This neural structure is essential to perception, behavior, thinking, and memory.

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**ON THE COVER:** A cultured neuron from the hippocampal brain region, important for converting short term memory to more permanent memory, and for recalling spatial relationships. The neuron has been engineered to overexpress the Shank 1B protein (aqua), which promotes the maturation and enlargement of dendritic spines and enhances presynaptic function. Courtesy of Morgan Sheng and Carlo Sala; modified from cover of Neuron (July 19, 2001).
As biomedical science becomes more interdisciplinary, research progress will depend on contributions from life scientists who are familiar with the tools and ideas of the physical and computational sciences and engineering. To help meet this challenge, HHMI and the National Institute of Biomedical Imaging and Bioengineering (NIBIB) of the National Institutes of Health (NIH) have joined forces to help start and sustain graduate training programs that integrate the biomedical sciences with the physical sciences and engineering.

HHMI will award up to 10 three-year grants of as much as $1 million each to support the development and early phases of the interdisciplinary programs. HHMI opened a competition for the grants in October 2004. All U.S. institutions that grant Ph.D. degrees in the biological sciences will be eligible for the three-year awards.

NIBIB, a new NIH institute with broad, interdisciplinary goals, will provide five additional years of support to the HHMI grantees through peer-reviewed institutional training grants.

The collaboration will create a new approach to the initiation, development, and maintenance of new graduate programs that provide up-and-coming biomedical scientists with relevant cross-disciplinary knowledge and skills. The project builds on work begun by the Whitaker Foundation, the National Science Foundation, and the Burroughs Wellcome Fund.

“This groundbreaking partnership between the NIBIB and HHMI will produce researchers who are skilled in biomedical disciplines, bioengineering, and quantitative sciences,” said NIH’s director, Elias A. Zerhouni.

“The HHMI-NIBIB partnership will capitalize on the different strengths of each organization. ‘HHMI can provide flexible support to catalyze the development of new, interdisciplinary programs,’ said HHMI President Thomas R. Cech. ‘The NIBIB will sustain these young programs once they are developed, as NIH does so well with traditional training grants.’

NIBIB Director Roderic I. Pettigrew said the new alliance would help future scientists to be

“better equipped to meet the complex challenges of 21st century medicine.”

The new NIH Roadmap and recent reports from the National Academies Convocation on Facilitating Interdisciplinary Research and the Association of American Medical Colleges’ Graduate Research, Education and Training Group emphasize the need for a new kind of graduate education that will prepare scientists to work across disciplinary lines to solve complex biomedical problems.

“We’re looking for training programs that provide strategies to eliminate or lower barriers between seemingly disparate scientific disciplines,” said Peter J. Bruns, HHMI vice president for grants and special programs.

The new graduate training program parallels HHMI’s commitment to bring together biologists, computer scientists, engineers, physicists, chemists, and mathematicians to conduct collaborative research at Janelia Farm, HHMI’s new research campus now under construction in Loudoun County, Virginia.

—JENNIFER BOETH DONOVAN

### Grants for Interdisciplinary Graduate Research Training

**HHMI-NIBIB INTERFACES INITIATIVE**

The Howard Hughes Medical Institute and the National Institute of Biomedical Imaging and Bioengineering are partnering to provide funds to initiate and sustain graduate training programs that integrate the biomedical sciences with one or more of the physical science, mathematical, computational, and engineering disciplines.

**PHASE I GRANTS**

*Three years, up to $1 million, funded by HHMI*

Register intent to apply by January 20, 2005
Proposal submission deadline: June 15, 2005
Awards announcement: November 2005

**PHASE II GRANTS**

*Five years, funded by NIBIB*

Competition: 2008

**ELIGIBILITY**

All U.S. institutions that grant Ph.D. degrees in appropriate science or engineering disciplines are eligible to apply. Collaborative programs between two or more institutions are acceptable.

More information: [www.hhmi.org/ref/interfaces/hhmi](http://www.hhmi.org/ref/interfaces/hhmi)
A Process of Discovery

Inda Buck is getting lots of requests to recall how she got “hooked” on the problem of understanding how the olfactory system recognizes thousands of different smells. After all, it has been only a few weeks since she and Richard Axel heard they’d been chosen to receive the Nobel Prize in Physiology or Medicine for 2004. Buck, like most scientists who go on to win the Nobel Prize, began her journey without knowing she was destined for that kind of success and recognition.

Buck began with an interest in psychology and a desire to help others. Her interests shifted to microbiology and immunology, but just as she was completing a postdoctoral fellowship in Richard Axel’s lab at Columbia University, she discovered olfaction. This “fascinating new world” prompted Buck to start all over. She began a second postdoctoral stint in Axel’s lab. The decision was unconventional all the way around.

As an HHMI investigator, Axel had the freedom to shift the research direction of his laboratory—something he has done several times in the course of a remarkable career. He also had the ability to support Buck as an HHMI associate as she began mapping the geography of this new world. After more than a few false starts, they published (in 1991) the fundamental paper that described the large family of genes responsible for encoding olfactory receptors. A thousand different receptors responding to a thousand different aromatic compounds!

That same year, Buck established an independent laboratory at Harvard University. By 1994 she was an HHMI investigator in her own right, a position she later transferred to the Fred Hutchinson Cancer Research Center in Seattle. During this time, Axel and Buck have continued their independent explorations of the olfactory system, working in a variety of model organisms.

Writing in Nature, correspondent Alison Abbott noted, “Colleagues ascribe Axel and Buck’s success in unearthing the roots of olfaction to their personal doggedness, underpinned by the steady, powerful support that HHMI gives its researchers.”

I could not have said it better myself. Over the past six years, six HHMI investigators have been honored with the Nobel Prize, and I am struck by the ways in which support from the Institute has enabled each of these scientists to strike off in new directions, to pursue new and challenging questions. What HHMI does is to identify scientists of exceptional talent and imagination and provide them with the freedom to exercise intellectual daring.

Even without this latest Nobel news, it has been a particularly exciting time for HHMI’s biomedical research program. Our investigators continue to generate profound insights into fundamental biological processes (see www.hhmi.org). Eleven investigators were elected to the National Academy of Sciences earlier this year and five to the Institute of Medicine. More recently, investigator Ronald Evans shared the Albert Lasker Prize for Basic Medical Research with Elwood Jensen and Pierre Chambon for discovering the role of nuclear hormone receptors in the regulation of biological processes, beginning with embryonic development and continuing throughout life.

We’re engaged in a national competition for new investigators, through which we plan to name 30 to 50 scientists from a pool of candidates nominated by more than 150 universities, medical schools, and research institutes. We are considering candidates from the full range of biological and biomedical science, with a focus on identifying a cadre of individuals who have demonstrated exceptional promise as independent researchers early in their careers. It’s a time when an investment by HHMI—and this competition represents an additional commitment of up to $350 million in research funds over seven years—can make a substantial difference in fostering high-risk, high-reward science. We’ll announce the new investigators in spring 2005.

Also this year, we identified the initial areas of research focus for HHMI’s Janelia Farm Research Campus—the identification of general principles that govern how information is processed by neuronal circuits and the development of enabling technologies, including imaging and computational analysis. These areas have helped us to frame the competition now under way for research group leaders. In a first for HHMI, this competition is open to scientists from around the world and across a wide range of scientific disciplines, including physics and engineering.

In thinking about this period of renewal and expansion for HHMI, I’m reminded of an observation made by Richard Axel: “Science is not a move to an end, rather it is a process of discovery, which unto itself should be a meaningful pleasure.” The coming months are likely to generate discoveries of their own as we identify new investigators and group leaders to take the Institute into the future.
The scent of verbena. Lemony and delicate in small whiffs, the fragrance can be overwhelming in clouds. Drusilla, William Faulkner’s implacable Civil War widow in The Unvanquished, wore sprigs of the flower in her hair, saying it was the only scent strong enough to be detected above the smell of horses and courage and so the only one worth wearing.

Smell is powerful. In an instant, it can transport us to another time and place, warn us of danger, plunge us into despair, or lift us to rhapsody. Of all our senses, smell is the one we tend to take for granted. Yet humans and all other animals rely on it in countless practical and even primal ways to identify our food, our predators, our mates.

The olfactory mechanics that make possible this exquisite ability to discern smells from the most subtle to the blatant have been the subject of study for Richard Axel and Linda B. Buck for much of their research careers. Now, the two HHMI investigators—Axel at Columbia University College of Physicians and Surgeons and Buck at the Fred Hutchinson Cancer Research Center—are enjoying, as newspaper headlines have noted, “the sweet smell of success.” On October 4th of this year, Axel and Buck received early-morning calls from Sweden informing them they had won the 2004 Nobel Prize in Physiology or Medicine for their discoveries of “odorant receptors and the organization of the olfactory system.”

Before 1991, when Axel and Buck published their seminal discovery of odorant receptors, little was known about the mechanics of smell. Researchers—Axel and Buck among them—were essentially groping in the fog, trying to find some solid information as to the biological underpinnings. Perseverance—and a good dose of ingenuity on Buck’s part—finally yielded the breakthrough that revolutionized the field.

ELEGANT EXPERIMENTS
In 1991, Buck was a postdoctoral fellow in Axel’s lab. She had embarked on a search for odorant receptors three years earlier, after having already completed a postdoctoral project in Axel’s lab on the neuronal system of the sea slug Aplysia.

Buck’s background was in immunology, and she had also been trying to develop a method to identify rearranged genes in the mammalian nervous system. “I was intrigued by the possibility that gene rearrangement or gene conversion might be involved in the generation of a varied set of odorant receptors or regulate their expression, as with antigen receptors in the immune system,” she says. “I became obsessed with finding the odorant receptors and stayed on in Richard’s lab to look for them.”

Buck and Axel initially adopted an “unbiased approach” with regard to the structure of odorant receptors, choosing to focus on two assumptions: that the receptor proteins would be selectively expressed by olfactory sensory neurons and, given the structural diversity of odorants, there would be a family of related, but varied, odorant receptors that would be encoded by a family of related genes.

Their efforts produced nothing at first. The tide turned when they added an additional assumption to their search. Based on scattered evidence from other labs, Buck made the decision to narrow her search to a family of proteins called G protein-coupled receptors (GPCRs), many of which were known to be involved in cell signaling. Making use of the recently developed gene amplification technology called PCR, or polymerase chain reaction, Buck says, she “decided to conduct an exhaustive search for GPCRs in the olfactory epithelium by taking a novel approach that involved using a number of different degenerate primers in a combinatorial fashion.”

Further analysis of the PCR products narrowed the search to one candidate. “When I cloned this PCR product and sequenced five of the clones, I found precisely what we had been looking for,” says Buck.

Buck’s go-for-broke instincts obviated the need to sort through thousands of genes and, according to Axel, “saved several years of drudgery.”

Scientific perseverance and a dose of ingenuity led to Linda Buck and Richard Axel’s discovery of odorant receptors.
“I had tried so many things and had been working so hard for years, with nothing to show for it,” says Buck. “So when I finally found the genes in 1991, I couldn’t believe it. None of them had ever been seen before. They were all different, but all related to each other. That was very satisfying.”

Buck found that, in contrast to the immune system, where genetic rearrangement and mutation create antibody diversity, each of the olfactory genes faithfully encodes a single receptor protein. The radical finding opened up the field of olfactory biology, providing the tools necessary to explore the mechanisms underlying odor perception at the molecular level. In the ensuing years, researchers—including Axel and Buck, who have worked independently since 1992—have filled in many of the blanks in our understanding of how we smell.

At every inhalation, air currents swirl up through the nostrils and past a thumbprint-sized patch of packed cells—some five million olfactory neurons, each neuron topped with hair-like cilia, each cilium lined with receptors. In the mouse, about a thousand genes encode an equivalent number of different receptor types. Humans have over 600 odorant receptor genes—representing a remarkable 2 to 3 percent of the entire genome—but perhaps because we no longer need a nose as keen as that of a mouse, about one-half of those genes are nonfunctional pseudogenes.

Still, with our 350 different receptor types, humans can detect an estimated 10,000 to 100,000 different odorants. “It is remarkable that we can smell so many different chemicals,” says Buck. “And it’s also amazing that even a slight change in the structure of the chemical can totally change the way it smells to us.”

With so few receptor types, how are we able to recognize the thousands of different odorant molecules of various shapes and sizes that we encounter? And how is it that in some cases a faint whiff is enticing while a strong burst is repelling? Somewhere in the arrangement by which odorants bind to receptors and initiate an electrical signal that travels along axons to the brain’s olfactory bulb (just above the back of the nose) and then on to the cortex, says Axel, lies “an intricate logic that the brain uses to identify the odor, distinguish it from others, and trigger an emotional or behavioral response.”

**Teasing Out the Logic**

In the years since their discovery of the receptor genes, Buck and Axel have made progress in teasing out that logic. In 1993–1994, Axel, at Columbia, and Buck, then at Harvard University, reported that neurons expressing the same kind of odorant receptor are scattered throughout the olfactory epithelium (surface tissue), but that their axons all converge at specific points in the olfactory bulb that are similar among individuals. This arrangement could, Buck theorized, not only enhance sensitivity but also ensure continued olfactory function should some part of the epithelium be damaged by infection.

By 1999, Buck and colleagues had uncovered “combinatorial receptor codes,” a finding that explains how we can distinguish a seemingly unlimited number and variety of odorants. The researchers showed that the odorant receptor family is used combinatorially to detect odorants and encode their unique identities. They found that a

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**To Learn More**

More information on Linda B. Buck and Richard Axel’s work may be found at the Nobel Prize site (http://nobelprize.org/medicine/laureates/2004/) and at these other Web sites:

**LINDA B. BUCK**
www.hhmi.org/research/investigators/buck_bio.html
www.fhcrc.org/visitor/nobel/buck/

**RICHARD AXEL**
www.hhmi.org/research/investigators/axel_bio.html
http://cpmcnet.columbia.edu/dept/neurobeh/axel/overview.html
Ten current HHMI investigators have won the Nobel Prize. In addition to Richard Axel and Linda B. Buck, the list includes these laureates:

- Günter Blobel, at the Rockefeller University, won the 1999 Nobel Prize in Physiology or Medicine for his discovery that certain proteins use intrinsic signals to govern their transport and localization in the cell.
- HHMI President Thomas R. Cech shared the 1989 Nobel Prize in Chemistry for the discovery that RNA in living cells is not only a molecule of heredity but can also function as a biocatalyst.
- Johann Deisenhofer, at the University of Texas Southwestern Medical Center at Dallas, shared the 1988 Nobel Prize in Chemistry for work that used x-ray crystallography to describe the structure of a protein involved in photosynthesis.
- H. Robert Horvitz, at the Massachusetts Institute of Technology, shared the 2002 Nobel Prize in Physiology or Medicine for identifying key genes that regulate organ development and programmed cell death.
- Eric R. Kandel, at Columbia University College of Physicians and Surgeons, shared the 2000 Nobel Prize in Physiology or Medicine for discoveries concerning signal transduction in the nervous system.
- Roderick MacKinnon, at the Rockefeller University, shared the 2003 Nobel Prize in Chemistry for research on the structure and function of cellular ion channels.
- Susumu Tonegawa, at the Massachusetts Institute of Technology, won the 1987 Nobel Prize in Physiology or Medicine for discovering the genetic principle for generation of antibody diversity.
- Eric Wieschaus, at Princeton University, shared the 1995 Nobel Prize in Physiology or Medicine for identifying 15 genes of key importance in determining the body plan and the formation of body segments of the fruit fly, Drosophila melanogaster.

single receptor can recognize multiple odorants and that a single odorant is typically recognized by multiple receptors, but that different odorants are recognized by different combinations of receptors. By virtue of this strategy, Buck says, the system has the potential to generate as many as a billion different codes.

The group also found that odorants with nearly identical structures have different receptor codes, a finding that explains why a slight change in its structure can alter a chemical’s smell. They also found that raising the concentration of an odorant recruits additional receptors into the response. This would explain why the appeal of verbena and other odorants varies with concentration.

More recently, Buck and her colleagues devised a genetic method for tracing neural circuits that allowed them to visualize how signals from different odorant receptors are organized in the olfactory cortex. They found that inputs from one receptor type are mapped onto clusters of cortical neurons at specific locations that differ for different receptors, but are the same among individuals. Surprisingly, while signals derived from different receptors are segregated in the olfactory bulb, signals from different receptors partially overlap in the cortex, and single neurons may receive combinatorial inputs from different receptors. Buck speculates that this could allow an initial integration of the components of an odorant’s receptor code and thus be important in the “reconstruction” of an odorant’s identity from its deconstructed features.

At the same time, Axel and his colleagues have turned to the fly brain to explore how olfactory information is encoded in higher brain areas. “There is a remarkable conservation of much of the logic of olfactory perception between insects and mammals, such that the basic principles of odor discrimination, we believe, have been conserved over 500 million years,” says Axel.

By studying the fruit fly in particular, Axel hopes to learn more about how odor can influence behavior. Fruit flies rely largely on scent to assess their surroundings and guide their social interactions. Certain odors, for example, play a critical role in flies’ mating behavior, as is also true in rodents and even in humans. By studying how the flies process olfactory cues and how those cues are translated into behavior, Axel’s group hopes to gain a better understanding of the neural circuitry involved. Visualizing the axonal projections of single neurons in the fly’s antennal lobe, which is analogous to the mammalian olfactory bulb, they found that the patterning of projections from the antennal lobe to the mushroom body (a cluster of neurons where learning is centered) is conserved among individuals, but differs for different antennal lobe neurons.

After hearing from the Nobel committee on their selection, Axel was quick to credit the many students and fellows in his lab who contributed to the work. “As scientists, we don’t

I had tried so many things and had been working so hard for years, with nothing to show for it. So when I finally found the genes in 1991, I couldn’t believe it. —LINDA BUCK

work in a vacuum;” he says. “We work together toward often common goals with a passion and an intensity. I’ve been blessed over the years with a remarkably strong and exciting group of students who did much of the work towards this end.”

It’s the process of collaboration and discovery, Axel says, that gives his life’s work meaning. “The joy of science,” he says, “is in the process, not in the end.”

Buck, who is one of only a dozen women to have won a Nobel Prize in the sciences, also appreciates the deep satisfaction that a career in research can give. She says she was “hooked” early on by the “monumental problem and wonderful puzzle” of olfactory perception, and she encourages her students to seek that same fascination in their own work, whatever that may be. “You want to do something that you’re obsessed with, that you just have to understand,” Buck says. “That’s where the joy comes from. That’s where the great discoveries come from.”

—MARY BETH GARDINER
Two leading obesity researchers discuss why our bodies need fat and how too much of it can be toxic.

**THE SPEAKERS:**

**Ronald M. Evans, Ph.D.**  
HHMI Investigator, The Salk Institute for Biological Studies

**Jeffrey M. Friedman, M.D., Ph.D.**  
HHMI Investigator, The Rockefeller University

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**Why are some people overweight and others lean?**  
**What can science tell us about how weight is controlled?**  
**Where will drugs to treat obesity come from?**

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**The Science of FAT**

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**HHMI’s 2004 Holiday Lectures on Science**

**LIVE WEBCAST:** December 2 and 3, 2004  
On demand: After December 7, [www.holidaylectures.org](http://www.holidaylectures.org)

**CHANNEL ONE BROADCAST:** January 12 and 19, 2005

**RESEARCH CHANNEL BROADCAST:** March 2005

**FREE ON DVD AND VIDEO:** April 2005  
[www.holidaylectures.org](http://www.holidaylectures.org)
a disease of abnormal, unfettered cell division, and understanding cell-cycle regulation in detail could open new therapeutic avenues for blocking cancerous growth.

He has identified a number of the key players and events that normally keep cell division under control. One of the most important is the gene

He chatted in his office in the sleek new 10-story research building opened last year at Harvard Medical School (HMS), where he is professor of genetics and medicine and an HHMI investigator. Elledge’s wife, geneticist Mitzi Kuro-da—also an HHMI investigator—has her lab in the same building. The couple moved to Boston in 2003 from Houston, where Elledge had done research at Baylor College of Medicine for 14 years. In addition to his Harvard appointment, he is affiliated with Boston’s Brigham and Women’s Hospital and the HMS-Partners Health Care Center for Genetics and Genomics.

Throughout his career, a pair of strong interests has shaped Elledge’s research. One of them is to uncover the complex interplay of genes and cellular signals that regulates the division and growth of cells. “I’ve always been interested in how cells decide whether to divide or not,” he remarks. It’s a fundamental life process and a front-burner issue for human health. Cancer is a disease of abnormal, unfettered cell division, and understanding cell-cycle regulation in detail could open new therapeutic avenues for blocking cancerous growth.

He has identified a number of the key players and events that normally keep cell division under control. One of the most important is the gene
Stephen Elledge believes in the idea that technological advancements have a big impact on science. “It’s like oiling the science machinery to make it go faster,” he says.

