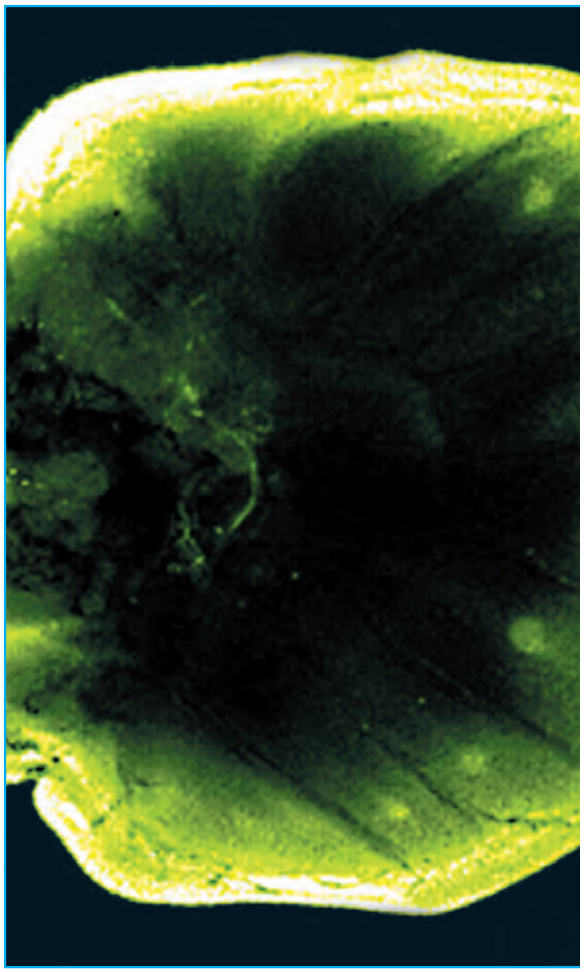
The images, which have frequently appeared on the covers of scientific journals, open a new window on development. Since Carroll's lab at the University of Wisconsin–Madison helped devise the versatile imaging technique, called multicolor confocal microscopy, about seven years ago, it has become an accepted tool of developmental biology.

A fertilized egg contains every genetic program needed by the adult, but scientists have long wondered how the genes are controlled and how each plays its part in the precise sequence needed to make an adult. Carroll and other biologists are helping to answer these questions by tagging gene products with antibodies and watching, moment by moment, as the genes turn on and off.

"Early in biology, we developed our concepts by studying final forms," Carroll says. "Now, instead of looking at what happens in adults, we base our descriptions on what we can see going on in the early stages." Because the gene precedes the protein and the protein precedes the structure, he says, "the activity of these genes, which we can visualize, foreshadows the physical history of the developing tissue."

To "see" proteins made by the various genes that guide development, Carroll and his colleagues create antibodies that link to those proteins. By attaching different fluorescent dyes to the antibodies, they can create multicolored images that show changes in individual cells over time.

The play-by-play drama of development is recorded in a confocal light microscope, which uses laser light and a computer-controlled scanning system to collect digital images of specimens labeled with fluorescent antibodies that detect gene activity. The confocal microscope eliminates stray light, says Stephen Paddock, the microscopist in Carroll's lab, and therefore allows imaging of individual cells at various depths in the sample. This capability is especially critical when researchers are studying embryos, where most cell differentiation takes place well below the surface.