Cdk2, which he isolated in 1991. He then discovered that it governs a critical step in the cell cycle often disrupted in cancer cases. A second key discovery was of CKIs (cyclin kinase inhibitors), such as p21 and the Beckwith-Weidemann syndrome gene p57, made with his colleague Wade Harper, also of Harvard. CKIs are the brakes on the cell cycle that prevent cell proliferation.

About 8 years ago came another landmark discovery: a short protein segment called the F-box motif, which Elledge showed has been conserved throughout evolution and therefore must be critical to cells. In fact, it plays an important role in destroying proteins that otherwise would pile up and halt cell division.

A second focus of Elledge’s work is the mechanism that enables cells to detect damage, or mutations, in their own DNA and then to take steps to prevent further trouble. Mutations, caused by exposure to DNA-damaging environmental agents or mistakes in copying DNA during replication, may be inherited and thus be harmful to many generations of organisms if not eliminated or repaired as they arise. When cells sense DNA damage, they can push an “emergency-stop button” that keeps them from dividing and passing on the genetic misinformation. They can also summon repair molecules to attempt to fix the problem. But the repair may be incomplete or inadequate, and the cell becomes more genetically unstable and susceptible to later damage, which can lead to uncontrolled growth and cancer. Elledge has made many contributions to unraveling the complexities of these crucial processes. (See related article on page 50.)

**BEAUTIFUL DESIGN**

Elledge’s penchant for invention surfaced early in his career, when he was a graduate student and postdoc, and has never waned. Over the years, he has devised, for example, new cloning methods, a technique to express copies of human proteins in yeast, and a system for rapidly making recombinant DNA constructs for genetically manipulating mice.

Elledge’s most recent technological tour de force, in collaboration with Hannon, is a set of tools that, for the first time, allows researchers to “silence,” or turn off, specific genes in mammalian cells—including human cells—one at a time. Similar techniques, known as RNA interference (RNAi), for silencing genes in lower organisms and thereby inferring their function, previously had failed when applied to mammals.

Elledge and Hannon created a library of retroviruses, each carrying a distinctive bit of RNA called a short hairpin RNA (shRNA) that can be introduced efficiently into all mammalian cells. Because each shRNA is designed to seek out and switch off a particular gene in a cell, this specificity not only promises genome-wide searches for gene function but also improves future prospects for turning off harmful genes in human infections, genetic diseases, and other disorders.

The two scientists’ breakthrough is being widely acclaimed by the biological research community, which stands to benefit in many ways. The shRNA library provides a long-needed simpler, faster, and cheaper alternative to breeding knockout mice, in which specific genes have been turned off through genetic manipulation of the embryo. “It takes as much as 12 to 18 months to generate a mouse with one knockout,” says Raju Kucherlapati, scientific director of the HMS-Partners Health Care Center and one of the inventors of the mouse-knockout technique. The Elledge-Hannon library method, he says, “has just phenomenal applications in virtually all areas of biology to discover gene functions.”

The initial library contains enough viruses to turn off about 10,000 of the approximately 25,000 genes in the human genome; next-generation libraries now cover all known genes.

Besides silencing genes and inferring their functions by how the cell behaves differently, the new libraries offer the reverse capability. That is, if researchers are interested in a particular cell process—say, how the cell resists the effect of a drug or grows under certain conditions—but don’t know which genes are responsible, the library can help pinpoint them. The shRNAs, in effect, can “interrogate” every gene in the genome to learn whether it is involved.

Colleagues note that the library, described in the March 25, 2004, issue of *Nature*, contains a couple of particularly ingenious features. Elledge and Hannon found a way to insert a small DNA sequence into each RNA-carrying retrovirus that gives the virus a distinctive tag, like a bar code. So when an experiment links a certain gene from the library to some cell process of interest, it’s easy to identify the gene by its tag.

In another clever maneuver, Elledge devised a way to shuttle any shRNA construct, which fits into its retroviruses like a tape cassette, into any other retrovirus. “This library of easily transferable shRNAs is a beautifully designed resource,” said a comment accompanying the *Nature* paper.

Today Elledge’s lab is a beehive of activity as he and his colleagues begin applying the breakthrough technology to a variety of problems. “I sit around making lists of all the projects I’d like to work on,” he says. And although he hadn’t previously focused on human disease generally, or cancer in particular, Elledge finds himself going in that direction. “I don’t know if it’s as you get older you’re more aware of mortality,” he says, “or if it’s just that once the RNAi revolution occurred I realized its most important applications would be to basic problems of human health.”

In any case, Elledge is in a big-picture frame of mind these days. “It’s the marriage of the human genome project with RNAi technology that allows you to think in this global way,” he says. “You begin thinking about finding the important genes for different biological processes, and you’re only limited by your creativity.”

—RICHARD SALTUS
Understanding Listeria

This pathogen endangers pregnant women, newborns, and those with weak immune systems. No wonder the U.S. Food and Drug Administration calls it a “bad bug.”

Butique fromage shops in Paris elevate raw-milk cheese to an art form, but pregnant women are advised to avoid those delights. Listeria monocytogenes is the reason—it’s a pathogen found in uncooked meat, raw vegetables, and foods like cold cuts and soft cheeses that are tainted after processing. Antibiotics clear up the infection, but because a pregnant mother’s symptoms can be mild, the pathogen often goes undetected, killing the developing fetus or leading to meningitis or other serious brain diseases.

What makes Listeria particularly troublesome is its unique ability to penetrate three barriers in humans: the intestinal barrier, the placental barrier, and the blood-brain barrier.

“When we started in 1986 we knew very little,” says HHMI international research scholar Pascale Cossart, who is also head of the Unit of Bacteria-Cell Interactions, INSERM U604, at the Pasteur Institute in Paris. “We sequenced and published the first gene of Listeria, and now the whole genome is finished.”

With colleague Philippe Glaser, Cossart headed a consortium of 10 laboratories that published the full genome sequences of L. monocytogenes and its non-disease-causing relative Listeria innocua in 2001. The information generated by that project is now helping to uncover new virulence factors. But it was Cossart’s experiments in classical genetics that paved the way to begin to uncover the molecular tricks by which L. monocytogenes crosses the three barriers and wreaks its destruction.

In a finding published earlier this year in the Proceedings of the National Academy of Sciences, Cossart and Marc Lecuit—the doctor on the Pasteur team—describe how the bacterium crosses the placenta to infect the fetus. It’s the first time anyone has explained how a pathogen crosses the so-called maternofetal barrier, and their work could now provide clues for other groups studying toxoplasmosis or cytomegalovirus, which share that capacity and hence also pose a threat to pregnant women and their babies.

The mechanism they describe relies on a single protein, internalin, which exists on the surface of the bacterium and interacts with a receptor on the surface of mammalian cells called E-cadherin. The “E” stands for epithelial, because E-cadherin is the molecule that causes cells to stick together in the epithelia of various tissues of the body, such as the intestine, liver, and brain. Cossart had earlier identified internalin by focusing on those L. monocytogenes mutants unable to enter cultured human epithelial cells. She found that only those lacking internalin could not do so.

“The surprise came when we started to look at the interaction between internalin and E-cadherin in different cells,” she says. “We can precisely study this interaction by using latex beads coated with internalin.” Whereas mouse cells seemed to be resistant to “infection” by internalin-bearing beads, human cells were not. What was going on? Mouse E-cadherin and human E-cadherin were previously thought to be identical and function similarly, but they are only 86 percent similar. One amino acid in particular, the 16th in the sequence, turned out to be critical for the ability to bind internalin, and hence to the difference in infectivity. “If you mutate that amino acid in humans, you lose that interaction; mutate it in mice, and you gain it,” explains Cossart.

Together, these results explain why the wild-type bacteria and the internalin mutants were equally bad at infecting the mouse. And they paved the way for Cossart’s group, in collaboration with Charles Babinet, also at the Pasteur Institute, to create a transgenic mouse model for listeriosis—a mouse that expressed human E-cadherin in its intestine but nowhere else. Then they were able to explore how the binding of
For the first time, researchers explained how a pathogen crosses the maternofetal barrier. The work could advance the study of other threats to pregnant women.

Working with placental tissue extracts from women who had been infected with *L. monocytogenes*, Lecuit (in collaboration with D. Michael Nelson at the Washington University School of Medicine in St. Louis) noticed that the bacteria accumulated in a layer of cells called syncytiotrophoblasts, which lie on the placental surface that bathes in the mother’s blood. These cells also express E-cadherin. When he took extracts of the same cells from the placental tissue of healthy women and exposed them in a dish to *L. monocytogenes* that either did or did not express internalin, he found that only those bacteria expressing internalin were able to infect the cells efficiently.

“With one single protein interaction, a pathogen can specifically target and cross two very different barriers,” says Cossart, who is now busy creating a population of transgenic mice that express human E-cadherin throughout their bodies, to see if the findings hold in vivo in the mouse model. And because E-cadherin is also known to be present at places in the blood-brain barrier, she has a hunch that a similar interaction may be happening there.

One final piece fits into the *Listeria* jigsaw. Cossart noticed that internalin can mutate, and that the presence of this mutation determined the effectiveness of the internalin/E-cadherin interaction. If the internalin gene mutates, the protein is truncated and is secreted by the bacterium rather than remaining attached to it. In other words, it loses its crucial binding capacity and the bacterium becomes unable to infect human cells.

This finding led Lecuit and Cossart, in collaboration with the Pasteur Institute’s Christine Jacquet, to survey the strains of *L. monocytogenes* isolated from infected women and from food in a single year in France, which they published this year in the *Journal of Infectious Diseases*. “We found that 100 percent of the strains responsible for fetal placental infections had full-length internalin, and 35 percent of the food strains had the truncated internalin which was secreted,” says Cossart. “That means there are many, many strains in food which will never infect humans.”

Mothers-to-be are not the only ones vulnerable to listeriosis, but their risk is 20 times higher than that of other healthy adults because of the natural suppression of their immune systems that allows their bodies to tolerate the growing fetus. Because their symptoms are so hard to detect, the emphasis, from a public health standpoint, has been firmly on prevention. Cossart thinks the findings of their survey could make the job of prevention a little easier by providing the basis of a test for distinguishing safe foods from dangerous ones.

—LAURA SPINNEY

internalin to E-cadherin triggers the swallowing up of the bacterium by enterocytes, the host cells that represent the first step in spreading the bacterium through the cells of the intestinal epithelium.

Cossart was delighted when a group at the German Research Center for Biotechnology in Braunschweig, Germany, led by Dirk W. Heinz, published the crystal structure of the internalin/human E-cadherin complex in *Cell* in 2002. “It clearly showed how amino acid 16 sits in a loop and interacts with one of the repeated units in internalin,” she says.

She and others also described most of the other steps in this infection cycle, including the mechanism by which *Listeria* and other pathogens form actin tails to propel them through the host cytoplasm. Her discovery of the ActA protein was a breakthrough in that respect. ActA is a surface protein of *L. monocytogenes* that recruits the Arp2/3 protein complex from the host cell to induce actin polymerization. When she looked at another bacterium, *Rickettsia conorii*—which is spread by tick bites and causes Mediterranean spotted fever—she found to her surprise that it also exploits Arp2/3. But it does so by using its own surface protein, RickA (a finding also published this year by Cossart, Edith Gouin, and colleagues, in *Nature*). Cossart thinks these structural differences between the two bacteria—the tails of *Listeria* consist of short, highly branched actin filaments, while those of *Rickettsia* are unbranched, more flowing, and hair-like—must spring from different properties of the bacterial proteins.

While she worked on the details, her mind remained on the bigger picture. Lecuit and Cossart wondered if the interaction between internalin and E-cadherin could also be responsible for the bacterium breaching other epithelia in the body—the maternofetal barrier, for instance. The answer turned out to be yes.
Bow WOW!

Unleashing the dog genome, scientists find all kinds of surprises.

It may be difficult to see the wolf lurking inside the diminutive Pekingese or Shih Tzu, but as any dog trainer will tell you, acknowledging the dog’s inner wolf is key to understanding it. Another clue might come from genetics, as scientists recently discovered.

HHMI investigator Leonid Kruglyak, Elaine A. Ostrander, and their colleagues at the Seattle-based Fred Hutchinson Cancer Research Center and the University of Washington have now documented the origins, both ancient and relatively recent, of purebred dogs. They analyzed the genetic variations in 96 short chromosomal sequences, called microsatellite loci, among 85 dog breeds and the gray wolf, widely believed to be the ancestor of domestic dogs.

DNA revealed a clear distinction between two subsets: 14 dog breeds of clearly ancient origin, and the rest of the breeds studied, which have more modern European origins. The ancient breeds showed a genetic pattern closely matching that of the wolf and included dogs of Asian (Shar-Pei, Akita, and Chow Chow), central African (Basenji), and Arctic (Siberian Husky) origin. The findings support a hypothesis put forth by some scientists that dogs were first domesticated from wolves in Asia and then migrated with humans to Africa and the Arctic, and even across the Bering Strait into North America.

The researchers also discovered that strict mating restrictions have created distinctive genetic patterns, making it possible to determine a dog’s breed from its DNA with greater than 99 percent accuracy. They reported their findings in the May 21, 2004, issue of Science.

The study’s sometimes surprising results, including the ancient lineage of the lapdogs Pekingese and Shih Tzu and the apparently more recent origin of some supposedly ancient breeds, have raised hackles among dog aficionados.

The study showed that breeds such as the Norwegian Elkhound and the Pharaoh Hound, which tradition holds have ancient origins, appear to be more recent. However, Kruglyak points out that the data can have two interpretations.

“What is equally consistent with the genetics,” he says, “is that there is an unbroken line from antiquity but there has been enough mixing with other breeds along the way that the ancient signature is now too faint to see.”

The analyses also showed a secondary, loose classification of the recently derived breeds into three groups: Mastiff-like dogs such as Bulldogs and Boxers; herding dogs such as Collies, Belgian Tervurens, and Shetland Sheepdogs; and hunting dogs such as terriers, spaniels, pointers, and retrievers.

Predictably, members of the American Kennel Club (AKC) and specific-breed clubs showed strong interest in the study, often donating their dogs’ DNA at shows where the researchers had set up booths.

Others are now trying to get into the act. “Since the study was published we’ve heard from a number of breeds that weren’t included,” says Ostrander, who spearheaded the Dog Genome Project and volunteered her own dog, a Border Collie named Tess, for the study. “We are now adding Dalmatians, along with
Irish Terriers, which is an important breed as they were a founder that contributed to multiple other breeds in existence today. The real payoff of the research, say Kruglyak and Ostrander, is not which breeds have the most ancient lineage but how it will extend the mutually beneficial bond that has so long existed between humans and dogs. “They are arguably our closest animal companions,” says Kruglyak. “Because people take good care of their dogs’ health, they are probably the second most-surveyed species (next to us).

As a result, there is a long list of several hundred diseases with a genetic basis that have been described in dogs. For example, cancer in dogs often manifests itself in almost exactly the same way as it does in humans, and the same therapeutics are used in the two species.”

Before the gene map was even completed, Ostrander and her colleagues had identified a gene responsible for a hereditary form of kidney cancer in [German] Shepherds by piecing together a pedigree of 20 generations in Norway and the United States. We never would have been able to do that for humans. For one thing, human families are too small and they have too few affected individuals in them. Dogs can have litters of five or more puppies and they have several litters, so we could have 10 or 20 offspring within a family to sample.”

Two veterinarians at Cornell University, Gregory M. Acland and Gustavo D. Aguirre, used earlier-identified segments of the dog gene map to help locate a gene that causes a form of blindness, progressive retinal atrophy, in Irish Setters. In the 10 years since a test for the defective gene became available, the number of Irish Setter pups born with the disease has fallen from 7 percent to nearly zero. Acland and Aguirre are now searching for genes that cause blindness in breeds such as Poodles and Cocker Spaniels.

Kruglyak says getting involved in the Dog Genome Project was a no-brainer: “The question of how you go within the last 100,000 years from the wolf to a population with such an incredible diversity of forms and behaviors and a huge range of morphological features is a geneticist’s dream.”

Now that the initial map is complete, the scientists are eager not only to add dog breeds but also to address some fundamental questions—such as what makes a dog a dog and not a wolf. To help answer those questions, Kruglyak and Ostrander have teamed with Robert Wayne, an evolutionary geneticist at the University of California, Los Angeles. Wayne and his colleagues study the genetics of canids, the broad group of animals that includes coyotes, jackals, and others, as well as wolves and dogs. It was Wayne who showed genetically that dogs probably followed humans into North America rather than having been redomesticated in the New World.

Basically, the team hopes that adding genetic information about other canids to the dog-genome data will reveal how wolves changed to become our “best friends.”

“The excitement right now in the Human Genome Project is to compare it to the chimp genome so we can understand what genes are unique, what makes us human,” says Wayne. “In the same way, we can think about dogs and how some genes have changed as wolves became man’s best friends. We can ask: Why the explosion in morphology? We can find genes that influence behavior, in addition to conformation, and find out what has changed in dogs to allow them to live with us.”

—KARYN HEDE
As you read these words, your brain is changing. Through a maze of pathways far more complex than any computer network, your brain is distributing copies of what you are seeing to more than a dozen separate processing centers, where words are being recognized, analyzed, and understood. Simultaneously, the neurons in your brain are undergoing subtle changes where they connect with each other, so that if you read these words again—whether a minute or a year from now—you’re likely to remember at least something of what they said.

Virtually any cognitive task requires that the brain function on many different levels, from the individual molecules in cell membranes to far-flung neural circuits that encompass many cerebral regions. But at the center of the action is the “synapse”—the nar-
row cleft between neurons where one brain cell sends signals to another. This neural structure is essential to perception, behavior, memory, and thinking. “Without synapses, the brain would be just a collection of isolated neurons,” says New York University neuroscientist Joseph E. LeDoux, author of Synaptic Self: How Our Brains Become Who We Are. “Synapses are the gateway for information flowing into the brain, the means of communication within the brain, and the means by which behavior emerges from the brain.”

The word “synapse” is more than a century old, having been coined in 1897 by the English physiologist Sir Charles S. Sherrington. (Actually, Sherrington wanted to call the junction between nerve cells a “syndesm,” but a classicist acquaintance persuaded him to combine the Greek syn, meaning “together,” with haptein, for “to clasp.”)

Through his study of reflexes, Sherrington helped demonstrate that the flow of information through synapses is a one-way affair: Nerve impulses pass from the axon of one neuron through a synapse to the dendrite of another neuron. But not until the 1950s did neuroscientists prove that synaptic transmission occurs through chemicals called neurotransmitters that are released by axons and detected by dendrites. As recently as the early 1980s, scientists knew very little about the detailed molecular mechanisms at the heart of synaptic processes.

Today, the synapse is rapidly yielding up its secrets. Researchers are identifying the chemical signposts that guide growing neurons to establish connections with each other. They are figuring out the molecular mechanisms by which brain cells change in response to experience. They are beginning to understand what goes wrong in diseases like Alzheimer’s and Parkinson’s and how the neural ravages of age might be countered. “Our rate of progress is exponentially fast,” says Morgan Sheng, an HHMI investigator at the Massachusetts Institute of Technology (MIT). “Ten years ago we only knew what some of the key components of synapses are. Now we know what most of them are.”

Rapid progress in turn has created unprecedented optimism. Researchers are confident that if they can figure out how synapses work, they can begin to piece together the molecular mechanisms of the mind. “I don’t pretend for one minute that a comprehensive understanding of how a synapse works will tell you about higher cognitive functions,” says Sheng. “On the other hand, the great strength of molecular studies is that they provide you with tools that you can use to go into the brain and ask systems-level questions.”

Many of these “systems-level questions” involve memory and the ability to learn new skills and information. But the ultimate prize is much greater. By understanding how synapses function and change over time, neuroscientists hope to shed light on the processes of reasoning, emotion, and maybe even consciousness. “A lot of us in the field think we’ve come to a real turning point,” says HHMI investigator Richard L. Huganir at the Johns Hopkins University School of Medicine, who studies the function of particular proteins in synapses. “We’re trying to go from the molecular details all the way up to the global effects of behavior. That’s an ambitious goal, but I think we’re getting there.”

OUT OF SYNC, LOSE THE LINK

Three floors above Sheng’s lab, postdoc Robert A. Crozier—like Sheng, a researcher at the Picower Center for Learning and Memory at MIT—is about to begin his first experiment of the day. Watching a television monitor mounted beside his elaborately rigged microscope, he searches for just the right cell in a slice of rat visual cortex, cut from a brain earlier that morning.

“You want to avoid the cells that have a sort of crispy look,” he says. “Those are cells that are dying. A healthy cell has a soft, fluffy appearance.” Choosing a viable candidate, he pops the pressure tube of a micropipette into his mouth and, spinning the fine-movement manipulators on either side of the microscope, positions the tip of the pipette against the wall of the cell. “This is the part you don’t see in the scientific papers,” Crozier comments. With a quick puff on the mouthpiece, the micropipette punctures the cell membrane, infusing the cell with a chemical that blocks the action of an enzyme known as protein kinase A (PKA). “That looks good,” he says. “At the beginning of the day, this is fun. If I’ve been doing it for a few hours and haven’t gotten a good recording, my language gets more colorful.”