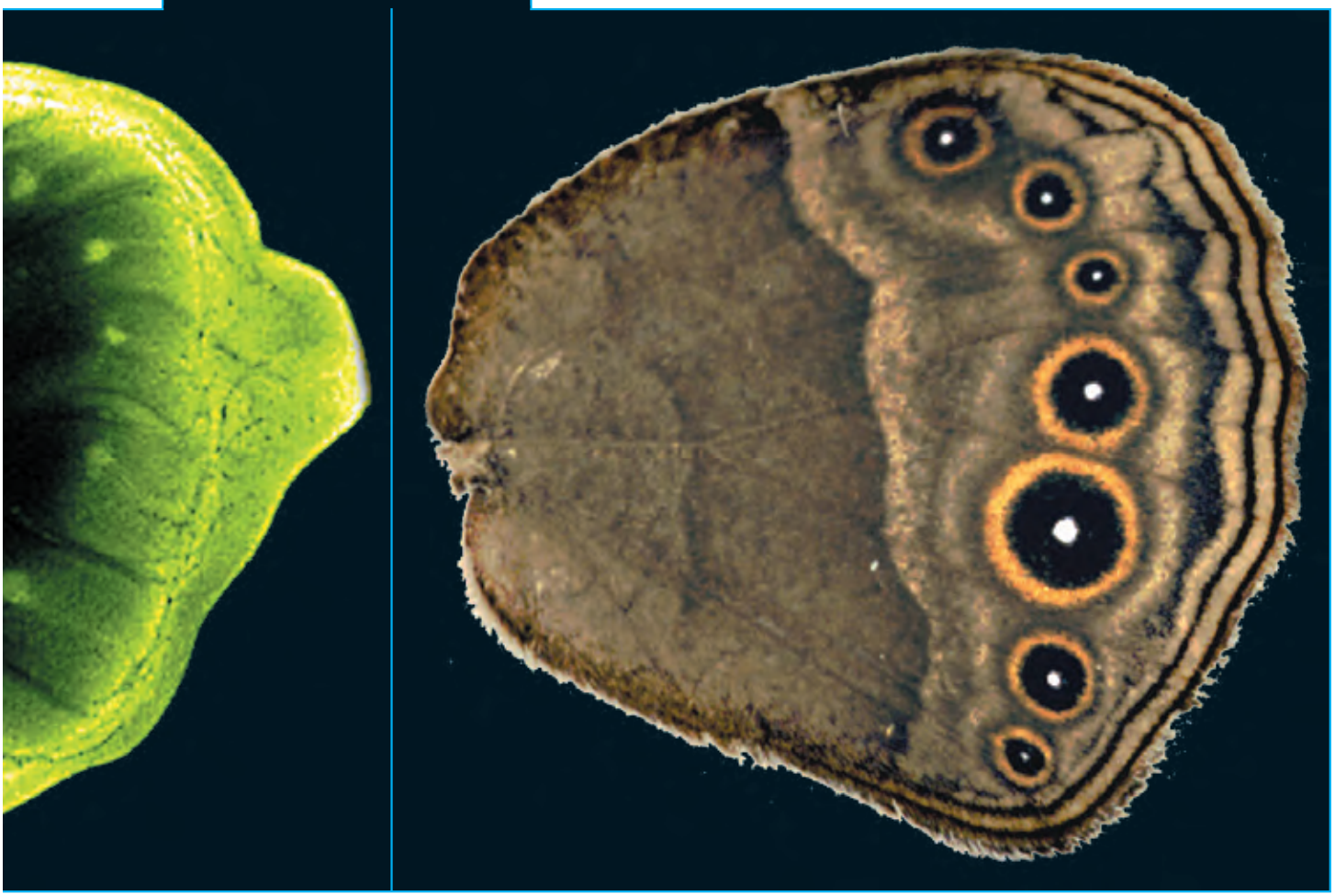


The visually arresting images show that a critical early stage of development involves orientation. "You need some way to take this mass of cells and subdivide it," says Carroll. "But there are no landmarks; cells are all the same to begin with." Yet some genes make proteins that establish the broad territories, front and back, top and bottom, left and right. As Carroll describes it, "You see progressive refinement. It's like going from country to state to city to zip code to street address." In anatomical terms, the final result of establishing this orientation is to allow cells to specialize—say, into skin, neurons or muscles.

The antibody probes can track similar gene activity in different organisms, providing new perspective on evolutionary relationships. That's what Carroll's lab has done to learn about one of its favorite genes, *distalless*, which controls the leading edge of development in limbs and other appendages of earthworms, sea urchins, butterflies, chickens, fish and crustaceans. The lab's studies have shown that this developmental process is widespread.

The elegant blend of antibody labeling, fluorescent dyes and confocal microscopy pioneered by Carroll and other biologists allows them to see development as a process. "By being able to study events as they happen," Carroll says, "as opposed to waiting for the outcome, we have the ability to get real-time pictures of animal development—to understand what's going on in what sequence. This process of building a complicated organism is highly orchestrated, highly regulated. To understand development, we've got to be able to investigate, analyze and visualize, in seconds and minutes, what the genes and cells are doing."

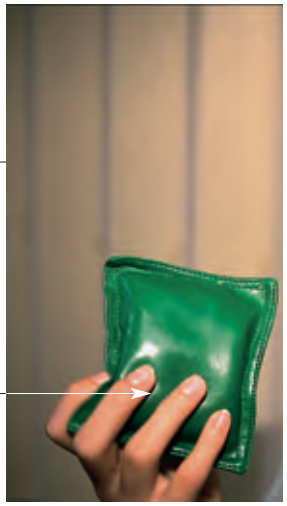
The multicolor confocal microscopy technique is promising but has only begun to describe the development process. It's still difficult to track through time and space the dozens of proteins that play critical roles. "We get a little lost beyond two or three gene products," says Carroll. "Probably the average cell is making 10,000 proteins. We understand certain aspects, but at a larger scale, we're lost." **—DAVID TENENBAUM**



The *distalless* gene, which directs the growing edge of a butterfly's wing, makes the distalless protein, which is linked to the greenish yellow dye. The left image shows the tiny disk of cells that forms the wing. Distalless is active at the growing edge and where the eye spots (specialized colored scales) later form. The right image shows the completed wing at lower magnification.

OBJECT LESSON

Explaining the Brain



■ By throwing a beanbag at a target, students discover how vision affects motor skills. When they wear goggles that shift their vision to the left or right, their brains have to relearn how to hit the target. The take-home lesson? Every time you learn something, your brain changes.

As any science teacher knows, getting students interested in neuroscience can be a challenge. When you start throwing around terms like cerebellum, corpus callosum, spinal ganglion and olfactory bulbs, many kids tune out. Now scientists at the University of Minnesota have come up with a friendlier approach. Called "BrainScience on the Move," it keeps students focused while it sparks their imaginations.

The program, supported by an HHMI grant, targets fifth through eighth graders by training their teachers. It begins in the summer when some 20 Minnesota teachers gather at the university's Twin Cities campus. There they attend "Brain U" for a weeklong crash course in neuroscience, including creative ways to teach it.

When they return to school, the teachers receive classroom visits from a university neuroscientist and a teacher from St. Paul's Science Museum of Minnesota. The visitors present a performance that resembles a magic show and leave behind a "brain trunk" stocked with videos, CD-ROMs, books and materials for six hands-on activities.

The approach has been effective for teachers and students alike, says Timothy Ebner, who heads both the project and the university's department of neuroscience. In program coordinator Carrie MacNabb's experience, "When you start talking to the students about this or that part in their heads and how it's involved in vision or how it's involved in remembering what they ate for breakfast—I have almost never seen a student who isn't completely hooked."

—SUSAN PERRY

■ To help introduce kids to basic brain facts—especially the names and functions of the brain's specific areas—the brain trunk contains *The Human Brain* (Philadelphia: Running Press, 1999), a kit with a 12-piece model of the human brain and instructions for classroom experiments and activities. In one activity, students conduct a blindfolded taste test that helps them discover how the brain processes information about food. In another, they use rhyming and other techniques to examine how the brain stores information.

DOUG KNUTSON (4)

■ Putting together a plastic model of the brain is one thing, but dissecting a real brain is even better. The Brain Trunk offers sheep brains for small groups of students to dissect, and worksheets help the students identify the various parts. Students must also answer questions, such as: How are the human brain and sheep brain similar? How are they different? What's the difference between the left and right sides of the brain?

■ To learn that sensory perception is relative, students put one finger in a container of hot water and one in cold water. After one minute, they put both fingers in room-temperature water. The finger that was soaking in warm water will feel cooler and the cold-water-finger will feel warmer in the room-temperature water. Experiencing the difference generates better and longer-lasting understanding than a didactic lecture on the subject.

New Technique Reveals Neuronal Calcium Channels in Action

By cleverly combining a laser-scanning microscope and a fluorescent dye, HHMI researchers have been able to analyze the opening and closing of porelike molecules, called calcium channels, in the membranes of nerve cells. These voltage-sensitive channels allow calcium influx into the cells, helping propagate nerve impulses triggered by neighboring neurons.