Electrophysiologists like Crozier have been probing the behavior of neurons for more than a century, since German physiologist Emil du Bois-Reymond began investigating what he called “animal electricity.” But today’s scientists are focused on targets that would have seemed impossibly precise to their forebears. PKA plays a pivotal role in the massively complex set of chemical reactions that occurs when a neurotransmitter crosses a synapse and is detected by a receiving dendrite. It opens additional receptors on the dendrite, helps alter the dendrite’s physical structure, and participates in the cascade of reactions that eventually leads to the synthesis of new proteins in the neuron.

Crozier’s work is part of a decades-long effort to understand what is known as “synaptic plasticity”—the changes...
In the 1980s, ignorance about synapses was so vast that it was hard to formulate meaningful questions. —MARK BEAR

that occur in synapses because of events we experience or thoughts we produce. Neuroscientists have long hypothesized that memories must be stored in the form of physical or functional changes in synapses. As LeDoux succinctly puts it, “We are our synapses.” But hard proof that such changes occur was surprisingly elusive. Not until the 1970s did researchers identify the first form of such plasticity, which they called long-term potentiation (LTP). When a rapid train of strong nerve impulses is sent down an axon, the synapses connecting that axon to the receiving dendrites of other nerve cells can be strengthened (or “potentiated”). For at least the next several hours, a nerve impulse passing through those synapses will generate a stronger response in the receiving neurons than was the case before the LTP occurred.

Crozier works with Mark F. Bear, an HHMI investigator at MIT who specializes in a complementary form of plasticity known as long-term depression (LTD). When a less rapid, weaker train of nerve impulses is sent down a neuron, the connection between that cell and receiving neurons can be weakened. “LTD was ignored for a long time,” says Bear. “But we were believers, and that conviction led us to persist in looking for a model for LTD.”

Bear’s interest in LTD dates back to his graduate school days at Brown University in the early 1980s, when he became interested in a classic experiment done two decades earlier by David H. Hubel and Torsten N. Wiesel. The two researchers sewed shut one eye of an infant kitten and showed that when the eye was reopened several weeks or months later, it did not respond to light. The kitten’s brain had rewired itself so that the eye no longer functioned.

What molecular mechanisms were responsible for such a remarkable neuronal change, Bear wondered. He started working on the problem, but in those days, he recalls, “our ignorance about the basic mechanisms of synaptic transmission in the brain was so vast that it wasn’t even possible to formulate very good questions.” Then, while doing a postdoctoral fellowship with Wolf Singer at the Max Planck Institute for Brain Research in Frankfurt, “everything changed,” he recalls. Researchers had discovered a particular kind of receptor in synapses that appeared to trigger LTP. Bear found that the recep-
tor also seemed to be playing a role in the weakening of synaptic transmission. When he blocked the action of the receptor with a chemical compound, he could dramatically reduce the loss of function when the eye of a laboratory animal was sewn shut.

As a new professor at Brown University, Bear’s first priority was to understand how this receptor was exerting its effects on vision. In 1992 he discovered, through experiments in brain slices, that appropriate stimulation of the receptor caused LTD. Perhaps LTD was contributing to the loss of vision in Hubel and Wiesel’s kittens.

As Bear’s lab at Brown grew, LTD remained a centerpiece of his research. With Brown physicist (and 1972 Nobel laureate) Leon N. Cooper, he developed a theory about how the loss of vision from one eye could produce a rewiring of the brain. They proposed that the neural reorganization resulted not from an absence of light but from a mismatch between the signals the brain was receiving from the open eye and those from the closed eye. To test the idea, Bear and his colleagues injected a long-lasting anesthetic into the eye of a kitten. When the eye was anesthetized, it sent no signals to the brain, so there was no mismatch between the incoming signals. Sure enough, when the anesthesia wore off, the anesthetized eye worked almost as well as before.

“The prevailing view used to be ‘use it or lose it,’” says Bear. But a more accurate conclusion may be “neurons that fire out of sync lose their link. It’s not the absence of activity that is important to induce blindness, but the presence of activity that is not useful.”

In 2003, Bear moved from Brown to MIT, where he and his coworkers succeeded in revealing a mechanism at least partly responsible for the loss of vision. When a neuron receives mismatched signals, synapses lose receptors just as they do during LTD. If the loss of receptors is sufficiently prolonged, Bear suspects, the synapse eventually will disappear.

**UNEXPECTED CONSEQUENCES**

These findings could have important implications for treating vision problems. For example, when children have visual imbalances such as the condition known as lazy eye, ophthalmologists typically patch the strong eye to help the weak eye get better. “But it’s usually a zero-sum game, because the strong eye gets weaker while the weak eye gets stronger,” says Bear. “There

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**At Columbia, Eric Kandel (l) and Steven Siegelbaum study diseases, neural development, behavior, and learning.**

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The challenge [now] is to synthesize reductionist and holistic approaches to create a unified view of mental processes. —ERIC KANDEL

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Prions on My Mind

In 2001, Eric R. Kandel walked into Susan L. Lindquist’s office and asked her a question out of left field. “Do you think prions could be involved in storing memory?”

Normally considered deformed renegades that mercilessly rob the brain of functions, prions might seem like unlikely candidates for safeguarding memories. “I nearly jumped out of my chair because I was speculating the same thing,” recalls Lindquist, a noted prion researcher who was then an HHMI investigator at the University of Chicago and has just stepped down after 3 years as director of the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts.

Kandel, an HHMI investigator at Columbia University College of Physicians and Surgeons and a 2000 Nobel laureate, told her about a puzzling prion-like property in a protein that seemed to play an essential role in long-term memory storage. Kandel’s previous research had described a memory-forming cascade in motion by CREB (cAMP response element binding protein), CREB functions as a molecular switch that turns short-term memory to long-term memory. A learning experience flips that switch, allowing proteins and untranslated messenger RNAs (mRNAs) to be dispatched throughout the cell body. However, he found that many mRNAs remain dormant until another learning experience acts as an alarm clock, arousing the mRNAs to build the proteins that strengthen just those synapses where long-term memory forms.

Kandel was intent on discovering what molecular mechanism acted as the alarm clock for dormant mRNAs. He further wanted to understand why that mechanism was set in motion at just those synapses where a specific, enduring memory forms. Like many neuroscientists, his lab used the foot-long snail Aplysia as a model organism because its exceptionally large neurons are relatively easy to study and manipulate.

Kausik Si, a postdoc in his laboratory, had pinpointed CPEB (cytoplasmic polyadenylation element binding protein) as the selective trigger that woke up the dormant mRNAs at those pre-loaded synapses. Kandel’s team wanted to know how CPEB maintains the continuing protein synthesis that stores a memory long after the learning experience has passed.

While investigating that question, Si noticed that the CPEB found in neurons differs from that of other cell types. Normally, CPEB has phosphorylation sites that activate mRNAs, which increases their levels of protein expression. In snails as well as in flies and mammals, however, the CPEB in neurons does not have those sites. Instead, it has a strange functional domain that is unusually rich in the amino acid glutamine and lacks any secondary structure.

Si knew of only one other protein type with a similar domain—prions. Prions cause a protein to alter its shape and then enforce a similar, self-perpetuating conformation on similar proteins. Si and Kandel wondered whether prion-like, self-perpetuating qualities in CPEB could be what keep a memory vivid over time. That’s why Kandel popped his question to Lindquist and later collaborated with her to pursue it.

Lindquist explains that the “funky” prion domains attach to one another, forming immobile, insoluble clumps. The rest of the proteins dangle along the side of the clump, like charms on a bracelet. This state can cause them to lose their beneficial function and gain a toxic function that leads to neurological disorders such as mad cow disease.

However, Lindquist had shown that the dangle sections of yeast prions can remain functional—and behave beneficially—in cultures. Theoretically, she reasoned, prion qualities could enhance a protein’s function if, for instance, it needs to be anchored to a site and work cooperatively to sustain a process—such as storing a memory at a synapse.

To test whether the Aplysia neuronal CPEB does act like a prion, Si attached its weird domain to a yeast protein. Using a color assay that Lindquist had developed, he watched the protein morph into a self-perpetuating prion before his very eyes. Then Si devised an assay for the whole Aplysia CPEB when expressed in yeast. It, too, appeared to behave like a prion.

Moreover, while Aplysia’s CPEB was very active in the prion clusters, it barely functioned in the nonprion state. Perhaps, the researchers hypothesized, CPEB needs to be in a prion-like state to sustain the perpetual protein synthesis necessary for storing memories. And perhaps that is why certain events—like repeating the times tables, practicing piano, and crashing a car—can become so unforgettable. They trigger enough CPEB production so that some copies of the proteins convert into prions to perpetuate themselves at the synapses where the long-term memory forms.

Kandel, Lindquist, and Si proposed this model of memory storage in the December 26, 2003, issue of Cell, accompanied by a Kandel and Si article on the role of CPEB in Aplysia long-term memory storage. “It’s a very nice finding,” Kandel says. “We’re now going back to the Aplysia nervous system, where we’ve shown that this protein is required for the maintenance of long-term memory. We want to find out if the prion domain causes self-perpetuation in neurons as it does in yeast, and, if so, if it is the mechanism that maintains memory in Aplysia neurons.”

Lindquist and Kandel speculate further that a similar prion mechanism might be involved in other contexts, such as developmental processes and cancers, where cells maintain a continuing function. This research therefore might lead to improved treatments for a range of disorders.
long and spindly, not short and solid as they are in unaffected individuals.

Bear enthusiastically accepted Warren’s offer, beginning a collaboration that has produced major advances in the understanding of fragile X syndrome. Bear’s lab has been able to show that the loss of fragile X protein essentially results in runaway LTD. Further, the receptors linked to this protein take part in a number of other neurological and metabolic processes that are strikingly askew in fragile X patients, who often have epilepsy, extreme anxiety, obsessive-compulsive disorder, and intestinal problems. “It was astonishing how far we could go in accounting for the symptoms of fragile X by assuming exaggerated signaling of this receptor,” says Bear. He and his collaborators have begun to explore whether blocking the receptor might allay some of the symptoms of fragile X syndrome, although because of the key role of the receptor in the nervous system, this approach warrants caution. Still, when mice prone to convulsions—a common feature of fragile X syndrome—are treated with blockers of the receptor, their condition is substantially improved. “Maybe if we intervene early enough we even could prevent some of the symptoms from occurring in the first place,” says Bear.

Bear had no idea, when he began his investigations of vision, that someday he would confront one of the most tragic of human genetic malfunctions. “It’s exciting and gratifying for me to see that the study of LTD may have relevance to diseases of the nervous system,” he says. “This is the best example in my work of how basic research can lead in unanticipated directions.”

LIKE CHRISTMAS LIGHTS

In his laboratory at the California Institute of Technology (Caltech), just down Ventura Freeway from the huge movie studios of Burbank and Pasadena, postdoc Gentry N. Patrick is also making movies—but his films chronicle the changes that go on every second inside our heads. Every two minutes, the half-million-dollar confocal microscope at Patrick’s side takes an image of the nerve cells growing in a dish mounted on the microscope’s stage. When Patrick combines the images into a time-lapse sequence, the cells’ dendrites glow and twitch like a string of Christmas tree lights in a steady breeze.

Patrick and his faculty adviser, HHMI investigator Erin M. Schuman, are probing one of the enduring mysteries of synaptic plasticity. How are the short-term impressions that fly through our minds every waking moment (the words of this sentence, the temperature in the room, a colleague’s voice) converted into long-term memories that we can recall days, months, or even years later? Many experiments have shown that the formation of long-term memories requires the synthesis of proteins in neurons, but the details of this process have remained largely unknown. Neuroscientists traditionally have held that protein synthesis occurs in the cell bodies of neurons and not in the dendrites or axons. But if long-term memories are encoded by changes in individual synapses, how do the necessary proteins get from the cell body to the particular synapses where they are needed?

Schuman and her colleagues at Caltech have been studying an iconoclastic idea: Maybe synapses change themselves in part by synthesizing proteins locally rather than waiting for them to arrive from the cell body. To investigate this hypothesis, they infect nerve cells grown in culture with a virus containing a bioengineered fluorescent molecule. Then they watch as the cells are stimulated with a growth factor or other compound. Wherever the nerve cell is actively making proteins (or, in a separate set of experiments, degrading proteins), the fluorescent molecule glows a bright green. “What we do is look at the essentials of how synapses work and change,” says Schuman. “We start with intriguing ideas and then follow them.”

Schuman says she “stumbled into” the study of local protein synthesis. As a new professor at Caltech in the mid-1990s, she and a colleague, Hyejin Kang, were studying a particular form of LTP in brain slices when, in one set of experiments that sought to block the potentiation with a chemical compound, they noticed that the blocking mechanism seemed to be occurring in the dendrites rather than in the cell body.

“We got completely captivated by the notion of local synthesis of proteins,” she says. “If the function of synapses is controlled by modification of proteins, then a way to change the protein complement of a synapse could be to construct or degrade proteins at the synapse.”

Schuman’s fluorescent images have provided the first definitive proof that protein synthesis occurs in dendrites.
transmitters, the dye is released too. The team has found that particular kinds of stimuli cause presynaptic neurons to undergo a long-term change that results in the release of greater amounts of dye, and presumably of the accompanying neurotransmitter. The researchers have even discovered a form of presynaptic LTD in which activation of receptors on the surface of the receiving neuron leads to a feedback signal that reduces release of neurotransmitter from the transmitting neuron.

That plasticity should occur in the presynaptic as well as postsynaptic neuron is not surprising, says Siegelbaum. As neuroscientists learn more about the function of synapses, they are recognizing more and more kinds of plasticity—some localized to particular parts of the brain, and some that occur throughout. “If you read about the brain in a textbook, it seems static,” says Siegelbaum. “But it’s actually a very dynamic thing.”

Understanding that dynamism at the molecular level is the most important goal in the neurosciences today, according to Thomas C. Südhof, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas who studies the generation of synapses and the release of neurotransmitters. It’s also a daunting goal—more than 1,000 proteins function in presynaptic synapses, typically as part of complex networks in which any given protein can serve more than one function.

“Understanding these protein networks and overlapping functions will be a major challenge,” he says. “The key, absolutely essential component, I believe, is to integrate different disciplines—physiology, genetics, structural biology, and biochemistry.”

In diseases like Alzheimer’s, the synapse itself may be the problem rather than a manifestation of a problem elsewhere. —ERIN SCHUMAN
responding to the cut whiskers, the synapses soon begin to respond more strongly to the remaining whiskers. This occurs not through the expansion of dendrites and axons, Svoboda explains, but through the growth of new spines between established dendrites and axons. The same thing probably happens in humans when we learn a new skill, like typing or playing the piano: The area of our brain responsible for our less-used fingers gradually expands as new spines link axons and dendrites that previously had few or no connections.

“Adult plasticity may mostly involve these local changes,” says Svoboda. “That could be the fundamental difference between the developing and the adult brain.”

Svoboda’s work has generated great excitement among neuroscientists because it addresses one of the central problems in the field: How do the actions of individual molecules and synapses contribute to changes in behavior? According to Susumu Tonegawa, an HHMI investigator at MIT (and director of the Picower Center for Learning and Memory there), “We are trying to understand not just the molecular and biochemical events taking place in the brain. We are trying to identify the neuronal circuitry and mechanisms underlying behavior and cognition. To do that, we need experiments that link processes at different levels of complexity.”

Tonegawa has been instrumental in developing the most widely used technique for exploring these links. During the 1970s and 1980s he was an immunologist working on the generation of antibody diversity, research for which he received the 1987 Nobel Prize in Physiology or Medicine. In the early 1990s he decided to switch fields and become a neuroscientist. In immunology, he often had studied genetically engineered knockout mice that lacked the gene for a particular protein. As a neuroscientist, he began to explore the effects of missing or altered genes on animal behavior.

A key challenge was to make the knockout technology more specific. “The proportion of genes that are expressed in the brain is huge compared to other biological systems—more than 50 percent,” he says. “So we had to add an additional technology where you can target gene manipulations to a specific type of cell or the specific phase of an animal’s development.” Through a manipulation of the introduced gene’s expression, Tonegawa and his colleagues developed a way to turn off a gene just in the forebrain, for example, and only in the adult animal. They then set about exploring the effects of these very selective genetic lesions.

One of the most provocative molecular pathways they have investigated involves an enzyme known as calcineurin—a regulatory protein that is active both in the immune system and in synapses, where it contributes to the growth of spines and to changes in synaptic strength. When the gene for calcineurin was knocked out in mice, they exhibited severe deficits in short-term memory. In exploring a maze for food, for example, the mutant mice would forget they had already fetched the food in a particular passage just a moment before and would explore it again. Further studies revealed other kinds of deficits. The animals were socially withdrawn; instead of sleeping together, as mice usually do, they would sleep in opposite corners.

Tonegawa and his colleagues believe these deficits bear a striking resem-
A good portion of the front of your brain appears to be devoted to networks that control other networks. —RANDY BUCKNER

blance to schizophrenia, a devastating disease that attacks approximately 1 in every 100 humans. People with schizophrenia also have problems with short-term memory and tend to withdraw socially. Even more suggestive, genetic studies of families with an especially high incidence of schizophrenia have revealed a link between the disease and the gene on chromosome 8 that encodes calcineurin.

Tonegawa and several colleagues are preparing to set up a small biotechnology company to begin looking for compounds that can alter the calcineurin pathway and possibly ameliorate the symptoms of schizophrenia. “The antipsychotic drugs that are on the market now mostly target dopamine receptors, and those drugs have serious limitations,” he says. “We are trying to intervene with a totally different target.”

A WINDOW ON THE MIND

“You’ll see a word appear in the middle of the screen,” postdoc Denise Head tells a visiting journalist who is snugly ensconced inside the magnetic resonance imaging (MRI) machine. “If it’s an abstract word, push the button on the left-hand side. If it’s a concrete word, something you can see or touch, push the button on the right side.”

In the adjacent control room, a computer monitor displays images of 12 virtual slices through the research subject’s brain. As the subject sorts the words into categories, the regions of his brain that are doing the work light up with the effort. “This is one of the best tests of memory we’ve found,” says Randy L. Buckner, an HHMI investigator at Washington University in St. Louis and leader of the experiment Head is conducting. “Putting a word in a category forces you to think about that word in the context of other words you know.”

Buckner and his colleagues, using a technique known as functional MRI, or fMRI, are watching the brains of humans as they think. When part of the brain is activated, oxygenated blood rushes to supply the firing neurons with energy. The scanner detects these changes in blood flow, opening a window on the mind.

Buckner has been investigating perception, brain changes during aging, and memory in particular. He and his coworkers have found, for example, that deciding whether a word is abstract or concrete typically activates specific areas on the left side of the brain that are associated with language, as would be expected with this task. But it also activates areas in the front of the brain that appear to control the process of making decisions about the nature of the word. Furthermore, some frontal regions are activated while comparing words, and a partially overlapping set of frontal regions is activated when comparing new faces with known faces. According to Buckner, “a good portion of the front of your brain appears to be devoted to networks that control other networks.”

Buckner and his colleagues are investigating this question in part by examining brain function in hundreds of individuals, including people with forms of dementia such as Alzheimer’s disease. By comparing brain function with memory capacity and the strategies people use to remember, the researchers hope to identify positive and negative mental changes that occur over time. This epidemiological approach may also suggest ways of intervening to stem memory losses. For example, Buckner and his coworkers have found in Alzheimer’s patients that certain kinds of memory processes, such as those involved in learning new skills, remain surprisingly intact.

More broadly, Buckner sees his images of the brain at work as one part of a more comprehensive view of the brain. “We need to correlate these observations across different levels of analysis, from the structural to the functional to the behavioral,” he says. “That way, we can ask causal questions about how one level relates to the others.”

UNIFIED APPROACH

Throughout the 20th century, scientists investigating the brain took one of two broad approaches, says Eric R. Kandel, an HHMI investigator at Columbia University College of Physicians and Surgeons. The reductionist approach sought to understand the brain in terms of its constituent parts—the cells and molecules of which it is composed. The holistic approach analyzed the brain’s ultimate effects: human behaviors and thinking. Study of the synapse helps to unify these two domains. “The synapse has been a very productive target for research,” says Kandel. “We have learned a great deal about receptors, receptor insertion into membranes, neurotransmitter release, and modulation of neurotransmitter release by endogenous inputs. Although we do not as yet have a complete understanding of how synapses work and how they are regulated for plasticity,...
It’s a spring day in Oklahoma, and the redbud trees are poised to burst into clouds of magenta. Against this backdrop, dressed in jeans and a plaid shirt, Charles T. Esmon looks like he’d be right at home stringing barbed wire on a ranch. But a few minutes into conversation with this easygoing HHMI investigator, it’s clear that his home is in the lab, not on the range.