Learning about the number and behavior of the channels could add important insights into how signals travel from neuron to neuron through the brain, and how such channel activity can permanently alter brain pathways in the neural processes underlying learning and memory. HHMI investigator Karel Svoboda and his colleague Bernardo Sabatini at Cold Spring Harbor Laboratory reported on the use of the new "optical fluctuation" technique in an article in *Nature* (November 30, 2000).

A major technical hurdle in understanding the behavior of calcium channels, says Svoboda, has been the widely used electrical-measurement technique called single-channel patch clamps, which uses microscopic electrodes to measure the electrical properties of individual channels. This technique cannot be applied to study the channels in intact synapses—the junctions between neurons across which triggering takes place.

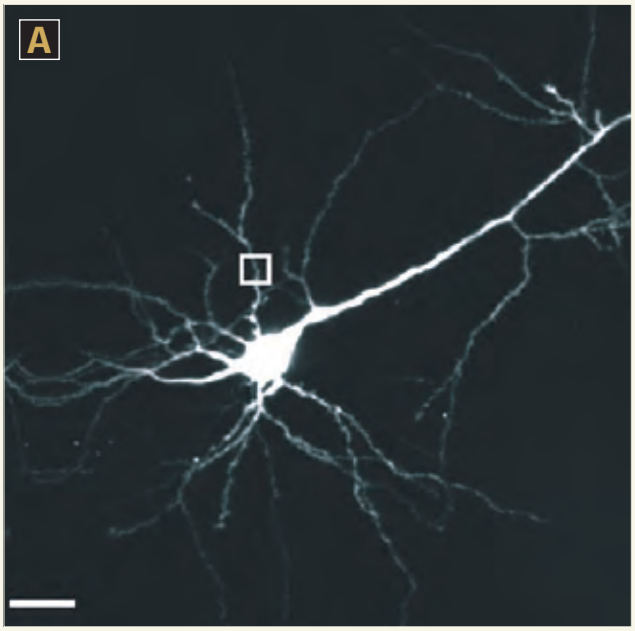
"The patch clamp technique can only be used in simplified preparations such as cell cultures," says Svoboda. "However, we are interested in synapses, which must be studied in intact nervous tissue because of the importance of the cellular network for their function.

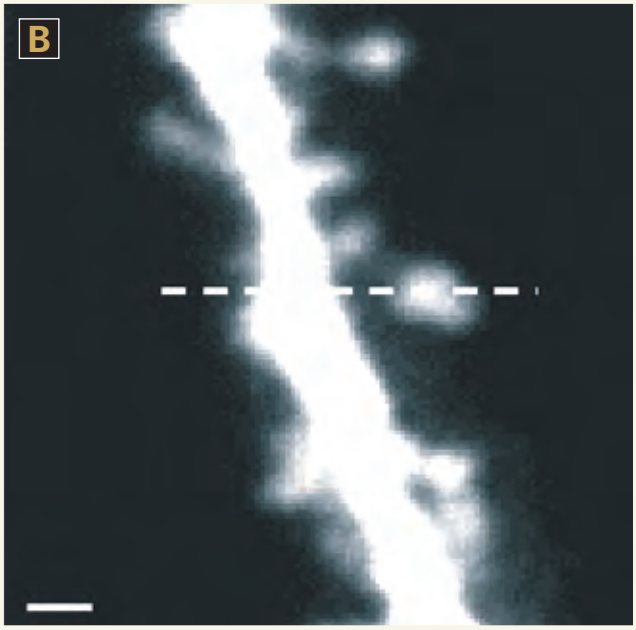
"For example, key aspects of normal synaptic function depend on the protective ensheathment of synapses by glial cells, which are not present in cell-culture preparations," he says. "Also, such synapses are far too small to be directly accessible by electrophysiological techniques." The synapses are located on tiny projections, or spines, that jut from the dendrites that branch off neurons.

Thus the scientists pioneered the optical

The new "optical fluctuation" technique reveals how calcium channels open and close in the membranes of nerve cells to help send signals through the body. The first image (A) shows a neuron that has been injected with fluorescent dye that is sensitive to calcium. The small square is enlarged in the second image (B), showing the neuron's dendritic spines, home to the signal-transmitting synapses.

The dotted line across the middle of this image corresponds to (C), which shows how an electrical action potential sent into the dendritic tree (arrow) causes calcium channels to open in dendrites and spines. Calcium flows into spines in a fluctuating manner, as shown in the histogram (D). Researchers can analyze the shape of the histogram to deduce the number and properties of the channels.





REPRINTED WITH PERMISSION FROM *NATURE*, 408:589-93, 2000. © NATURE PUBLISHING GROUP

fluctuation analysis technique, in which they inject calcium-sensitive fluorescent dye into target neurons in slices of rat brain, and then use a laser scanning microscope to stimulate and measure the light that the dye emits. The scientists trigger the calcium channels in the neurons to open by applying an electrical "action potential" that produces a telltale flow of calcium into the cell, causing the dye to fluoresce.

By repeatedly applying the action potential in the same way and measuring the fluctuations in light from the cell, Svoboda and his colleagues are able to measure the calcium fluctuations in the synapses caused by the opening and closing of calcium channels. They can analyze the fluctuations over many trials to deduce the number and properties of the channels.

"The process is like flipping coins," Svoboda explains. "Let's say you have ten coins. On any given series, you might get anywhere from one through ten heads. On average, you'll get five heads but the fluctuations in the number of heads from trial to trial can actually tell you how many coins there are and what the probability of heads is. We essentially used that trick to find out how many channels there are in the spines and what their properties are."

"For example," he continues, "we could figure out the probability of a channel opening in response to an action potential." The scientists' studies revealed that the typical dendritic spine held only about ten channels, ranging from 1 to 20, and that the channels opened with a high probability on each triggering. "It was really surprising that an individual synapse only works with such a small number of channels," Svoboda says.

The new technique is yielding insight into a key mechanism by which nerve impulses propogate, he adds. "The spine channels are functionally important because they serve to amplify synaptic currents through a sort of current-boosting mechanism, and they also couple synaptic activity to downstream biochemical events," Svoboda explains. "These events include those that modify the receptors themselves, leading to changes in the efficacy of synapses, which are a basis for long-term plasticity in the brain.

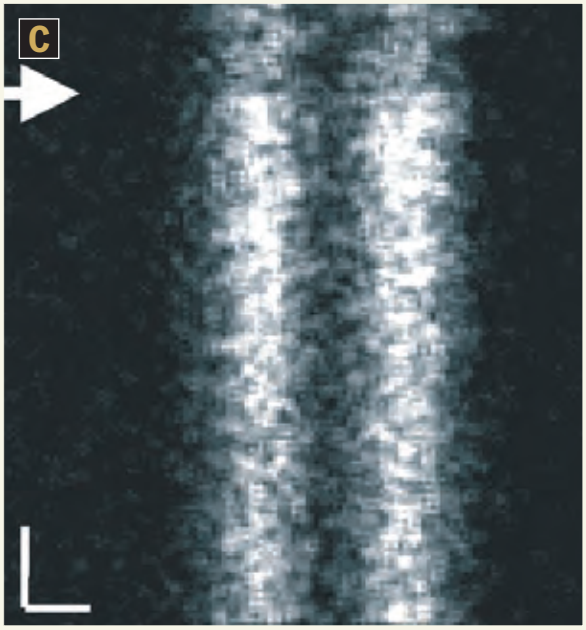
"Voltage-sensitive calcium channels in particular have been implicated in coupling to gene-transcriptional events," he adds, "somehow activating signaling to the nucleus that leads to the onset of transcription, which is required for the modification of synapses."