Moreover, the Illinois native says there’s nowhere else he’d rather live and work than in his adopted state, a place better known for rodeo stars than for scientific standouts. To him, the setting is not the least bit stultifying—it is, in fact, downright stimulating.

Esmon’s record stands as proof. In his 28 years at the University of Oklahoma Health Sciences Center and the Oklahoma Medical Research Foundation (OMRF), where he heads research in cardiovascular biology, Esmon has made major contributions to understanding blood clotting and associated disorders, uncovered links between the body’s natural anticoagulant system and the immune system, and laid the foundation for the first drug to effectively treat septic shock—the most common cause of death in this country’s intensive care units. In 2002, Esmon was elected to membership in the National Academy of Sciences, making him one of only four scientists with Oklahoma ties ever to be elected to that prestigious group. He’s also the only HHMI investigator in the state.

MORE CREATIVE

Being far from either coast, at an institution that’s well-equipped but not wealthy, “we’ve had to be more creative,” says Naomi L. Esmon, who has been her husband’s research partner for more than two decades. “We don’t instantly look for a way to use the high-tech stuff because we figure the Harvards and the Johns Hopkinses can afford all that equipment and they’re going to go into it full-tilt. We would rather deal with the ideas than deal with the technology, even if that sometimes means doing things the old-fashioned way.”

Such circumstances “keep Chuck thinking strangely,” says Naomi, and she means that in a good way. When he is forced to come up with innovative solutions to research problems, he’s likely to notice connections that others might miss.

“He’ll take information from biophysics—such as binding curves and enzyme kinetics—and just by listening to clinicians be able to put it all together, going from, say, crystal structures to biology and clinical applications,” says Naomi. “And it goes both ways—we’ll see something in the biology that sends us back to doing the basic enzymology.”

Brown University professor Steven Opal, a physician and infectious disease specialist who has collaborated with Esmon, says he “is one of those rare individuals who can understand patterns within complex systems and integrate information in an orderly manner. He makes sense out of the intrinsic entropy that is human biology.”

In Esmon’s early work, “thinking strangely,” along with a few fortunate twists of fate, led to the discovery that the blood vessel lining plays a key role in regulating blood clotting. At that time, in the mid-1970s, the idea that any-
thing in the lining might control what happened within the blood was unimaginable, he recalls. “It was somehow thought that a blood vessel was just a wonderful, Teflon tube—a magical, inert surface that blood just didn’t see.”

But something about that view didn’t make sense to Esmon: “Normally, blood doesn’t clot when it’s inside you. But if you shed blood into a tube, it all clots, and it clots solid. If you think about it, clotting to completion means it’s not regulated, so something is tremendously different in vivo than in vitro. The most obvious difference between what happens to blood in a tube and what happens in you is the ‘in you’ part, and the difference is the blood vessels.”

Esmon had become intrigued with protein C, a recently discovered anticoagulant that somehow prevented or limited blood clotting in the body but had no such effect in test tubes. Perhaps some process in the endothelial cells that line blood vessels spurred protein C into action, Esmon reasoned. But, although studying cultured endothelial cells might yield answers, “we didn’t have any endothelial cells and there was almost no cell culture being done here at the time,” he recalls.

Seeking a low-cost alternative, Esmon happened upon a paper by an East German researcher who collected pigs’ ears from a meatpacking house, connected tubes to blood vessels in the ears, and perfused the severed ears to study the workings of another type of activator. With no shortage of meatpacking plants in Oklahoma, Esmon got a slew of pigs’ ears and set up similar experiments to test the idea that thrombin—an enzyme that usually promotes clotting—might switch roles when bound to the endothelium and prevent clotting by activating protein C. Thrombin seemed a likely candidate, as researchers at the University of Iowa had recently found that it bound reversibly to endothelial cells.

The experiment yielded encouraging results, but it scared the daylights out of visitors, Esmon recalls. “The pigs’ ears were kept in a room with a glass door, near the entrance to the research area. So when you’d walk by, you’d see 20 pigs’ ears lined up with these things going in and coming out and dripping—it really looked like something out of a Frankenstein movie.”

**FACTOR V**

The macabre setup soon could be dismantled, however, thanks to a serendipitous event. Hidding his bets against failure in the riskier protein C work, Esmon also was studying factor V, a protein critical for blood clotting. He wanted to follow the process by which subunits of the protein separate and come back together, and he thought a CD/ORD (circular dichroism and optical rotary dispersion) machine would reveal the details. He had no such instrument, but Whyte G. Owen, a friend from graduate school days, had access to one at the University of Iowa, so Esmon scheduled a visit.

“Whyte called me just before I flew off and said the machine was working,” Esmon recalls. “By the time I landed, the machine was broken.” So much for factor V. But as luck would have it, Esmon had tucked a vial of protein C into his pocket, just in case there was time after the CD/ORD experiments to test his thrombin-activation hypothesis with a model Owen was using to study a control mechanism in the heart.

Using a technique first described in 1897, Esmon and Owen flowed protein C and thrombin, separately and together, through vessels in a beating rabbit heart. “If we incubated thrombin with protein C outside the heart, nothing happened,” says Esmon. “If we put protein C through the heart, nothing happened, and if we put thrombin through the heart, nothing happened. But if we put the combination through, we had a really, really potent anticoagulant…. We got about a 20,000-fold rate enhancement,” suggesting that something in the blood vessels made the difference. Later work by Esmon, Owen, and Naomi Esmon confirmed that thrombin binds to a specific receptor in the vessel wall, thrombomodulin, which speeds up protein C activation.

Details of the protein C pathway were falling into place, but the researchers wondered just how important the pathway was to human health. The first hints came from a baby born completely deficient in protein C. Almost immediately after birth, the infant developed large clots in the small blood vessels where protein C activation normally occurs. Doctors tried to intervene with clotting inhibitors, but nothing worked, and the baby died.

“With that patient discovery, we knew darn well that protein C was physiologically very important to humans,” says Esmon.

Another medical observation helped round out the protein C picture: Healthy people rarely develop blood clots, even after surgery, but surgical patients with cancer, atherosclerosis, diabetes, and certain other diseases must be treated aggressively to prevent life-threatening clots.

“The one thing that’s common in these conditions is some sort of underlying inflammation—an infection or disease process, for example,” notes Esmon. Perhaps clots are more common in people with these diseases because inflammatory mediators turn off the clot-preventing system, he and his colleagues speculated, and their hypothesis turned out to be true.

“Over a number of years, with a lot of collaborators,” he says, “we’ve been able to show that the system shuts down in diabetes, over atherosclerotic plaques, in vein-bypass grafts (at least, in experimental animals), and in some types of autoimmune disease.”

That body of research has influenced other scientists as well, says Opal. “Esmon’s work has made us all refocus our attention on the mechanisms of inflammation and cellular signaling induced by the coagulation system and on the role of endogenous anticoagulants as immune modulators.”

Finding a link with inflammation prompted Esmon and OMRF colleague Fletcher B. Taylor to perform a set of experiments with implications for treating septic shock, a response to massive bacterial infection that sends blood pressure plummeting and can lead to organ failure and death.

Esmon and Taylor tried infusing lab animals with small amounts of thrombin, in hopes of activating the natural anticoagulant system, and then injecting them with *Escherichia coli* bacteria in amounts that normally would produce lethal septic shock. The procedure was not without its critics: In spite of the earlier work showing how thrombin activates protein C, the idea of preventing the widespread clotting associated with septic shock by squirt- ing in an enzyme that ordinarily promotes clotting was “by no means well-accepted,” Esmon recalls. But the experiment worked. “Most of the animals lived, and most didn’t even get sick.”

Esmon and Taylor went on to show that the same effects—keeping inflammation at bay as well as preventing clotting—could be achieved by treating the animals with activated protein C extracts. This work set the stage
for Eli Lilly and Company to develop the drug Xigris, which is widely used today to treat severe sepsis.

“Now we’re interested in understanding more about how this system works,” says Esmon. Xigris is the only effective drug for treating sepsis, but it doesn’t rescue all patients—about half of those treated with the drug still die.

Typically, the drug is given for four days, after which the patient’s protein-C-activating mechanisms should be able to take over. But that doesn’t always happen. “It turns out that some patients have almost no capacity to activate endogenous protein C, while in others that system is more or less okay,” says Esmon. So he and colleagues are developing an assay for circulating activated protein C that they hope will pinpoint patients who need prolonged treatment with Xigris. They’re also trying to document the extent to which the protein C system is compromised in a wide range of inflammatory conditions, from atherosclerosis and diabetes to inflammatory bowel disease. They plan to use that information to predict which patients are at greatest risk for developing blood clots and to monitor the effectiveness of prevention and treatment efforts.

RECEPTOR’S TRICKS

The latest chapter in the protein C story revolves around a new character, the endothelial protein C receptor (EPCR), which Esmon’s lab identified and continues to study. Originally found on the surface of vascular endothelial cells, EPCR enhances protein C activation by snaring the protein and making it more accessible to the thrombin-thrombomodulin complex. But EPCR has a few other tricks in its repertoire. One of them, discovered by Esmon’s postdoctoral fellow Jun Xu, is its unusual ability to travel from the cell surface to the nucleus, toting activated protein C and altering the expression of certain genes in the process. This behavior suggests that EPCR may have a developmental function, which current experiments with mice are aimed at clarifying.

Studying the structure of EPCR has yielded still more surprises. The versatile molecule turns out to look a lot like the major histocompatibility complex class I family of molecules, which play a major role in the inflammatory response. “So it begins to look like EPCR might be involved in the immune system directly,” says Esmon.

In a project headed by Naomi Esmon, researchers are exploring the involvement of protein C and EPCR in a condition known as lupus anticoagulant/antiphospholipid antibody syndrome, which occurs in some people with autoimmune diseases such as systemic lupus erythematosus. Patients who have the syndrome produce antibodies that inhibit blood clotting in a test tube but increase the risk of blood clots in the veins, arteries, and placenta (thereby causing recurrent miscarriages). The researchers have shown that this happens because these patients produce antibodies that prevent activated protein C from doing its anticoagulant work, particularly on membranes that have been oxidized—a common occurrence in inflammation. Current work focuses on developing an assay for the inhibitory antibodies, which could help identify lupus patients at risk for developing clots and be used to monitor their treatment, and on probing the role of EPCR in the syndrome.

Earlier in life, Esmon thought about going to medical school. He elected instead to pursue research. On the whole, though, his career can be characterized as interlinking both interests. From crystal structures and mouse models to the bedside and back, the Esmons’ projects weave disparate pieces into ever-clearer pictures of complex physiological processes, just as Naomi Esmon described in explaining how her husband’s need for “thinking strangely” takes their work in so many interesting directions. With a mind that darts here and there like the bright fish he keeps in saltwater aquariums at home, Esmon never lacks inspiration. But it’s not just his brilliance that has made him successful, says Tim Mather, who worked in Esmon’s lab as a graduate student and now is a faculty member in the cardiovascular biology research program at OMRF. “He’s not only a fountain of ideas, but he’s also very generous with his ideas,” Mather says. And Esmon encourages the same attitude in his lab group by welcoming suggestions from everyone and never shooting them down. “He’s like a very indulgent older brother,” Mather observes. “He wants to see you succeed.”

As a result, there’s a spirit of openness and cooperation in the Esmon lab that surprises some postdocs who’ve worked in more cutthroat environments. “There’s just enough competition to keep things interesting, but you never feel that you have to lock up your notebooks,” says Deborah Stearns-Kurosawa, who was a postdoctoral fellow in Esmon’s lab and is now on the OMRF faculty.

For Esmon, the work is not only interesting but also immensely satisfying, he says. “If you start out doing the basic biochemistry, and you get some people’s lives saved, it’s a very good feeling.”

Naomi and Charles Esmon—partners in marriage as well as in the lab—with Shadow (l.) and Lilly.
The Case Against
Interphase, prophase, prometaphase.
*Memorizo, memorizas, memoriza.*
Metaphase, anaphase, telophase.
*Memorizamos, memorizais, memorizan.*

Biology must seem a lot like Spanish to high school student Ahava Vogelstein. She learns them both in much the same way—by rote. And the language of science can sound as foreign as any when you’re bored and the concepts seem irrelevant.

Biology invites us to explore all of life, but Ahava seldom gets to explore anything. Rarely is she stimulated to ask “What if?” and to seek the answer through experimentation. This particularly frustrates her father, a cancer researcher who believes science should be taught the way it’s conducted.

“The essence of scientific thinking is that it’s experimental,” says Bert Vogelstein, an HHMI investigator at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University. “It boils down to four simple questions: What is the question I want to answer? What experiment can I construct to answer that question? What are the experiment’s results? Do these results answer the question?”

Instead, Ahava often learns passively, memorizing phases of cell division, for instance, as if she were conjugating a Spanish verb. Such information—educators call it “content”—is fundamental, but it can seem pointless if not presented in the proper context. “Some of Ahava’s teachers have been superlative,” says her father, “but in many classes there is not enough emphasis on why we would want to know this stuff and how we have come to know it.”

**THRILL OR CHILL?** Researchers who love their work want their children to experience the thrill of science, too. Some hope their kids will pursue it as a career. They pay extra attention to their children’s science education, monitoring its progress—or lack thereof.

Parents in the profession are admittedly tough critics, but their observations carry special weight.
and not just because of their scientific knowledge. Although many researchers have never taught in a classroom, most found their inspiration there. They know how it feels when a teacher kindles a student’s interest, and they understand what’s lost when the sparks get doused instead.

All too often, science lessons extinguish children’s natural curiosity, say HHMI researchers who have witnessed this firsthand. While emphasizing with overburdened teachers and appreciating outstanding ones, they share Vogelstein’s frustration with rote instruction and a curriculum that doesn’t spur students to think experimentally.

Statistics bear out their concerns. In the Third International Mathematics and Science Study, American 12th graders ranked near the bottom in science proficiency compared with age-mates in other countries. On last year’s American College Testing (ACT) science exam, only one in four high school students proved ready for college-level biology. That’s not entirely surprising, observed Richard L. Ferguson, ACT’s chief executive officer, because fewer than two-thirds of those tested had taken more than two years of high school science, including physics.

“Far too few college-bound high school students today are taking even the basic coursework necessary to prepare for college, let alone pushing themselves by taking higher-level courses,” Ferguson said upon releasing the scores in August 2003.

In other words, far too few find science palatable, let alone irresistible.

The problem is exacerbated, many educators say, by federal policies pushing for rapidly accelerated progress in the so-called three Rs—reading, writing, and arithmetic—at a time when school budgets are desperately strained. “The nation shortchanges our students by not considering science as important as other core subjects,” says physicist Gerald F. Wheeler, executive director of the National Science Teachers Association. The No Child Left Behind Act of 2001 places strict accountability pressures on schools by mandating (among other things) statewide proficiency testing in grades 3 through 8, with significant penalties for schools whose students fall short. The first round of testing begins in 2005, with science exams starting two years later. Given those deadlines and the dire shortage of funding, some primary and middle schools have turned away from science in a kind of academic triage.

Whatever the subject area, a rigid focus on standardized tests comes at the expense of real learning, warns biomedical engineer Richard Schwarz of the University of Texas at Austin, who has taught high school math and science in three states. AIDS researcher Bruce D. Walker, an HHMI investigator at Harvard Medical School, observed the chilling effect of one teacher’s preoccupation with exams in his daughter’s high school science class.

“I couldn’t believe it,” says Walker. “One of the basic formula for hooking kids on science. Set the stage for inquiry with a spirit of fun and adventure. Engage their hands and minds with experiments that prompt questions and analytical thinking. Link everything learned to the world at large so they can recognize it outside the classroom, whether in the night sky or on the evening news.

It takes a potent catalyst, though, to make the formula work: a skilled, enthusiastic teacher with ample resources and administrative support.

The spirit of a motivated teacher is contagious, says Ann Chester, an HHMI program director and mother of three who serves as assistant vice presi-
For enticing future biologists with another kind of zoo, Christopher A. Walsh, an HHMI investigator at Harvard Medical School, cites his daughter’s kindergarten teacher, who taught even him something new. “The teacher raised monarch butterflies and had the kids draw pictures of each stage of development,” recalls Walsh, who thereby learned the difference between chrysalis and cocoon. “Then, after the butterflies emerged, the kindergartners nurtured chick eggs. She showed them how to use a flashlight to see the embryo in the egg.”

Effective instructors “begin every lesson with the students and their experience and include examples from the print media, movies, or TV,” says Deanna Buckley, a research associate at the University of Texas at Austin and past HHMI grantee who has taught science in high school. It also helps to use real data, says Buckley, who suggests hand-held GPS units as a real-world tool for data collection, storage, and analysis.

HIT OR MISS | Inspiring science teachers are out there, but getting one is like winning a door prize—an unexpected treat. “We should be able to say that fun science classes are the rule rather than the exception,” says Chester.

William Newsome’s sons, for instance, grew up in Palo Alto, California, a coveted school district in a notoriously budget-challenged state, but the science classes were hit or miss. “Some were good, because the teachers got the kids thinking,” says Newsome. “But others were quite dull. One was just, ‘Here’s the stuff you have to learn and regurgitate on the test.’”

Cell biologist Kathleen Gould, an HHMI investigator at Vanderbilt University in Nashville, says science education is “minimal” at the elementary school her daughters, Jessica and Sarah, attend. “It’s up to the individual teacher,” she says, Jessica hit the jackpot last year with a 1st-grade teacher who loves nature. “They had puppies and a terrarium, and Jessica talked about it a lot at home,” Gould says. Sarah never even mentioned what her 4th-grade class was doing in science, but she remained curious about science outside of school.

Gould and her husband, also a researcher, do their best to encourage that interest at home. “We discuss science often as a family,” she says. “Sarah in particular asks about reproduction, cloning—things she reads in the paper. Living out in the woods, we hike a lot, and we talk about the plants we see. We get the kids chemistry sets and crystal-growing sets. It’s a lot of fun, although maybe I have more fun than they do.”

Chester, like Gould, has served science with dinner since her children (now 17, 18, and 21) have more fun than they do.”

Training gaps may, in fact, lead to neglect of science in the classroom. Teachers weak in this area, particularly those who must teach many subjects in the lower grades, may be reluctant to devote class time to it. “Few elementary teachers have a strong background in science, and some don’t like to be asked questions that they don’t know the answer to,” Buckley says.

Vogelstein proposes a far-reaching solution: Use computers to replace much of the science instruction teachers now handle. The free-thinking cancer researcher, who admits to having ditched most of junior high because he didn’t like school, says computers can be programmed to get kids to think, be engaged, and to inspire students, you should be able to reproduce that experience for them.”

“A good part of the attraction of science is the inquiry and discovery—getting your hands dirty—and to inspire students, you should be able to reproduce that experience for them.”—ANN CHESTER
answer questions, construct experiments, do the experiments (“in silico,” as he puts it), and interpret the results, all at the student’s own pace and all through emphasis on the scientific method. “This sort of personal interplay between individual and experiment can’t easily be realized in a standard classroom setting,” he says. “The teacher would still have an essential role, but it would be to teach the students how to learn for themselves, using computers, the Internet, and whatever else is available to them.”

Some might welcome the chance to step back, if given the resources to try such an approach. Science teachers tend to be deeply committed to education, but also “overburdened, underpaid, demoralized, and unsupported,” says Richard Schwarz, a teacher turned researcher.

“Teachers have a thankless job,” concurs Chester. “You might say they’re doing doggone okay, given what little they have to work with.”

Nevertheless, she says, “a teacher must figure out how to work within the existing system, even when it is prescribed and limiting.”

Through efforts both inside and outside the schools, she and others are trying to help.

**TOUCH A BRAIN, HOLD A HEART | AIDS researcher Walker made an unusual donation to the high school his daughter and son attend: a PCR machine his lab at Harvard no longer needed.**

PCR, or polymerase chain reaction, amplifies the number of copies of a DNA segment to produce a sufficient amount for testing. “The students are doing PCR,” Walker says. “They’re learning all sorts of new techniques.”

He also invited one of the school’s science teachers to spend a summer at the lab learning about the biology of HIV. Now Walker and the teacher are developing a high school genetics course focusing on HIV. “We will teach them genetics by looking at viral evolution and evolutionary dynamics in this real-world pathogen,” Walker says. Members of the senior biology class will visit the Harvard lab for a research day. “We’ll start with basic talks about HIV, then tell them some of the questions we are facing and have them go through the assay methods we use to answer those questions.”

Newsome and his Stanford graduate students take the human brains they study into Palo Alto’s middle schools, where 7th graders don plastic gloves to examine them. The annual spring event, called the Brain Project, was born at a parent-teacher conference in 1995 when Newsome promised his son’s science teacher that he would lead a few classes on the brain.