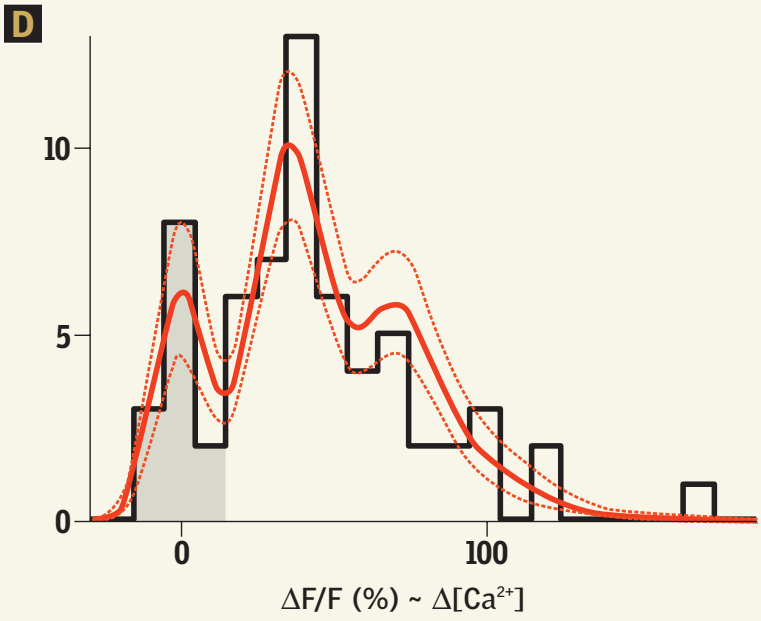
In further studies, Sabatini and Svoboda have discovered how the signals from the calcium channels are distributed in the dendrites and their spine tips, or "heads." The scientists used a chemical activator, or agonist, which switched on receptors called $GABA_B$ that are known to activate the calcium channels. "When we add $GABA_B$ receptor agonist, which presumably activates all $GABA_B$ receptors along the dendrite, only the calcium channels in the spines are modulated by these activated receptors," Svoboda says. "So this is good evidence that these spines are actually separate signaling compartments and that the signaling environment in the spines is very different than in the dendrites."

Further detailed studies should yield significant new information about the nature and location of such key biochemical processes underlying neuronal function and plasticity, according to Svoboda. "We know that the synapse activates the spine, the calcium comes in and activates enzymes or receptors, and all of this biochemical action happens in the spine head," he says. "None of these signaling events appear to take place in the dendrite."

What's more, adds Svoboda, researchers should be able to use the optical fluctuation technique to study any proteins that control calcium flow in the membranes of neurons. Fortunately, he says, such proteins include some of the most important receptors involved in transmitting biochemical signals within synapses.

—DENNIS MEREDITH





An Investigator's Right-Hand Man in the Laboratory

Laszlo Jakoi knows a lot about balance. In his 14-odd years in the lab of HHMI investigator Joseph Nevins, he has learned how essential it is for the body to balance the demand for cell growth against both programmed cell death, or apoptosis, and the abyss of uncontrolled growth known as cancer.

The cells' lessons about order, adaptability and resilience have served Jakoi well, not only in planning and carrying out experiments but also in his role as lab manager. For 30 years and counting, he has served as the right-hand man for HHMI investigators at Duke University Medical Center—most recently with Nevins, whose lab studies a family of proteins called E2F that serve as checkpoints in the cell cycle, dictating how and when cells multiply.

Jakoi's importance to the lab cannot be overestimated, says Nevins, because he keeps tabs on all the lab's current activities while also serving as its living memory. "For the most part, the people doing the work in any academic laboratory are students and postdoctoral fellows," Nevins points out. "By definition, they are all here for a limited period of time. In the years that Laszlo has been here, there have probably been five or six generations of people that have come through the lab. The constant turnover of people, techniques and reagents creates the potential for chaos. It's the nature of the business. Laszlo has maintained the continuity of the lab through each of those generations."

Where is that DNA sample that the former postdoc was working on three years ago? Ask Laszlo. How do you isolate rabbit antibodies for that next series of experiments? Ask Laszlo. No time to run that DNA shift assay this week? Laszlo will help.

"I see myself as a facilitator," says Jakoi. "I do work on my own experiments, but I also run the day-to-day operation of the lab. I do most of the ordering, see that the equipment is working, prepare reports and administer the lab safety program for the group."

The task that may most endear Jakoi to his coworkers is running interference with the numerous sales representatives who come to the lab. "You wouldn't believe how much time this saves for the postdocs," Jakoi says with a grin. And since he has an intimate knowledge of just about every kind of experiment the lab has ever carried out and every assay it has used, he can help evaluate almost any new product for it.

Nevins says that a combination of tact and an ability to exert a "subtle authority" is at the heart of Jakoi's balancing act. In a laboratory full of bright and motivated individualists, such as one finds in most any successful academic research setting, Jakoi successfully directs, instructs and collaborates in his own quiet way.

"You sort of keep an eye on what's going on," Jakoi agrees. "I may see somebody presenting data at the lab meeting and have the inclination that the technique wasn't properly done. I can say, 'Hey, I can help you.' " Frequently, his colleagues don't wait to be asked. "More often than not," says Jakoi, "because of the positive relationships we have in the lab, people come up to me and say 'would you please help me with this?' because they know I know how to do it better or they have so much to do and need help."

Nevins says that in virtually every lab where the principal investigator is established and in mid-career, you will find someone like Laszlo Jakoi who manages the day-to-day operations for a chief whose numerous external commitments are critical to the lab's success but regularly cause him or her to be away.

"HHMI labs are like islands," says Pamela Phillips, who directs HHMI's research operations nationwide. "Lab managers are the central office's link to what's going on in the labs. They help us to stay connected and to trust that everything will be taken care of in a decentralized environment. They're just invaluable."

—KARYN HEDE

Laszlo Jakoi is the manager and "living memory" for the laboratory of Joseph Nevins.

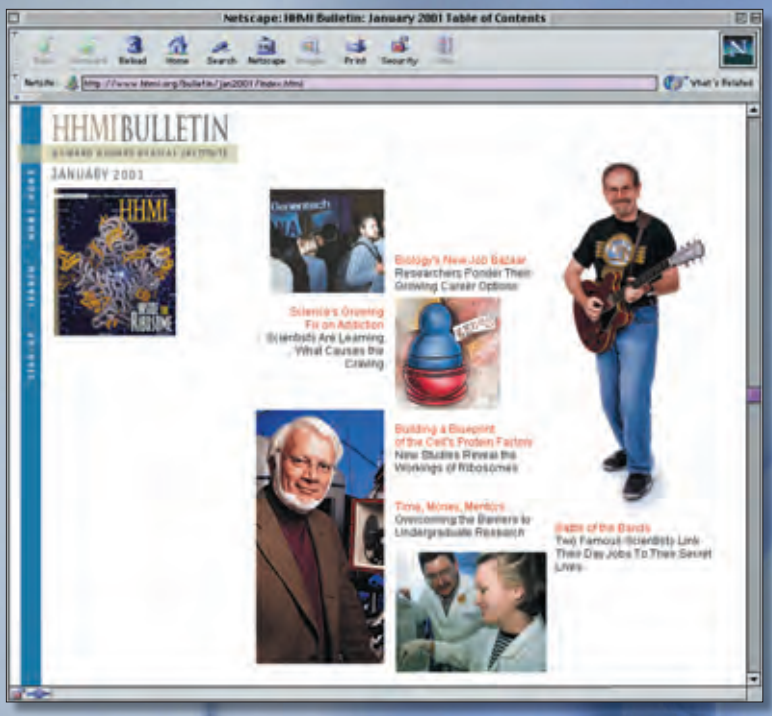
WILL McINTYRE

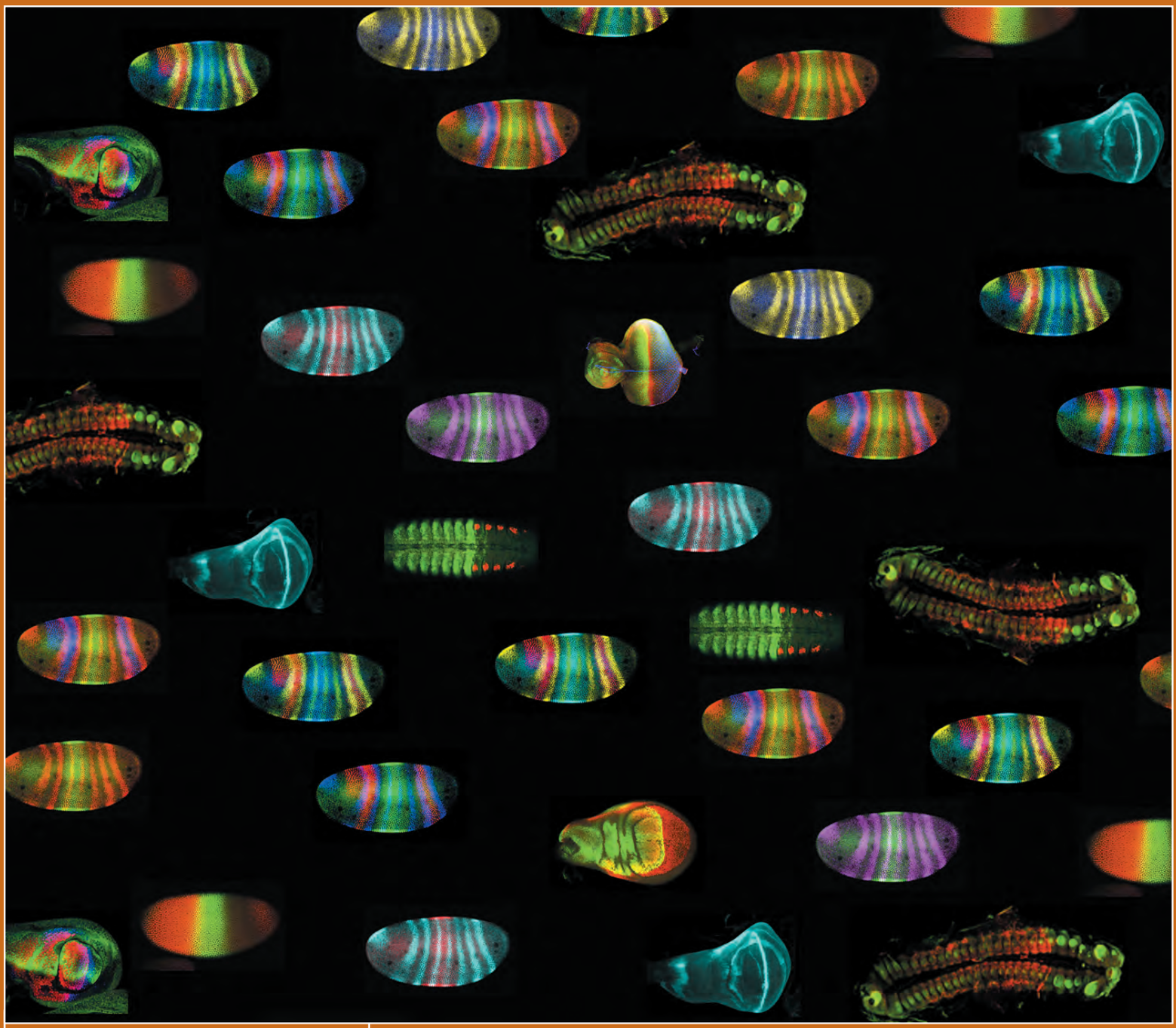
The *Bulletin* Goes Online