“It’s a winning situation all around,” Newsome says. “The kids are really into it. Teachers realize that people doing research value their efforts to help kids. Graduate students learn how to educate the public. And I think it’s important for all scientists to know how to communicate with the public.”

Chester’s outreach project targets disadvantaged students and their teachers throughout West Virginia. She created the HHMI-funded Health

**Making the Case**

Science education can be a hard sell these days, squeezed between budget constraints and the national rush to bone up on the three Rs. How does one justify the need to improve it?

Science is essential, of course, to enlightened decision making. “We’re faced with scientific decisions on a daily basis,” says Ann Chester, assistant vice president of health sciences at West Virginia University. “Even our choice of a car impacts the globe. If students are trained to think in a scientific manner, the result is more likely to be for the greater good of the greater number of people.”

At least half the major bills facing Congress have a scientific or technological component. “We vote for candidates who take stands on these issues,” notes Stanford neurobiologist William Newsome. “In order to be responsible citizens, people need to understand the science involved, even if they’re never touched by it directly.”

A recent government report cites another reason: an “emerging and critical problem” that threatens the nation’s economy and security. The report, released in May by the National Science Board, warns of a “troubling decline” in American students preparing for careers in science and engineering, as the number of such jobs continues to grow and fewer foreign-born workers are available to fill them because of heightened security rules and global competition.

“[T]he United States has always depended upon the inventiveness of its people in order to compete in the world marketplace,” the report’s authors state. “People with skills in science and engineering are of vital importance to the nation’s health, security, and prosperity.”

Scientists who entered the workforce this year made the pivotal decision to take challenging math and science classes fully 14 years ago, in middle school, notes the report. “If action is not taken now to change the trends [behind the declining enrollment in such courses today], we could reach 2020 and find that the ability of U.S. research and education institutions to regenerate has been damaged and that their preeminence has been lost to other areas of the world.”
Sciences and Technology Academy in 1994 because, she says, “I’d been trying to inspire kids to go to college in a place where many kids had never seen a college, let alone known the freedom such an education brings.” Every summer, her program brings teens and teachers to the university to explore inquiry-based activities. The ultimate hands-on experience, she says, is their visit to the cadaver lab.

“We get them in there with the med school faculty and let them touch a heart,” says Chester. “They're wearing gloves, but their noses are bare and the smell is frankly overwhelming. I remember one girl, from a really poor family, who was recalcitrant about getting up close. She just kept saying it stank. A physician asked her, ‘Would you like to hold a human heart?’ He placed it in her hands, and her eyes lit right up. She carried the organ all around the room, whispering, ‘Look—it—I’m holding a human heart.’”

**CLASS ACTS** Chester’s summer institute is among hundreds of programs across the country where teachers can bolster their science skills. The National Science Teachers Association, pushing for a more inquiry-based curriculum and a national teacher-competency standard, offers a variety of professional development workshops. HHMI and other organizations sponsor analogous programs, ranging from intensive live-in academies to individual mentorships with scientists.

To study seed genetics, Mario Godoy-Gonzalez and his students take science to the field.

Teachers are increasingly taking advantage of these offerings, even when it means giving up summer vacations with their families. And the results can be dramatic.

Mario Godoy-Gonzalez is one of 14 primary and secondary teachers named by the National Science Teachers Association this year as Outstanding Science Educators. He teaches high school in Royal City, Washington, an agricultural town where many families have roots in rural Mexico. Godoy-Gonzalez, born in Chile, tailors his curriculum to the local community. To teach genetics, for instance, he has developed a unit using microchips encoded with wheat DNA to compare sequences from different seed varieties and determine which characteristics will make some more vigorous than others. In another project, his students investigate hazards faced by farm laborers who cannot read the English-language warnings that accompany agricultural chemicals.

Godoy-Gonzalez has won honors and grants for his imaginative biology lessons, which draw on everything from haiku to herbal lore. Biology is not his primary subject; he was hired to teach English as a second language. He also teaches math, world history, and physical science, all bilingually.

He actually disliked science as a kid. “We never did real science. In my high school it was, ‘Read the book, answer the questions, take the test.’ After high school, I never took another science class. Science was scary to me,” he says.

That changed soon after he was assigned to teach it, though. Godoy-Gonzalez attended a life sciences institute for teachers, sponsored by HHMI—and while devising projects to turn kids on to science, the instructor himself got hooked. He continues to build hands-on experience in the summers, working with laboratory researchers through the Science Education Partnership Program at the University of Washington. And he has found a lasting mentor in Steve Verhey, a molecular biologist at Central Washington University.

What a difference enthusiasm makes, with a little help from summer school and some scientific friends. Godoy-Gonzalez’s love for science is contagious, whether he’s speaking Spanish or English.

And yes, he teaches languages in much the same way, with poetry, folklore and … okay, maybe just a little bit of rote.

“It’s less a matter of scientific knowledge than of reaching out to make emotional contact with kids. The critical thing is how effective teachers are at engaging kids in the classroom.” —WILLIAM NEWSOME
Normally, we don’t think about walking. Our legs fall automatically into a left-right rhythm, one foot in front of the other. But a spinal cord injury, stroke, or degenerative disease like Parkinson’s can rob us of this basic skill. To help people with these conditions, scientists need to better understand how the nervous system coordinates walking.

To that end, HHMI investigator Thomas M. Jessell at Columbia University College of Physicians and Surgeons and Martyn D. Goulding at the Salk Institute for Biological Studies have described how one discrete part of walking—the timing of left-right movements—is regulated. They studied mice, but similar mechanisms likely operate in humans. The collaborators stress that their finding, described in the May 13, 2004, issue of Neuron, is just a tiny step in overcoming paralysis. Yet their insights, derived from combining genetic and physiological (functional) techniques, may empower scientists to take giant leaps toward that goal.

Jessell and Goulding asked this question: How does the right leg know what the left is doing? The answer lay in a local neural network in the spinal cord that works independently of the brain. This network, known as a central pattern generator (CPG), orchestrates simple walking movements. (Another CPG network regulates rhythmic breathing.) CPGs include interneurons, intermediary cells that signal motor neurons to fire in alternating patterns.

Until recently, scientists knew little about the CPG or its integral interneurons, partly because interneurons are notoriously more difficult to study than their larger, more accessible, and more easily identifiable cousins, the motor neurons. Researchers can insert a probe to measure a particular neuron’s electrical activity and perform microsurgery by snipping a neuron’s long axon to discover its function in a far extremity. The more elusive interneurons, though, are too tightly packed among other neurons to target selectively with a physical probe, and their axons are too short to snip.

Jessell realized he could use the equivalent of genetic microsurgery to molecularly dissect the interneuron circuitry: Silencing specific molecular signals, known as transcription factors, can prevent specific cell types from forming in a developing organism. About six years ago, his lab began focusing on V0 interneurons, a class of interneuron that potentially could regulate walking coordination.

Meanwhile, Goulding’s lab, using physiological studies, was finding that these V0 interneurons connect motor neurons on the left and right sides of the spinal cord. He and Jessell joined forces to investigate whether V0 interneurons coordinate the left-right timing pattern of leg movements, or the flexion-extension pattern, or both.

Prior genetic research by Alessandra Pierani, then an HHMI postdoctoral student in Jessell’s lab, had led to a way to create mice that lacked V0 interneurons, and Goulding then isolated the spinal cords from the newborn mice to study their neural functions. He found that the motor neurons in the cords without V0 interneurons showed completely normal flexing patterns, but their left-right timing was significantly off. After additional experiments ruled out other explanations for the perturbed timing, the researchers concluded that V0 interneurons control the timing of left-right activity, while flexing patterns are controlled independently.

“We’ve developed a model for discovering which neuron types are most critical for a specific function,” Goulding says. “It’s an important conceptual advance, and now others can use this tool to go further.”

Scientists can use these and other genetically modified mice to build a “wiring” diagram of the neurons in the spinal cord and brain. Researchers can then observe the effects of potential drugs to study neural functions—and to develop therapies. Eventually, combining these findings with stem cell techniques pioneered in the Jessell lab might lead to new therapies to regenerate or restore neurons in the spinal cord.

“Learning more about how the nervous system controls motor neurons can help us understand how to get the spinal cord working again after injury,” Jessell explains. “Our general hope is then to build a more comprehensive understanding of neural circuitry in the brain.”

Goulding adds that his mother, a polio victim, was paraplegic, so he is keenly aware of the implications of such work for those who long to walk again.

—CATHRYN DELUDE
In on the Ground Level
Talented student helps paint the big picture in comparative genomics.

Few students get to witness the birth of a scientific discipline, but in Krishna M. Roskin’s case, he even participated in the delivery. As a graduate student at the University of California, Santa Cruz (UCSC), Roskin helped to advance the nascent science of comparative genomics as he worked with a team of more than 20 researchers in six countries on the rat genome sequencing project. The team’s results were published in the April 1, 2004, issue of Nature.

With the genetic sequence of the brown Norway rat in hand, the team was the first to compare three mammalian genomes (rat, mouse, and human), triangulating data in their search for molecular-scale similarities and differences between species as well as the mechanisms of evolutionary change over the past 12 to 24 million years.

“It was really great to move toward understanding the major evolutionary events and to begin to get a handle on that,” says Roskin, who performed many of the computations for the analyses.

Roskin, who was home-schooled while his family traveled throughout Central America and Europe, never imagined that one day he would study genomes. His passions were computer science and mathematics, which became his majors in college. Roskin’s life story took a propitious twist in 2001, his junior year, when David Haussler, an HHMI investigator who directs the UCSC Center for Biomolecular Science and Engineering, contacted him.

“I got this grandiose e-mail saying, ‘How would you like to join the greatest scientific project of all time?’” Roskin recalls. He found the offer too intriguing to resist, so he joined the project as an undergraduate and then stayed on for graduate school.

First, Roskin helped develop the UCSC Genome Browser for visualizing the map of the human genome, which was roughly decoded in February 2001. Then he worked on the mouse genome draft sequence, published in December 2002. Roskin made the oft-quoted estimate that 5 percent of the mouse and human genomes had been conserved, or preserved, over evolutionary time. The estimate ignited interest in these genomic regions, which are believed to contain much more than just protein-encoding genes; two-thirds of the sequences are thought to encode other kinds of genes as well as elements that regulate transcription and other functions.

“There’s now a big hunt for what we think are the regulatory elements, but we couldn’t learn much from just two species,” Roskin says. Then came the project to sequence the rat genome. By this time, Roskin no longer had to work 18-hour days at the computer, as he was faster and more efficient in his computations. “I learned to pace myself—it was like a marathon run,” he says. Teams of researchers at several institutions regularly sent him sequence data, and his job was to put it all into a coherent picture and then make comparisons.

At times, Roskin says, he felt like a cartoon switchboard operator madly trying to cope with a deluge of calls. “Sometimes it seemed like I would never get all the numbers to fit together.” But he had some expert collaboration. For example, the lead research teams held a conference call each Friday, often quizzing Roskin on his methods. “It was a very ‘dynamic’ process,” he recalls, “but mostly fun.”

The biggest challenge for Roskin was joining with Haussler to try to convince everyone that particular genomic regions called “ancestral repeats” were neutral sequences that could be used as baselines for measuring rates of evolutionary change. Once that was satisfactorily proven, the teams used ancestral repeats to determine that mice and rats have experienced more genetic change than have humans, evolving about three times more rapidly.

Surprisingly, they found that the rates of evolution—changes in single bases as well as insertions and deletions of longer sequences—also varied between regions of each genome. Chromosomes also revealed differences in their rates of change. “It was interesting to see that each chromosome has its own personality and is unlike its neighbors,” says Roskin. “We don’t know why there are such big differences.”

This mystery has captivated Roskin, who hopes to explore it further as he works toward his doctorate in computer science. He’s now taking biology courses, including one on chromatin structure, to better prepare for computational questions he wants to answer. “I’m curious whether the places in the genome with lots of evolutionary change will correlate with a certain position in the nucleus,” says Roskin. “Are they more accessible to mutagens and radiation? Will there be a consistent pattern? Data coming out on that would be really exciting.”

—KAREN F. SCHMIDT
A Heart’s Critical Start

When a young researcher studying heart defects found a new mutation, nobody guessed what the gene was about to reveal.

Throughout her graduate-student days at the University of California, San Francisco, as she studied heart defects in zebrafish, HHMI predoctoral fellow Emily C. Walsh enjoyed a hip-hop dance class called cardiofunk. She vowed to apply that name to the first mutant she discovered. Her chance came when, in the course of raising a known line of mutants, she noticed there actually were two types. Both had abnormal heart valves, but the unknown mutant also had a defective heartbeat.

“It was sheer luck that I was paying attention,” she recalls.

Walsh was coming to the end of her predoctoral studies in Didier Y. R. Stainier’s lab, so her boss handed over the cardiofunk mutant to a new recruit, HHMI physician and medical student fellow Thomas Bartman. As an M.D., Bartman had treated many infants with congenital heart defects—between 1 and 2 percent of babies are born with such problems—and his goal as a researcher was to explore the effects on valve development. This method brought cardiofunk to Walsh’s attention, but it fell to Bartman to find the gene responsible for the mutant’s missing valve. Using positional cloning, a technique that identifies chromosomal regions the mutant always inherits, he took two years to identify the gene.

“When we found the gene that was causing the problem we were rather surprised,” he says. “It wasn’t a gene that was expressed in the EC. Actually, it’s an actin gene in the muscle layer of the heart.”

Because actin is a protein involved in muscle contraction, the finding suggested that the embryos’ underlying problem lay in flawed pumping of the heart rather than in the inability of the EC per se to form a valve. Sure enough, when Bartman went back to study even younger embryos—at 36 hours of development rather than 48—he found that, although the valve had not yet begun to form, the heart’s ability to pump blood was already defective. Further experiments confirmed that the healthy formation of the valve depended on an undisturbed heartbeat in the earliest stages of development, a finding published by the Public Library of Science’s PLoS Biology in May 2004.

“It made us wonder if the heart malformations we see in children could be due to problems that they have with heart function during the first weeks of development,” says Bartman. No systematic studies have been done to test this hypothesis, because it is impossible to peer into the developing heart of a human embryo. (The human heart starts beating at 23 days and the EC forms at about 28 days, often before a woman realizes she is pregnant and when the embryo is too small to be visualized in detail by ultrasound.) But anecdotal evidence from doctors indicates that, in families with inherited defects in heart function, some affected individuals also have structural heart disease—suggesting that the same mutation might be responsible for both.

If this is true, it could mean that when an environmental insult (say, medication taken by the mother) interrupts the early heartbeat of the fetus, even temporarily, structural deformities could form later on. It also could mean—once more is known about the mechanism of this association—that limiting such environmental insults could prevent congenital heart defects in a sizeable part of the human population.

—Laura Spinney
Decoding Key Cancer Toeholds

Scientists solve the structures of cancer-related cell-growth receptors.

Collective embarrassment” is how Genentech’s Mark X. Sliwkowski describes the feeling. For years, researchers at his company and elsewhere could not figure out the three-dimensional structure of the epidermal growth factor (EGF) receptors—HER1, HER2, HER3, and HER4. “A lot of big-name labs tried,” he says. “We burned through quite a few post-docs trying to get this done. It was hard.”

Stakes were high because this family of receptors regulates cell growth and differentiation, and it is implicated in several human cancers. Figure out how to specifically jam those receptors, and the result may be a blockbuster cancer-fighting drug.

The field burst open in August 2002 when HHMI investigator Daniel J. Leahy “came out of the blue,” as Sliwkowski puts it, and published the structure of the region of the HER3 molecule that sits outside the cell’s membrane. Leahy was a newcomer to the EGF-receptor field, but his experience working with other receptors provided an edge. A month later, researchers in Australia and Japan independently solved the structure of a fragment of the extracellular region of HER1 with growth factor bound to it. A University of Pennsylvania School of Medicine team led by Mark A. Lemmon then teamed with Leahy to publish more on HER1’s structure.

Leahy’s group at the Johns Hopkins University School of Medicine added another piece to the puzzle in February 2003 by solving the structure of the most well-known receptor, HER2, whose overexpression signals a particularly aggressive and deadly form of breast cancer. They also showed how it binds with Herceptin (trastuzumab), Genentech’s celebrated drug that extends survival—by months—of women with advanced breast cancer whose tumors overexpress HER2.

That paper answered questions that had baffled scientists. Why, for example, does HER2 seem to be the most important EGF receptor? The reason is that it is the only family member that holds its partnering, or dimerization, arm open and ready for action. Because the others remain closed until a relevant growth factor appears, HER2 is a favored growth-signaling partner.

In April 2004, Leahy collaborated with Genentech scientists to publish in Cancer Cell the structure of the extracellular domain of HER2 as it is bound to the firm’s experimental drug Omnitarg (pertuzumab), with the group’s schematic gracing the journal’s cover. That structure showed how Omnitarg inhibits HER2’s function and blocks growth-factor signaling.

Herceptin and Omnitarg bind to different domains of the HER2 receptor and they work differently. In patients whose tumors have high levels of the HER2 protein—20 to 30 percent of women with breast cancer—Herceptin awakens the immune system to attack tumor cells with HER2 on their surface. Omnitarg, on the other hand, binds directly to HER2’s dimerization arm to shut down growth signaling, and it appears to be active in an additional group of cancers—those in which HER2 is activated but not overexpressed.

Genentech is conducting phase II studies of Omnitarg in patients with several cancers—ovarian, lung, prostate, and some breast cancers—of the latter type. In earlier studies, the drug appeared safe for patients with advanced disease, and in some of them it produced a measurable response.

EGF-receptor crystals are particularly tricky to make. They require mammalian cells—a more expensive and time-consuming proposition than the bacterial systems commonly used to grow crystals—and the receptors carry tangles of attached sugars. To grow crystals, the molecules need to be packed in an orderly array, but, as Leahy explains, it’s hard to pack a crate of tomatoes with the vines still on. So he and his team figured out a way to remove the vines and pack a nice tight crate.

The researchers used four or five tricks to grow the crystals and solve the structure. “We were developing the methodology to solve another problem,” Leahy admits, “but it turned out to be very applicable [to the EGF receptors].”

Leahy’s lab recently solved the structure of the remaining EGF receptor, HER4. His team is also looking beyond these receptors’ extracellular domains, or antennae, to crystallize the whole molecule—the region outside the cell, the part within the cell membrane, and the part that sits inside the cell awaiting the outside signal that sets the internal-growth machinery in motion. Now an HHMI alumni investigator, Leahy says dealing with all three components “puts a whole new level of complexity on the table.” —Cori Vanchieri
In Search of a Thousand Solutions

The power of medical training, a global view, and an open mind.

When Maria-Estel Garcia was very young, a professor of math in her hometown of Puebla, Mexico, set forth a challenge that seemed magically powerful. "Imagine," he said, "if you do a single math problem every day, at the end of three years, you’ll have the solutions to a thousand problems!"

"That seemed fantastic to me," Garcia says, and she embraced that challenge. It was some time before she realized her sly father was not just passing along a love of puzzles but also coaxing a little extra homework out of his daughter.

Today, Garcia is 22 and freshly graduated from the University of California, Berkeley, where she was one of five finalists for the University Medal, Berkeley’s top honor for a graduating senior. Hoping to begin studies in medicine and public health in 2005 after a work stint in Brazil, Garcia remains intent on puzzling out the world’s problems, one patient at a time. The traditional premed courses she incorporated into her major in Latin American studies are a good start toward this goal, but she insists that, while they are necessary, they are not sufficient.

"I don’t believe you can simply look at the body as a mechanism for disease," she says. "If you want to heal someone’s illness, you need to take intangible factors like culture and belief system into account, too."

Garcia speaks quickly, between sips of an iced mocha outside a coffee bar on a leafy Berkeley street. Her dangling earrings dance when she laughs or shakes her head to clarify a point. Poised yet exuberant, she looks and sounds like many of the other bright new graduates strolling past on their way to a promising future.

But Garcia’s personal history and her decision to get involved in public health programs in Guatemala and Brazil during her undergraduate years have given her insights that most would-be medical students lack, says John Matsui, who directs the HHMI-sponsored Biology Scholars Program at UC Berkeley. Matsui founded this mentoring project 12 years ago to draw more students from diverse ethnic and socioeconomic backgrounds into science, and Garcia, he says, is a star alumna.

"Maria’s already a crack scientist," Matsui says, "and she also sees the connections between science and society. She has a global view of everything she does."

That view came early. Born to a Mexican father and Puerto Rican mother (both teachers, they met as university students in Berkeley), Garcia spent her earliest years in Mexico. When the family returned to Berkeley in 1988, Maria-Estel was 7 years old.

To this day, Garcia is keenly aware that moving to a new country and learning a new language and culture can be challenging even under the best of circumstances. She not only incorporates that sensitivity into her work, at times it is her work, at least in part. In hopes of learning more about how migration to the United States affects families, she traveled with a professor and classmates during her freshman year to conduct research interviews in the Mayan town of Zunil, a tight-knit community of 15,000 in the lush green highlands of Guatemala.

"In Zunil, to talk about AIDS prevention, you must talk to women and men separately, using round-about language," Garcia says. "There I was telling a 14-year-old married woman that she had to ‘protect herself’ from her husband, who’s been in the United States and may or may not have been faithful to her. It was very hard."

During that visit and two subsequent trips, she spent hundreds of hours interviewing residents, primarily women, and found migration’s effects on the region to be both surprising and sweeping—for better as well as for worse. The most obvious benefit, she learned, is that money earned in America goes much farther in Zunil; it has fueled a housing boom and an improved standard of living. But because crossing the border can be costly, sometimes illegal, and perhaps dangerous, workers who finally make it to the United States often travel solo and can afford few visits home. When they do return to their families, some bring the world’s ills with them. When she visited in the year 2000, Garcia discovered that eight residents of sexually conservative, geographically isolated Zunil had died of AIDS.

Two years later, Garcia found herself in Brazil, interning with a community health organization that uses poetry and theater to teach crucial public health messages to adolescents. The contrast between the two Latin American countries couldn’t have been more striking, she says.

"In Zunil, to talk about AIDS prevention, you must talk to women and men separately, using round-about language," Garcia says. "There I was telling a 14-year-old married woman that she had to ‘protect herself’ from her husband, who’s been in the United States and may or may not have been faithful to her. It was very hard."
In Brazil, the message was much more direct—though, in its own way, just as difficult to convey. Garcia worked with young people who incorporated their own stories into a play about childhood sexual abuse, with the aim of teaching other children how to stand up for themselves and say no.

“Hearing them talk about some of these issues—things I’d never heard adults talk about openly, let alone teens—really opened my eyes to the power and importance of culturally appropriate medical care,” Garcia says. “Ultimately, medicine is not straight-up science. It’s about how you apply that science, how you apply what you learn.”

Ten years from now, Garcia hopes to be applying what she has learned for the benefit of a health clinic in some underserved community in the United States. She expects to treat whoever walks in the door and to treat them not only with medical therapies but with an open mind.

“When you encounter somebody from a different culture, there’s no way you can know the subtleties of their experience or be certain you understand what they’re trying to say,” she says. “But the point is to make the attempt to understand and to realize that, if someone is resistant to a medicine or treatment, you shouldn’t just insist that they’re wrong.”

—DEBORAH FRANKLIN

Teaching the Teachers
To help engage students before they tune out of science, an HHMI program gets teachers to think like scientists.

On a steamy afternoon this past summer, a group of seventh-grade teachers in white lab coats huddled over microscopes in a Virginia classroom, comparing the architecture of plant and animal cells. Intent on their observations, they seemed unaware of the pungent smell of their onion specimens. Down the hall, eighth-grade teachers ran a bottle-full of murky, malodorous water through sand and charcoal to filter out some of its impurities and then heated the sample to evaporate others. Next door, a group of sixth-grade teachers clustered around a set of Bunsen burners. They had just combined an acid and an alcohol and were curiously sniffing the vapors, trying to identify the familiar scent. It didn’t take them long to realize their good fortune—while their colleagues had to contend with onions and dirty water, this group got to enjoy the aromas of wintergreen, banana, and pear wafting from their test tubes.

Thirty-four science teachers, representing every middle school in Virginia’s Loudoun County Public School system, gathered for a two-week course that introduced them to new science concepts and techniques for sharing what they had learned with their students. The course served as the kickoff for a long-term program sponsored by HHMI to bring new science curricula into the county’s schools—focusing first on the middle schools because researchers have found that is a time when many students begin to lose interest in science. The workshops for teachers are part of an HHMI plan to invest $1 million annually in the public schools in Loudoun County, where the Institute’s new Janelia Farm Research Campus will open in 2006.

The summer science course’s methodology was scientific in its own right, having been designed and conducted by the Cognitive Learning Institute (CLI), a group of scientists dedicated to the study of how students learn by applying what is now known about the way the brain processes and stores information. According to CLI’s president, Keith Verner, the course aimed to apply these concepts to both science teaching and student thinking skills throughout middle school.

Sixth-grade teacher Susan Kretzler said that this “neurocognitive approach” geared to the middle school student, together with the laboratory experiences, makes the course unique.

The teachers spent mornings in the classroom, learning about things like how memory works. After lunch, they moved into the labs, where the intent of the hands-on activities was not only to get the teachers thinking like scientists—asking questions and working out ways to answer them with the tools of science—but also to stimulate their ability to learn new concepts and integrate the more theoretical learning from their morning sessions.

The laboratory activities introduced the teachers to sophisticated equipment and methodologies to explore the concepts at the core of their curriculum. “My head is just buzzing with all the things you can do with this,” sixth-grade teacher Sharon Lanham exclaimed as her group discussed potential applications for the digital spectrophotometers they had just used to determine the properties of a solution. By the end of the two weeks, each of the teachers had worked through three new lessons for their classrooms. These were based on the neurocognitive approach of the course and were carefully integrated so that students build on skills and concepts learned not just earlier in the school year but throughout middle school. And although teachers from each grade level had their own set of laboratory experiences during the course, plenary discussions, together with collaborative work on lesson designs, ensure that all teachers will be aware of what their students are learning in colleagues’ classes.

But the program does not end there. The group of teachers will return to the course for the next two summers to expand their portfolios of science experiences for students. And when the teachers present their new lessons during the coming school year, experts from CLI will be available both for scientific consultation and to monitor the lessons’ success. In that way, the pedagogies may be further refined before being taught to a new group of teachers next summer.

Beyond that, CLI leaders hope the experience in Loudoun County ultimately will serve as a model for the rest of the country.

—JENNIFER MICHALOWSKI

Virginia teachers experience equal and opposing forces firsthand.
Jeff Pinard, Fighter

A patient who searched for his own genetic flaw is still battling cystic fibrosis, and prevailing.

I was babbling incoherently. … My organs were failing. … The doctors thought they were going to lose me. My only chance was to be put into an artificial coma so that all of my energy could go toward fighting the infection. So for three weeks I was in a coma. The infections were so severe and so resistant to antibiotics that the ICU staff had to keep switching to stronger drugs. But I’m a fighter and wasn’t ready to go.”

That’s how Jeff Pinard, who is 34 and has been battling cystic fibrosis (CF) his entire life, remembers a particularly bad bout with the disease in 1999.

Eight years earlier, Pinard was the hero of a story in an HHMI publication titled Blazing a Genetic Trail; the article described how he spent a summer working in a genetics lab at the University of Michigan to help find his own genetic flaw. Pinard was then a sophomore at the university and started working as a systems engineer for an electric utility. Jeff got married in 1998 and bought a house. But he kept being hospitalized for pancreatic infections of his lungs and pancreas, he kept up with his daily treatments—for example, having his chest pounded for at least an hour a day to loosen the sticky mucus in his lungs. Yet, he was full of life and enthusiasm for his research.

That article has been inspiring letters to the Institute ever since. Not untypical is this one, received a few months ago:

I am a science teacher in Texas. Every year as part of my eighth-grade genetics unit, we read the article from the HHMI Web site about Jeff Pinard. … My students always enjoy the article and always wonder what happened to Jeff. Does anyone there have any information about him?

Indeed we do. Jeff has been remarkably open about his life and is even reachable at his e-mail address, jpinard@comcast.net.

Soon after the article was published, Jeff became ill several times and was forced to drop out of college. Reluctantly, he went home to his parents in Grand Rapids, Michigan, where he started working as a systems engineer for an electric utility. Jeff got married in 1998 and bought a house. But he kept being hospitalized because of pancreatic problems that caused excruciating pain. These problems remain unsolved, despite a variety of treatments.

In 1999, what was first thought to be a “routine pancreatic infection” turned into the emergency noted above, which led to his three-week coma.

“One day they let my coma medicine accidentally lapse a bit too much,” Jeff recalls. “In my half-awake state, I tore out all of my ventilating equipment and the IV in my neck that was for blood transfusions. My poor dad was there alone, and he freaked out. Ten doctors and nurses got there in about a billionth of a second, he said, and I woke up. Well, they decided to try letting me breathe on my own, and I did” This was the first step in a terribly difficult recovery.

“The doctors and my bosses then agreed that I needed to retire to try to extend my life span. As much as I enjoyed the work, the stress of it was wearing down my health. So I am now on full-time disability and fairly stable.”

According to his mother, Diane Pinard, “fairly stable” means that Jeff has good days and bad days. He can walk around but is seldom free from pain and needs frequent periods of rest throughout the day.

The constant illness took a toll on his marriage too, which broke up within a few years. But he met someone else and remarried in 2003. “Jennifer is very understanding of my cystic fibrosis and is always there for me,” he says. “I spend 12 to 30 days a year in the hospital, usually over two or three hospital stays, and always opt to get out early to continue healing at home. I can do my IV antibiotics there after I’m stabilized.”

The gene whose mutations cause CF was identified in 1989 by Francis S. Collins (then an HHMI investigator at the University of Michigan and now director of the National Human Genome Research Institute at the National Institutes of Health) and Lap-Chee Tsui (then a genetics researcher at the Hospital for Sick Children in Toronto, Canada, later an HHMI international research scholar, and now vice chancellor at the University of Hong Kong).

The CF gene is recessive, meaning that only people who inherit mutations from both parents develop CF. There are many such mutations of the CF gene—scientists have found hundreds of them—and some produce “atypical” disease, such as male infertility or pancreatitis, rather than full-blown CF. It all depends on the error in the protein, called CFTR (CF transmembrane conductance regulator), that is produced by the gene.

Jeff has two different kinds of mutations in his CF gene. One of them, being milder, accounts for his relative resistance to the disease; he has managed to survive despite numerous medical crises. In addition, “he is such a determined young man,” says his mother. “He has taught me so much about the value of life and not complaining about what life dishes out.”

—MAYA PINES
Traffic Control
Research team works out signaling essential to fighting off infection.

Jason G. Cyster studies a moving target. In the immune system, he explains, cell migration “is just the way the system works.” Immune cells called lymphocytes essentially survey the whole body, move out of circulation into infected tissues, migrate into the lymph nodes to become activated, and head back out again to produce antibodies or to fight infection in cell-to-cell combat.

With his team at the University of California, San Francisco, HHMI investigator Cyster has focused on deciphering lymphocyte traffic patterns and learning what regulates them. Understanding how to control these cells’ movements might lead to more successful vaccines and better therapies for transplant rejection, chronic inflammatory diseases, and lymphomas. In a recent paper—published in the January 22, 2004, issue of Nature—Cyster’s group described the biological mechanism, based in the lymph organs, of a new class of immunomodulating drugs.

“The lymph organs are important for survival and for vaccination to work,” Cyster says. “If you don’t have them, you are essentially dead.” from a severely weakened immune system. The two main types of lymphocytes, B and T cells, interact in the organs and tissues of the lymphoid system—lymph nodes, spleen, tonsils, and Peyer’s patch in the gut.

Once activated, B cells produce antibodies that can neutralize viruses and bacteria or mark infected cells for destruction. T cells come in two main types: helper T cells, which help activate B cells, and killer T cells, which return to infected or inflamed tissue and destroy it. Killer T cells can wreak havoc when a patient receives an organ transplant because they attack the “foreign” tissue. Immunosuppressant drugs like cyclosporin keep the destructive T cells at bay, but such drugs inactivate all lymphocytes, including the helper T and B cells, and make patients highly susceptible to infections.

A new immunosuppressant compound called FTY720 may offer a better solution. Developed as a potential therapeutic by the Novartis Institutes for BioMedical Research in Basel, Switzerland, FTY720 holds activated lymphocytes inside the lymph nodes, preventing them from circulating back to infected or transplanted tissues. But because the lymphocytes can still be activated and amplified inside the lymph nodes, the B cells can still produce antibodies, which are released into the bloodstream. This means that patients treated with this compound, or a similar drug, may stand a better chance of fighting off minor infections.

Drug companies studying FTY720 and related compounds did not know how they held lymphocytes hostage inside the lymph nodes, Cyster says. His group and key collaborators worked to fill in the missing pieces. Previous research showed that FTY720 activated cell-surface signaling receptors, called sphingosine-1-phosphate (S1P) receptors, in the lab dish. By studying strains of mice that lack S1P receptors on all their circulating cells, including lymphocytes, Cyster and his colleagues were able to show that one of the receptors, the subtype S1P1, was needed for lymphocytes to leave lymph nodes.

It turns out that activated lymphocytes temporarily quell the S1P1 receptors on their surface, thereby shutting off the S1P signal and allowing them to stay in the lymph tissue long enough to interact with other lymphocytes and to expand their numbers. Then, the receptors are cranked back up, and the cells can migrate out of the lymph node and into circulation by following a chemical trail of S1P, which is at high levels in the blood.

The research team found that FTY720 caused the cells to attenuate their S1P1 receptors indefinitely, holding them in the lymph node until the drug was no longer given. Pharmaceutical companies working on FTY720 and its chemical cousins hope this unique mechanism will provide a safer immunosuppressant for transplant patients and possibly even for treating chronic autoimmune diseases like multiple sclerosis.

After working out the biology of the drug,
Post-Soviet Science

For Estonia and Lithuania, joining the EU may prove to be a mixed blessing for research.

What a difference a decade makes. Ten years ago, the tiny Baltic nations of Estonia and Lithuania were behind the Iron Curtain. On the first of May this year, along with eight other countries, they became full-fledged members of the European Union (EU).

The EU now comprises a border-free marketplace of 25 countries working together to improve economic and social conditions. For science in Estonia and Lithuania, however, this new status may prove to be a mixed blessing.

EU membership makes Estonian and Lithuanian scientists eligible for research and infrastructure grants, and it paves the way for potentially lucrative collaborations with researchers in other member countries. Yet membership also forces the Baltic scientists to compete on a level playing field with their German, French, and British counterparts, among others, whose scientific infrastructure and economic underpinnings are much stronger.

EU membership calls for government support of science far beyond what Estonia and Lithuania are prepared to spend. The EU has set a goal that its members direct 3 percent of their gross domestic product (GDP) to science by 2010. Estonia and Lithuania each now spend less than 0.8 percent.

“It is fine to encourage governments to spend 3 percent of their GDP, but where are they going to get it?” says Saulius Klimašauskas, an HHMI international research scholar and head of the Laboratory of Biological DNA Modification at the Institute of Biotechnology in Vilnius, Lithuania.

Compounding the situation, Estonia and Lithuania’s scientific institutes are reeling from the loss of their exemption to the EU’s 18 percent value-added tax on equipment and supplies purchased with outside grants such as HHMI international research scholar support. As of May 1, 2004, the day they became full members of the EU, these two countries suffered the equivalent of an 18 percent budget cut.

A country of 3.6 million people bordering on Poland, Belarus, Latvia, and Russia, Lithuania has a GDP of $40.9 billion, which ranks it 78th in the world marketplace in terms of purchasing power parity. Estonia, with a population of 1.3 million, has a GDP of $17.4 billion and places 113th on the same scale.

Although Estonian scientists did well in the special competitions for EU candidate countries, those days are over; some of these researchers are now concerned that, because regular EU funding focuses on larger projects done by consortiums, Estonia will suffer a serious disadvantage.

“The EU’s emphasis on consortiums tends to favor the big countries,” says Mart Ustav, an HHMI international research scholar and chair of microbiology and virology at the Institute of Molecular and Cell Biology at Estonia’s Tartu University. “You need considerable infrastructure and resources to join, and make a difference in, integrated projects. You need huge facilities with very sophisticated equipment. But we are small groups doing science with our hands; we are not competitive.”

EU membership also means an infusion of infrastructural funds, but Ustav and fellow HHMI international research scholar Pritt Kogerman, a senior research scientist at Estonia’s National Institute of Chemical Physics and Biophysics and an associate professor at Tallinn Technical University, wonder how the money will be used. “Even if our government wants to spend it on research, and the EU says ‘No, it has to go for roads,’ then it has to go for roads,” Kogerman suggests.

However, while such constraints apply to...
Estonia and Lithuania alike, the countries’ scientific prospects differ.

When the Soviet Union collapsed, Estonia did something that Lithuania did not. The newly independent Estonian government dismantled the Soviet system in which a government-funded Academy of Sciences ran the country’s research institutes and placed responsibility with the universities instead. “This was an important step,” says Rein Vaikmäe, research policy adviser to the Estonian minister of education and research. “Coming from the earlier system, where the government funded everything and there was no competition, we needed to change completely. It helped us get rid of some very weak research organizations.”

Some scientists resisted the change because integrating the research institutes and universities added teaching to their responsibilities. However, “thinking about how small this nation is, we could not afford to waste resources,” Vaikmäe says. “It was hard, but it was the right solution.”

The new government also established the Estonian Science Foundation, which introduced the Western practice of competing for grants. “We started to learn how the world of research funding actually works—that you have to go through peer review, that quality counts, that publishing counts,” Vaikmäe recalls.

Like the move to merge research institutes and universities, Estonia’s switch from automatic government funding to competitive grants was a full swing of the pendulum, from nothing but government support to 100 percent competitive grant funding.

“Coming over from the old system to the new was quite painful, but most of our scientists have become quite effective at it,” says Vaikmäe. In fact, while it was still an applicant for EU membership, Estonia was one of the most successful candidate countries at getting Framework Program grants, a primary source of EU research funding.

Nearby Lithuania, meanwhile, with more than twice Estonia’s population, faces greater obstacles as it seeks to leverage EU membership into improvements in its research arena.

“Our research system and our education system are not competitive,” Romualdas Kalytis, head of the science division in the Lithuanian ministry of education and science, says flatly. “We should have made big changes in the beginning of our independent life, the way Estonia did, but we didn’t. Now we have problems between academics and the government. We are fighting with each other instead of establishing good research policy.”

Algimantas Pauliukonis, director of the Institute of Biotechnology in Vilnius, thinks Lithuania is competitive in some fields. “In DNA-protein interactions and restriction enzymes, for example, our research is high quality and completely competitive.” All three of HHMI’s Lithuanian international research scholars work at the Institute of Biotechnology, which has spun off two thriving companies: Fermentas Life Sciences, which produces restriction endonucleases, nucleic acids, nucleotides, and oligonucleotides that supply 10 percent of the U.S. market for such products; and Sicor, which makes protein pharmaceuticals.

Saulius Klimasauskas calls EU membership “a positive change” for Lithuania because the EU’s system of doing science “is more efficient than ours.” He also hopes EU infrastructural funding will help Lithuanian scientists purchase the major equipment they need, such as a nuclear magnetic resonance machine, to be globally competitive.

But he and fellow HHMI scholar Ceslovas Venclovus are concerned about the EU’s emphasis on applied research. “They think it is better to have quick returns and attract companies to make money and pay taxes,” Venclovus says. “We hope they understand that, without the fundamental science, they cannot do good applied science.”

Kalytis and numerous others in the Lithuanian government hope EU membership will help convince scientists not to emigrate to the United States or Japan. “If we can’t stop emigration, at least we can keep them in the EU,” he suggests. “Mechanisms must be invented to stop this brain drain.”

Mart Saarma, an Estonian who now heads the University of Helsinki Institute of Biotechnology, agrees. “One or two Estonians in the U.S. is a very small thing,” says Saarma, who is a member of the Estonian Academy of Science and still teaches part-time at Tallinn Technical University, a 45-minute ferry ride from Helsinki.

“Those same one or two researchers could make quite a difference to science in Estonia.”

One of the principles underlying HHMI’s international program, which currently supports 132 scientists in 29 countries, is a commitment to helping promising scientists remain in their own countries. An HHMI grant lured Venclovus back to Lithuania and has enabled many others to return to, or remain in, their own countries. And Tamás Freund, a Hungarian, says that his first HHMI grant “played a major role in making a decision to stay in Hungary [another new member of the EU] in spite of prestigious job offers from the West.”

— JENNIFER BOETH DONOVAN

Janelia Tour Congressmen Frank Wolf (left) donned an Institute hardhat in September for a tour of the construction site at HHMI’s Janelia Farm Research Campus. Just elected to his 13th term—and the senior member of Virginia’s delegation in the House of Representatives—Wolf serves the state’s 10th District, which includes Loudoun County, home to Janelia Farm.
Trashing Misfolded Proteins

When proteins don’t fold properly, their fate is sealed. But how the cell gets rid of such useless baggage has remained something of a mystery.

Normally, when proteins are manufactured in the cell’s fluid portion, called the cytosol, many are transported into the endoplasmic reticulum (ER), a membrane-bound network where proteins are folded and sent on to their final destinations. It’s there that they undergo quality control—only properly folded proteins are allowed to proceed. Those that don’t pass muster because of, say, a mutation, are moved out of the ER and back to the cytosol, where they are degraded. But little has been known about the mechanics of this process, called retro-translocation.