Did you read in a previous issue of the *Bulletin* about how researchers are unraveling the mystery of protein synthesis? How about the stories on drug addiction, new career options for biomedical scientists or undergraduate science education? If you missed them, you can now read them online at *www.hhmi.org/bulletin*.

The *Bulletin*'s new "e-zine" adapts print stories for the Web and often broadens them with new material. An online version of "Battle of the Bands," a story about two HHMI researchers who play rock music, enables readers to hear their songs. "Science's Growing Fix on Addiction," which was limited to four pages in print, links to a biography of James Olds, who pioneered the mapping of the brain's reward centers. Online readers of "Biology's New Job Bazaar" can click to Web sites that highlight scientific jobs and career advice.

New technology is also making it possible to enhance the written word with animations, video and other features, many of which the *Bulletin* will introduce soon. Of course, readers who want the magazine in printed form—like the copy you're holding now—can continue receiving it for free. New subscribers are welcome (but they do need to go online to sign up at *www.hhmi.org/bulletin*).





Sean Carroll and his colleagues used multicolor confocal microscopy to create these images, which reveal the embryos of different organisms taking form early in their development. **See story, page 34.**

NONPROFIT ORG.
US POSTAGE
PAID
SUBURBAN MD
PERMIT NO. 6561

Howard Hughes Medical Institute
4000 Jones Bridge Road
Chevy Chase, Maryland 20815-6789
301.215.8855 www.hhmi.org