Now, researchers have uncovered a group of interacting molecules that help funnel mangled proteins to the cell’s garbage disposal. While much remains to be learned about these and other players involved in retro-translocation, scientists say they ultimately may help to shed light on diseases such as cystic fibrosis.

Several years ago, Yihong Ye, a postdoctoral fellow in HHMI investigator Tom A. Rapoport’s laboratory at Harvard Medical School, discovered p97, an enzyme that was pulling proteins out of the membrane. But p97, being in the cytosol, somehow had to hook up with a channel to accomplish that task. Recently, Rapoport and his colleagues identified that necessary component, which they called Derlin-1. They also identified a second protein, VIMP, involved in the process.

“VIMP is the link between Derlin and p97,” Rapoport says. “There’s still a missing link, though—something that recognizes a misfolded protein on the other side and that brings it into the channel.”

That link might be US11, a viral protein identified by Rapoport’s team and, independently, by another group of Harvard researchers. US11, in conjunction with Derlin-1 and VIMP, tricks the cell’s disposal system into treating immune proteins as garbage to be removed. A cellular molecule, similar to US11, also might help turn on the retro-translocation system when it recognizes a misfolded protein, Rapoport concludes. He and

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You Must Remember This

A recent study has shown that people with Alzheimer’s disease, often unable to remember people, places, and events, do retain a form of memory—called “implicit memory”—that enables them to perform simple procedures like tying their shoes or combing their hair. The study also showed not only that patients’ abilities to do such tasks can improve with practice but also that along the way they use a region of the brain that is involved in higher learning—and that had been thought to decline with age.

HHMI researcher Randy L. Buckner and his colleagues at Washington University in St. Louis gave a series of words to several groups of individuals—young adults, healthy older adults, and those in the early stages of Alzheimer’s disease—and asked if the object represented was living or nonliving. All groups answered more quickly with practice, an example of implicit learning. Brain scans showed that, surprisingly, the subjects’ brain activity during these exercises was greatest in the frontal cortex, which is associated with sophisticated functions such as strategic thinking and problem solving.

The researchers certainly didn’t expect to see changes, much less large, positive ones, in that region for the Alzheimer’s patients, says Buckner. “This study showed us that cognition in the higher-level brain regions can be modified in healthy adults and Alzheimer’s patients alike.” The researchers reported their findings in the June 10, 2004, issue of Neuron.

LARGE Expressions

Researchers studying congenital forms of muscular dystrophy have found that expressing high levels of a sugar-adding enzyme can restore normal muscle function in mice.

The scientists, led by HHMI investigator Kevin P. Campbell at the University of Iowa Carver College of Medicine, expressed the gene for the enzyme called LARGE in cells from patients with several different types of muscular dystrophy. Normal function of alpha-dystroglycan (an important protein needed for structural support in muscle) was restored in these cells.

“It was surprising that when we used cells of patients having defects in several different glycosylation enzymes, LARGE was able to bypass those defects and rescue the phenotype,” says Campbell. “Expression of LARGE produced functional dystroglycan that could bind to its extracellular ligands.”

Campbell sees the potential for new medical approaches: “A drug that stimulates LARGE might be able to restore alpha-dystroglycan function and be used as therapy for these kinds of muscular dystrophies.”

The Campbell team’s work appeared in the July issue of Nature Medicine. In a related paper in the June 25, 2004, issue of Cell, Campbell and his colleagues outlined LARGE’s role in processing alpha-dystroglycan.

Family of Man

We may have more in common with rats, mice, chickens, and dogs—evolutionarily speaking—than previously thought. In scanning genome sequences of these creatures, researchers turned up something unexpected: long patches of DNA that have been passed down unchanged over hun-
his colleagues reported their findings in the June 24, 2004, issue of Nature. Because “we still don’t understand the process very well and we have not yet identified all the players involved,” Rapoport says, he has many unanswered questions—including whether there are other pathways for retro-translocation.

Smallpox’s Manhattan Project

The effects of a smallpox-virus release by terrorists, though potentially disastrous, could be minimized by a broad collaboration to develop new treatments and vaccines, according to a recent National Academies report by a panel of leading scientists. “We recommended a vigorous antiviral and drug-discovery program,” says HHMI investigator Stephen C. Harrison of Harvard Medical School, lead author of the report. “We concluded that such an effort could be mounted—largely through the pharmaceutical industry, but with heavy participation by academe, the NIH, and the Centers for Disease Control and Prevention—because there are identifiable drug targets on the virus.”

The panel considered research needs on three levels: following through on identifying the virus’s key enzyme targets; expanding scientific knowledge of its basic cell biology, including how the virus infects and replicates; and clarifying virus pathogenesis—that is, how smallpox kills (the disease was eradicated before a great deal was known about that process).

Harrison believes the experience gained over the past 20 years in addressing the AIDS epidemic will prove invaluable in combating smallpox. “The work on HIV has given us an intellectual model and a context for what we need to know in dealing with other infectious agents,” he says. At the same time, fields such as biotechnology and structural biology, and our understanding of infectious diseases, have matured.

The report, based in part on a two-day workshop at the National Academies in June 2003, appeared in the August 3, 2004, issue of the Proceedings of the National Academy of Sciences. HHMI investigators Michael E. O’Donnell at the Rockefeller University and Peter Walter at the University of California, San Francisco, were among the coauthors.

The team of scientists, led by HHMI investigator David Haussler at the University of California, Santa Cruz, found perfectly matching regions of 200 to 800 DNA bases in the genomes of humans, rats, and mice. The same stretches of DNA are 95 percent and 99 percent identical in the chicken and dog genomes, respectively.

Such results “cannot be due to just chance,” says Haussler, whose team found 481 such stretches of DNA bases in humans, rats, and mice. These sequences must have been under relentless selective pressure not to change in the evolution of vertebrates, he says. “We still want to know what these conserved regions are doing, and what molecular mechanisms would require such conservation.” Haussler speculates that most of these “ultra-conserved” DNA regions probably don’t code for proteins but instead may play a regulatory role.

The researchers reported their findings in the May 28, 2004, issue of Science.

Pox Boxer

Scientists led by HHMI international research scholar Gurasegaran Karupiah and colleagues at the John Curtin School of Medical Research at the Australian National University have identified the immune-system malfunction that causes some mice to be more susceptible to mousepox, a viral cousin of smallpox.

It turns out that mice that resist mousepox generate three types of regulatory proteins, or cytokines, that are released by immune-system cells to mount an immune response. These cytokines are interferon gamma (IFN-γ), interleukin-2 (IL-2), and tumor necrosis factor (TNF). Susceptible mice make hardly any of these molecules.

When the researchers removed some of the cytokine molecules from resistant mice, they found that only IFN-γ was critical to a good immune response. Therefore, “we think we can use IFN-γ as an antiviral against poxviruses,” says Karupiah. “By combining IFN-γ with other cytokines and antivirals, we can stimulate the immune response of the individual to fight the infection and stimulate immune memory.”

While Karupiah hopes these findings can provide insights into how to improve protection against smallpox, he stresses that the value of the results can extend beyond poxviruses. “We want to use this as a model to understand infections in general,” he says.


Squeeze Play on DNA

DNA needs to be tightly squeezed together before a cell divides, but some of the molecular details behind the process have remained sketchy. Now, scientists studying proteins called condensins have cleared up one part of the mystery.

HHMI investigator Carlos Bustamante, at the University of California, Berkeley, and his colleagues knew that condensins were somehow involved in compacting (“condensing”) DNA and were needed for chromosomes to maintain proper cell division. To see if they could learn more about the condensation process, the researchers intermittently applied force to a single molecule of DNA with condensins attached, alternately stretching it and relaxing the pressure. They found that the DNA continued to bounce back, again and again, to a condensed form in a stepwise fashion.
Evolutionary Insights

Researchers have discovered that the mechanism for transporting infection-fighting antibodies from mother to young evolved independently at least twice, taking different paths in different species. While they’ve identified a protein receptor, FcRY, that moves antibodies within an egg to a developing chick, they’ve observed that it is a very different molecule from FcRn, its counterpart in mammals, including humans.

HHMI investigator and evolutionary biologist Pamela J. Björkman and her colleagues at the California Institute of Technology have been especially intrigued by FcRn’s close resemblance to a set of immune-system proteins called the major histocompatibility complex (MHC). These proteins spot foreign peptides, such as those from invading bacteria, and trigger immune-system T cells to action.

“We were interested in finding out how far back in evolution an MHC-related Fc receptor appeared, which might give us some idea of the primordial function of the MHC fold—peptide binding or antibody transport,” Björkman says.

The researchers decided to look for clues in other vertebrates. Earlier studies had shown that an avian antibody was transferred from hens to chicks through an unknown receptor. When they isolated this receptor, which they dubbed FcRY, they discovered it resembled an unrelated protein, phospholipase A2 receptor, which is found in muscle.

Björkman concludes that evolution apparently has used two completely different molecules, FcRY and FcRn, to accomplish similar functions. “This implies that the transport of antibodies from mother to young evolved independently in mammals and in birds and reptiles,” she says.

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These results, though revealing, are only preliminary. “We’d like to know more about the condensin-DNA interactions,” says Bustamante, “and see these and other processes involved in manipulating DNA and chromosomal structure in more detail.”

The scientists reported their results in the July 9, 2004, issue of Science.

Circuit Riders

Researchers have discovered three proteins that work together to control the number of energy-producing mitochondria within the cell. The work may prove especially important for those who have or are at risk of developing type 2, or adult-onset, diabetes.

Previous studies by former HHMI postdoctoral fellow Vamsi K. Mootha at the Whitehead Institute for Biomedical Research, as well as by others elsewhere, showed reduced mitochondrial gene expression in the muscles of diabetics and in those predisposed to the disease.

More recently, Mootha and his colleagues used microarray analysis to monitor the expression of more than 10,000 genes at once. Then, using advanced computational and comparative-genomics tools, they discovered that the transcriptional coactivator PGC-1 alpha (which affects mitochondrial gene expression in muscle), together with two other proteins, forms a regulatory circuit that instructs the cell to make mitochondria.

“No one had seen this circuit before,” says Mootha, now an assistant professor of systems biology at Harvard University. “A drug that could activate the circuit may be one way of combating diabetes.”

Mootha and his colleagues reported their findings in the April 27, 2004, issue of the Proceedings of the National Academy of Sciences.

Resistance Fighter

Gleevec, the highly touted medication for chronic myeloid leukemia (CML), fails about 15 percent of the time as patients develop genetic mutations and resistance. But a new compound, BMS-354825, now under development by Bristol-Myers Squibb in Princeton, New Jersey, may remedy that situation.

HHMI investigator Charles L. Sawyers and colleagues at the Jonsson Comprehensive Cancer Center at the University of California, Los Angeles, have shown that BMS-354825 eliminated Gleevec resistance in mice with CML. And in subsequent test-tube studies, the compound blocked the proliferation of cancerous bone-marrow cells obtained from human Gleevec-resistant CML patients.

In CML, a chromosomal switch during blood-cell development forms a mutant kinase enzyme, BCR-ABL, that fuels an out-of-control proliferation of white blood cells. Gleevec’s mode of action is to stop CML by binding to and disabling BCR-ABL while it is still inactive.

But some mutations change the kinase’s flexibility, locking it into the “on” position, which renders Gleevec ineffective. BMS-354825, however, binds to the active form of BCR-ABL instead. Combining Gleevec and a kinase inhibitor such as BMS-354825 thus might be the answer for CML, says Sawyers.

The findings were reported in the July 16, 2004, issue of Science.
**Hormone Work Wins Lasker**

Ronald M. Evans, an HHMI investigator at the Salk Institute for Biological Studies, won the 2004 Lasker Basic Medical Research Award. He shared the award with Pierre Chambon of the Institute of Genetics and Molecular and Cellular Biology in Strasbourg, France, and Elwood V. Jensen of the University of Chicago and University of Cincinnati College of Medicine for research illuminating how hormones act on receptors within the nucleus of the cell to control gene expression.

Evans discovered a large family of hormone receptors (PPARs, or peroxisome proliferator-activated receptors) that help control sugar, salt, calcium, and fat metabolism in the body, and thus are primary targets for treatment of diseases such as cardiovascular disease and cancer. Evans’ work led to the identity of a new hormone implicated in the formation of fat cells. Study of the new hormone is giving insight into the problems arising from excess weight and obesity, which may open avenues for treatment of type 2 diabetes and atherosclerosis.

The Albert Lasker Medical Research Awards were created by philanthropists Albert and Mary Lasker post-World War II to raise public awareness of the value of biomedical research to a healthy society. Often called “America’s Nobels,” the awards are considered some of the most prestigious and coveted honors in medical science.

**Five Elected to Institute of Medicine**

Four HHMI investigators and one member of HHMI’s scientific review board were named members of the Institute of Medicine of the National Academies in October 2004. The investigators are Mark M. Davis, Stanford University School of Medicine; Helen H. Hobbs, University of Texas Southwestern Medical Center at Dallas; Charles J. Sherr, St. Jude Children’s Research Hospital; and Arthur Weiss, University of California, San Francisco. The board member is Tony Hunter, of the Salk Institute for Biological Studies.

**David Baker**, an HHMI investigator at the University of Washington School of Medicine, shared the 2004 Foresight Institute Feynman Prize Award with Brian Kuhlman, a researcher at the University of North Carolina. The two won the award in the category of theory in advances in nanotechnology for their development of RosettaDesign, a program used in the design of stable protein structures.

**W. Emmett Barkley**, director of laboratory safety at HHMI, was selected by the National Safety Council to receive the 2004 Distinguished Service to Safety Award in recognition of his outstanding service to HHMI, to the council, and to the safety profession.

Barkley also won the 2004 Arnold G. Wedum Distinguished Achievement Award, the highest honor given by the American Biological Safety Association.

**Carolyn R. Bertozzi**, an HHMI investigator at the University of California, Berkeley, received the 2004 Iota Sigma Pi Agnes Fay Morgan Research Award. Given annually, the award honors a woman chemist or biochemist, 40 years of age or below, for research achievement in her field.

**Suzanne Black**, an HHMI-supported teacher at Inglemoor High School in Kenmore, Washington, is one of two science teachers in Washington to receive the 2004 Amgen Award for Science Teaching Excellence.

**Carlos Bustamante**, an HHMI investigator at the University of California, Berkeley, received the 2004 Hans Neurath Prize, given for a singular or original discovery in the field of protein science.

**Six HHMI investigators and one international research scholar were recently elected fellows of the American Academy of Microbiology. The investigators are Kevin P. Campbell, University of Iowa Carver College of Medicine; Stephen J. Elledge, Harvard Medical School and Brigham and Women’s Hospital; Joseph Heitman, Duke University; Dan R. Littman, New York University School of Medicine; Erin K. O’Shea, University of California, San Francisco; and Arthur Weiss, University of California, San Francisco. The research scholar is Pascale Cossart, Institut Pasteur in Paris, France.**
Two HHMI investigators were among five researchers who received the 2004 Prince of Asturias Award for Technical and Scientific Research. Recognized as one of the world’s most important lifetime achievements in science, the award was given this year to researchers at the forefront of the fight against cancer.

The award went to Joan Massagué, an HHMI investigator at Memorial Sloan-Kettering Cancer Center, for his work identifying factors that trigger cell transformation and those that inhibit cell proliferation, and to Bert Vogelstein, an HHMI investigator at the Johns Hopkins University School of Medicine, for his discoveries that describe the roles of some of the most common cancer genes. The other co-recipients include Judah Folkman of Children’s Hospital Boston and Harvard Medical School, Robert Weinberg of the Whitehead Institute for Biomedical Research and the Massachusetts Institute of Technology, and Tony Hunter of the Salk Institute for Biological Studies, who is a member of HHMI’s scientific review board.

The Prince of Asturias Foundation awards, given in areas as diverse as the social sciences, international cooperation, and the arts, aim to extol “scientific, technical, cultural, social, and humanistic work carried out by individuals worldwide.”

Five HHMI investigators and one medical advisory board member were elected fellows of the American Association for the Advancement of Science. The investigators are Richard H. Ebright, Rutgers, The State University of New Jersey; Joseph Heitman, Duke University Medical Center; Richard L. Huganir, the Johns Hopkins University School of Medicine; Morgan Sheng, Massachusetts Institute of Technology; and Bruce D. Walker, Massachusetts General Hospital. The board member is Gregory A. Petsko, Brandeis University.

David Ginsburg, an HHMI investigator at the University of Michigan Medical School, received the 2004 ASCI Award from the American Society for Clinical Investigation for advancing our understanding of the molecular basis of blood clotting, and for his education and mentoring of future researchers and clinicians.

Two HHMI investigators were elected in 2004 to the American Philosophical Society. The investigators are H. Robert Horvitz, Massachusetts Institute of Technology, and Donald F. Steiner, the University of Chicago Pritzker School of Medicine.

Ronald R. Hoy, an HHMI Professor at Cornell University, received the Association of Neuroscience Departments and Programs’ 2004 Education Award, given for outstanding efforts in neuroscience education.

Thomas M. Jessell, an HHMI investigator at Columbia University College of Physicians and Surgeons, won the 2004 Robert J. and Claire Pasarow Foundation Award for his contributions toward understanding the basis of neuropsychiatric disease.

Simon W.M. John, an HHMI investigator at the Jackson Laboratory in Bar Harbor, Maine, received the 2004 Lewis Rudin Glaucoma Prize in recognition of his work on the genetic basis of primary congenital glaucoma.

Louis M. Kunkel, an HHMI investigator at Children’s Hospital Boston, won the 2004 William Allan Award from the American Society of Human Genetics. The award recognizes far-reaching scientific contributions to human genetics over a sustained period of inquiry.

Pedro Labarca, an HHMI international research scholar at the Center for Scientific Studies in Valdivia, Chile, received the Chilean 2004 National Prize in the Natural Sciences for his contributions to research and education in the field of neurobiology.

Thomas Litwin, director of the HHMI undergraduate science education program at Smith College, is one of 20 academic environmental scientists in the U.S. to receive a 2004 Aldo Leopold Leadership Fellowship, which provides intensive training in communicating science to non-scientific audiences.

Roderick MacKinnon, an HHMI investigator at the Rockefeller University, received the 2004 Max Tishler Prize from Harvard University for his discoveries related to the structure of ion channels. MacKinnon also won the 2004 Bijvoet Medal for Outstanding Research from the Bijvoet Center for Biomolecular Research in The Netherlands.

Emmanuel Mignot, an HHMI investigator at Stanford University School of Medicine, received the 2004 W. Alden Spencer Award from the College of Physicians and Surgeons of Columbia University in recognition of outstanding research contributions in neural science.

Christopher Miller, an HHMI investigator at Brandeis University, was selected to receive a 2004 John Simon Guggenheim Memorial Foundation Fellowship for his work on the structure of potassium and chloride channels.

Two HHMI-supported researchers are among the 23
Fuchs Honored for Pioneering Research

Elaine Fuchs, an HHMI investigator at the Rockefeller University, was selected to receive the 2004 Dickson Prize in Medicine. Awarded annually by the University of Pittsburgh School of Medicine, the honor highlights “paradigm-shifting” biomedical research by a scientist or physician in a still-unfolding career.

Fuchs explores the mechanisms by which skin stem cells both self-renew and commit to proliferate and differentiate along a particular lineage. Adult skin must be able to repair wounds, replace cells lost in normal wear and tear, and hairs—which arise from cells within the skin—must be regenerated periodically throughout life. Fuchs hopes to extend an understanding of the normal biology of skin stem cells to an understanding of how these processes go awry in human diseases of the skin, including genetic diseases and skin cancer.

MacArthur Fellows for 2004. Known popularly as the “genius” grants, the awards from the John D. and Catharine T. MacArthur Foundation encourage recipients to exercise their creative instincts for the benefit of human society. Before joining the faculty at Harvard Medical School and Massachusetts General Hospital, Vamsi Mootha was supported twice by HHMI, first as an HHMI-NIH research scholar and later as a physician postdoctoral fellow at the Whitehead Institute for Biomedical Research. Now on the faculty at Stanford University, Julie Theriot was an HHMI predoctoral fellow at the University of California, San Francisco.

Sean J. Morrison, an HHMI investigator at the University of Michigan Medical School, won the 2004 Presidential Early Career Award for Scientists and Engineers, an award given by the U.S. government to honor and support promising scientists and engineers at the outset of their independent research careers.

HHMI investigators Erin K. O’Shea and Jonathan S. Weissman, both at the University of California, San Francisco, were co-recipients of the Protein Society’s 2004 Irving Sigal Young Investigator Award.

Julio Ramirez, research director of the HHMI undergraduate science education summer program at Davidson College, was one of eight educators named in 2004 to receive the Director’s Award for Distinguished Teaching Scholars from the National Science Foundation.

Rebecca R. Richards-Kortum, an HHMI professor at the University of Texas at Austin, received the 2004 Sharon Keillor Award for Women in Engineering from the American Society for Engineering Education and the 2004 Piper Professor Award from the Minnie Stevens Piper Foundation.

Matthew P. Scott, an HHMI investigator at Stanford University School of Medicine, won the 2004 E.G. Conklin Medal from the Society for Developmental Biology for his “distinguished and sustained research” in developmental biology.

Joan A. Steitz, an HHMI investigator at Yale University School of Medicine, received the 2004 Howard Taylor Ricketts Award from the Society for Virology.

Thomas C. Südhof, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas, was selected to receive the 17th annual Bristol-Myers Squibb Freedom to Discover Award for Distinguished Achievement in Neuroscience Research.

Roger Y. Tsien, an HHMI investigator at the University of California, San Diego, won the 2004 Keio Medical Science Prize from Keio University, Japan’s oldest private university.

Peter Walter, an HHMI investigator at the University of California, San Francisco, was elected an associate member of the European Molecular Biology Organization.

From the University of Chicago for outstanding accomplishments in medical science. She also won the 2004 RNA Society Lifetime Achievement Award.

Lab to Gridiron Calvin Carlyle, 25, has two loves, football and science. When he was a student at Oregon State University, he did microbiology research in an HHMI undergraduate science education program and played safety for the Beavers the year they won the Fiesta Bowl. Now he’s in the NFL, a cornerback on the practice squad of the Baltimore Ravens. Eventually Carlyle the scientist, the football player. “Now he’s enjoying his football career. You don’t get to be paid to play,” he explains.

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Like begets like. Dogs never give birth to cats, and apple trees won’t sprout from acorns. That is life’s central dogma, arising from the dependable passage of DNA intact and in the same order from one cellular generation to the next. However, tens of thousands of times a day, the DNA in virtually every cell in every living organism, including humans, suffers genetic damage, chemically induced mutations, and simple mix-ups of one form or another.

Some of these genetic mishaps come about during the course of natural cell division; others arise from external sources such as pollution or medication. Too many mutations or alterations and the cell simply dies.

That’s good because it helps to ensure that only healthy cells divide. If a cell with damaged DNA survives, it may convey permanent mutations, perhaps resulting in life-threatening illnesses. Many common cancers, including lymphomas and leukemias, can develop from damaged DNA. Some researchers suspect that Alzheimer’s disease and even aging itself may be the cumulative result of damaged DNA.

Despite the abuse our DNA endures, our individual genomes usually stay basically intact because DNA has a remarkable capacity for repair. Our cells have built-in, highly efficient machinery that finds and fixes “genetic typos.”

Scientists used to think there wasn’t much reason to worry about the integrity of DNA—they believed that innately it was shielded from harm and fundamentally sta-
ble. This immunity, it was reasoned, allowed genetic information to pass reliably from generation to generation.

The damaging effect of UV radiation and X-rays on genetic materials was already recognized in the early part of the 20th century, and the realization that cells sometimes could correct genetic mutations after damage emerged in the 1930s. Nonetheless, says Stanford University geneticist Philip C. Hanawalt, one of the pioneers in the field of DNA repair, “For many years after the discovery of the double-helical DNA structure, we thought the genetic material must be incredibly well-protected and not subject to chemical alteration.” But now, Hanawalt says, “We know that damage to DNA occurs all the time and that DNA repair is essential to maintain the genome.”

In recent years, genetic investigators have discovered more and more about the many ways DNA can be damaged. Researchers have learned much about the complex genetic machinery that cells deploy to fix broken, cut, mutated, and misplaced genetic materials. Out of that evolving understanding has emerged a deeper awareness that DNA is truly dynamic and that responses to genetic damage are nearly as fundamental to life—and health—as is the genetic code itself.

Errol C. Friedberg of the University of Texas Southwestern Medical Center at Dallas wrote the major textbook on DNA damage and repair and has identified a number of the genes responsible for various forms of DNA repair. “The field has exploded,” he says. “What used to be the field of DNA repair is now cellular responses to damage. It spills into other fields all over the place.”

“We know that damage to DNA occurs all the time and that DNA repair is essential to maintain the genome.”

**Bases Astray**

Even during the normal process of cell proliferation, a few hundred of the 6 billion nucleotide bases—the A-, T-, C-, and G-designated...
humongous job.” —STEPHEN ELLEDGE

chemical entities that unzip and synthesize new DNA during replication—are chemically altered or end up in the wrong place. Given the complexity of the process, the fact that some bases go astray or get damaged should not be surprising. Imagine what it would take to duplicate every item in a typical home and then identically arrange the new versions at another location. Mistakes, spills, and breakage would be virtually inevitable. Because the genome contains exponentially more items than any home could hold, “duplicating a cell is like duplicating a small city,” says HHMI investigator Stephen J. Elledge at Harvard Medical School and Brigham and Women’s Hospital. “It’s a humongous job.”

Fortunately, in most environments, base pairs go astray relatively few times during replication of the cell. According to HHMI investigator Paul Modrich at Duke University Medical Center, the error rate during the cell-division cycle typically amounts to no more than 100 to 1,000 mistakes per copy of the entire genome. That would be something like one typo in every 1 million to 10 million keystrokes on a computer keyboard.

Why worry about such a small number of errors? The reason is that in genomic replication even a single typo can be disastrous. “Even a single base change can lead to an inherited disease or predispose a cell to tumor development,” says Modrich.

Not only can mistakes occur during normal cell cycling, but DNA faces threats to its integrity all the time. Exposure to UV radiation, X-rays, and other environmental insults such as tobacco smoke, as well as oxidative free radicals and certain medications, can break DNA strands apart or cause a base to become chemically altered. A 1968 study by James E. Cleaver, a professor of dermatology at the University of California, San Francisco, alerted the scientific world that, without repair, these broken ends and mutations could undermine the stability of the entire genome, with untold results (see sidebar).

Modrich and others followed Cleaver’s finding with the discovery that certain defective repair mechanisms were associated with a common hereditary form of colon cancer. Eventually, it became clear that failed DNA repair lay behind many other types of cancers as well as a variety of inherited neurodegenerative disorders. “We now know that a number of cancer syndromes involve defective repair mechanisms,” Modrich says.

DNA repair mechanisms evolved “a long, long time ago,” Stephen Elledge says, “when we were single cells floating out in the ocean.”

“Duplicating a cell is like duplicating a small city. It’s a humongous job.”

“...Highly specialized repair mechanisms deal with different types, sizes, and regions of damage. Some repairs chop out the bad base or bases on one strand and then patch them, using the intact strand as a template. (That’s called either base excision repair or nucleotide excision repair, depending on how the lesion gets detected and which pathway is used to remove it.) Other types of repair fuse broken ends back together, using a variety of tricks for uncoiling and splicing the strands. Still others use recombination mechanisms in which sections of a broken strand are repaired by using information located on an undamaged homologous chromosome. And other mechanisms chemically change a mutated base back to its original form without breaking the DNA strand. Each repair mechanism requires its own combinations of genes and enzymes.

Several separate repair systems may be required to complete a particular repair task. HHMI investigator David G. Schatz at the Yale School of Medicine notes that “each sys-
into play. At that point, says Schatz, a so-called checkpoint-pathway system acts "as a foreman on the job who says that the caretakers—the carpenters who rushed in to repair the genomic scaffold—didn’t do the job right and the cell has to die."

The checkpoint genes—perhaps most famously the p53 gene, termed a "tumor-suppressor gene"—initiate a ritualistic process of cellular dismantling called apoptosis, resulting in cell suicide. In normal skin cells, for example, sunburn leads to extensive apoptotic responses, and the skin sloughs off its dead cells as peeling, a necessary process for healthy remodeling of the skin and ridding the body of UV radiation-damaged cells that could mutate and become cancerous.

MECHANISM FOR SURVIVAL

Given the presence of DNA repair responses in even single-cell organisms such as yeast, it is likely such processes arose very early in the development of life, perhaps even simultaneously with life itself. "Repair mechanisms evolved a long, long time ago," says Elledge. "When we were single cells floating out in the ocean, we were constantly bombarded by UV light and X-rays. We needed a repair mechanism for survival."

Comparable systems therefore are found in all organisms, making it possible to study repair mechanisms in a wide variety of model systems—for example, by observing the effect of knocking out different parts of the damage-control response in yeast, mice, and other species. The findings can then be applied to exploring the more complex human genome.

Many of these animal studies shed light on fundamental mechanisms underlying diverse diseases and conditions. For instance, studies of the p53, BRCA1, and BRCA2 genes, which are part of the same checkpoint pathway, have shown that when cells with damaged DNA fail to be killed, a cascade of events may follow, setting off unchecked proliferation of damaged cells. "If you get rid of activation of p53," says Elledge, "there's no apoptosis, and that's one of six or seven things that happen in the evolution of cells that are eventually going to turn into a tumor."

While unchecked damage may be the first step in ultimately producing cancer, sometimes a cell will purposely initiate a form of DNA damage for the health of the organism. While working in the MIT laboratory of David Baltimore (who is now president of the California Institute of Technology), Schatz discovered two genes, RAG1 and RAG2, that encode a protein complex—a kind of "molecular scissors"—to make cuts in DNA. The snipped ends of the DNA are joined to DNA segments in other parts of the chromosome, through recombination and end-joining repair processes, to form novel genetic combinations that encode B-cell anti-

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**Early Research**

Early insights into DNA repair came from the study of children with xeroderma pigmentosum (XP), a rare, genetically inherited disease. For youngsters with this condition, brief exposure to even normal daylight rapidly leads to skin cancer and other malignancies. Some call this ultraphotosensitive group “moon children” because on the occasions when they venture outside they must wear head-to-toe UV-proof suits. No cure for XP exists, and few patients survive to adulthood.

In 1968, James E. Cleaver, a professor of dermatology at the University of California, San Francisco, discovered that XP patients lack an essential mechanism, known as excision repair, that in normal individuals acts to correct solar damage to the skin’s DNA. He showed that, absent this damage-repair mechanism, the skin cells of moon children continue to replicate their UV-damaged DNA. Those cells can then mutate into cancerous forms, leading to tumors or neurodegenerative disorders.

Cleaver’s finding surprised many geneticists. The traditional view—that DNA’s tightly bound double-helical structure remained stable and largely inviolable throughout the life of the cell—had already been challenged, but for the first time it was apparent that, without proper repair, propagation of cells with damaged DNA could lead to grave harm. —M.W.
bodies and T-cell receptors. These altered cells can then sense and lead the attack on invading pathogens.

HHMI investigator Frederick W. Alt at Children’s Hospital, Boston, and Harvard Medical School; André Nussenzweig at the National Cancer Institute; and his brother Michel C. Nussenzweig, an HHMI investigator at the Rockefeller University, have been studying the machinery underlying the breaks and recombinations that enable an immune response. DNA is tightly bound in compact bundles, making the mechanics of its unwinding especially hard to study.

“The process [of breaking off and unwinding ends and then reattaching them in the right place],” says Michel Nussenzweig, “is like going into a huge tangle and making a break, and holding the ends together while you untangle and then repair it. It’s incredibly complicated.”

Lymphocytes somehow carry out this DNA chopping-and-rejoining process without recruiting their complete DNA checkpoint machinery and initiating apoptosis. But while the reshuffled DNA enables the immune cells to recognize and respond to foreign invaders, the chopping off and unwinding of DNA ends leaves them at least temporarily vulnerable to unwanted linkages with other loose ends. The ends float freely and may splice together with ends located in other chromosomes, sometimes with unfortunate results; certain genetic translocations of this type lead consistently to B-cell lymphoma, one of the most common forms of cancer. “The system rolls the dice,” says Michel Nussenzweig, “because the value of immune protection is so high.”

**UNEXPECTED THERAPIES**

Better understanding of faulty repair pathways that lead to genomic instability may yield new insights into treating cancer. “The more information we have,” says Alt, “the more chance there is of stumbling onto unexpected therapies.” Knowing that a person carries a defect in a repair mechanism might result in recommendations for beneficial lifestyle changes. For instance, up to 80 percent of women who inherit a damaged version of BRCA1 or BRCA2 will develop breast cancer before age 70. Studies are under way to see if treating these women with the drug tamoxifen before they develop cancer may have preventive benefits.

When people do develop diseases, it actually may be possible to tailor more effective treatments early on for individuals by using knowledge of their DNA repair deficits. “We could segment patients based on who will respond to a particular agent,” says Elledge.

Already, scientists in the United Kingdom have found that the fully functioning version of BRCA1 makes breast cancer cells 10 to 1,000 times more resistant to one type of drug, which works by damaging DNA within cancer cells. However, the gene makes the cells over 1,000 times more sensitive to a second type of drug that works by blocking cell division. Screening breast cancer patients for the functioning of BRCA1 before treatment could make choosing a more effective drug more likely.

Another strategy is to make tumors more sensitive to a therapeutic agent by knocking out part of their repair pathway. Yet another is to manipulate elements in the checkpoint pathway to initiate apoptosis in cancer cells. To address as many of these options as possible, Elledge is providing pharmaceutical companies with checkpoint-pathway genes he has identified; subsequent studies should show whether they might be effective targets for antitumor drugs in one way or another.

Investigators are also looking into gene-therapy techniques to help mimic natural DNA-repair mechanisms; such methods might correct the errors behind inherited disorders such as Huntington’s disease, cystic fibrosis, and sickle cell anemia. A team led by Yale’s Peter M. Glazer has already been able to introduce a specific DNA sequence into a target gene, where it corrects a mutation, in extracts of human cervical cancer cells. The researchers are now pursuing a similar strategy in animal models of the disease. “If you can bind something to the gene, maybe you can use that to change the gene,” says Glazer. “If you change the gene to a new sequence, it is permanently fixed.”

The routine therapeutic use of such genetic interventions remains a distant hope, however, because the DNA damage-control mechanisms still essentially remain a mystery. “Life,” says Friedberg, “is necessarily a delicate balance between genomic stability and instability—and of mutation and repair.” By understanding the mechanisms that keep life in balance, the possibility for repair of damaged human cells—perhaps even repair of the repair system itself—may come closer to reality.
Tracking Cancer in Real Time

A conversation with Owen Witte.

It was, that the sites where the leukemia grows are not where we traditionally had sampled, and that the kinetics of leukemia’s growth can be monitored by this technique. It gives us a better understanding of the pathway of the pathogenesis, and it may give us insights into how to interrupt that pathway as well.

We know that the immune system will rally against cancer. Is this what you’re trying to see?

Witte: If the immune system were so good at rallying against cancer, why do so many patients still die from it? Cancer patients are treated with a variety of therapies. Some get better and stay better, but many others don’t. We’re attempting to quantify the immune response against cancer so that we can understand and amplify that response. And in patients where there is no immune response, we want to use vaccines or other modalities to try and activate it. When we treat a patient with one or another drug therapy, usually the outcome is measured by very crude indicators, such as life or death, the progression of the cancer, or the volume of the tumor. But we’d like to know what is happening to the tumor and the immune response in real time.

By using a PET scan with a patient taking Gleevec for a solid-tumor type called GIST, for example, one can see within a few days that the level of glucose metabolism shows a dramatic decrease in the areas of the tumor. Because tumors thrive on glucose, this means the patient is having a great response. But a significant number of patients don’t actually respond, even after a month of therapy. We often aren’t aware of that, given the means we have today, so we keep treating and treating. But with a PET scan, we can see much more quickly whether a drug is working, and if not we can switch the patient to another drug.

It sounds like this technology could revolutionize drug discovery.

Witte: The ability to see inside the body of a patient or an experimental animal in a kinetically meaningful manner relative to disease progression is a very important concept because the disease isn’t a beginning or an end. It’s a pathway, and the more you understand that pathway the better off you’re going to be in trying to change it. Ultimately, these techniques will greatly reduce the time it takes to evaluate new cancer treatments, improve the detection of responders versus nonresponders, decrease the cost of bringing a drug to market, and, in the process, dramatically improve the lives of patients.

What’s the next step?

Witte: We plan to investigate different types of inflammatory and autoimmune conditions, using PET probes linked to specific antibodies that define different cellular components of the immune system. If you think about cancer as too many cells and cells in the wrong place, autoimmunity is exactly the same thing.

Could that work for bacterial and viral infections too?

Witte: Bacteria, viruses, fungi, anything you can genetically manipulate to put in these reporter genes, you can study in this manner. The number of applications of PET scans will be limited only by the cleverness of the chemists, biochemists, molecular biologists, and clinicians who get together to make a new probe or go in a new direction.

—LINDA MARSA
Full Court Press

Chance favors the prepared mind. It isn’t surprising that this expression, from Louis Pasteur, is one of Stephen M. Cohen’s favorites. Its wisdom has served him well.

Juggling many different responsibilities as the Institute’s vice president and chief financial officer, Cohen, 52, has to be prepared to take advantage of appropriate opportunities as they come along. He manages a range of programs, including the budget, finances, human resources, information technology, internal auditing, purchasing services, building services—even the cafeteria and the fitness center. Each department requires its own expertise, and Cohen has the rare ability to shift mental gears when moving from one area to another.

“One of the things I love about my job,” he says, “is that I get to see the entire Institute, which is like two companies—an investment-management company with $13 billion to manage and a scientific-research organization with 300 labs all over the country. The challenge for me is to engage meaningfully with area specialists in each of these fields. I have to have the ability to integrate departmental priorities and imperatives with a larger business-managerial agenda.”

Ultimately, he adds, “I have to be sure [such integration] serves the Institute, which has a largely academic mission—although the organization is more like a corporation than a university.”

Cohen came to HHMI in 1997 from the Yale University School of Medicine, where he had served as associate dean for administration since 1989. Previously, he was Yale’s director of finance (from 1981) and a senior financial analyst (1977–1981). He also was a senior financial analyst on the New York Stock Exchange in 1977 and an operations analyst for Citibank from 1975 to 1977.

Cohen’s formal education included no medical or scientific training; his present literacy results from on-the-job training, enthusiasm, and that “prepared mind.” A native of Harrisburg, Pennsylvania, he majored in English and history at Penn State, receiving a B.A. in 1974. Two years later, he was awarded an M.B.A. in finance and accounting from New York University’s Stern School of Business.

To gain new knowledge and share ideas, Cohen hosts periodic meetings with his counterparts from places like Harvard and Stanford. Structured as seminars, the meetings explore topics such as e-commerce in the life sciences, investment accounting, and information technology. “We treat these events as consultations to HHMI,” Cohen says. “Even though our guests get a lot out of them, we structure the agenda to make sure we get answers to the questions we are asking.”

Most mornings Cohen arrives by 8:15 a.m., and he tries to leave by 6 p.m. He’s not one to work well into the evening, although he relishes an occasional late night. “You want a job where you occasionally stay until 9 p.m.—then it’s a good engaging job,” he says. “But you don’t want to stay until 9 every night.”

“I do a lot of work at home,” Cohen acknowledges, but there are limits. “I do writing and conceptualization there—creative stuff. What I don’t do at home is crunch numbers, or e-mail.”

He and his wife Debbie Friedman, an online teacher for the State University of New York, have two teenage sons: Adam, 15, and Josh, 13. The family is active in several Jewish community organizations and has visited Israel many times.

When it comes to exercise, Cohen’s “one activity” has been regular basketball for the past four years with the same group of men. They work out two mornings a week at a friend’s personal half-court gym. “I love this sport,” he says, and he plans to stay with it, despite what he calls his “questionable” knees and numerous past injuries.

Two years ago, during one of his basketball games, Cohen took a break. He found himself on the bench with another regular, Rome Hartman, a writer and producer for 60 Minutes correspondent Lesley Stahl.

Turning to Hartman, Cohen asked: “Are you working on any interesting stories lately? I’ve got a great one for you. What’s the most important institute you’ve never heard of?”

“Tell me,” Hartman replied.

That chance encounter started a chain of activities that brought 60 Minutes to the Institute. Last fall, CBS’s popular TV newsmagazine cast HHMI in a quite-favorable light during a segment on the Institute reported by Stahl and featuring President Thomas R. Cech and several HHMI investigators. Downplaying his own role, Cohen says the program resulted from months of effort by HHMI’s communications and public affairs staff, whose hard work “made it a success.”

“Steve is high energy, and it shows in everything he does,” says David A. Clayton, HHMI’s vice president and chief scientific officer. Clayton saw another of Cohen’s traits at a recent Institute reception, where “Steve revealed his abilities as a terrific comedian with great timing, and we got the full story on his department in the bargain.”

According to those who have seen him at HHMI parties, Cohen also is reputed to be an enthusiastic dancer.

“Unsubstantiated,” he declares.

—MARLENE CIMONS
George W. Thorn

1906–2004

George W. Thorn, a towering figure in the history of Howard Hughes Medical Institute and in American medicine, passed away on June 26 in Beverly, Massachusetts, at the age of 98. Thorn played an integral role in the creation and development of the Institute, and his prominent career as a physician, clinical innovator, and academic leader spanned seven decades.

An early adviser to Howard R. Hughes, Thorn helped guide the formation of HHMI, first in helping to select the group of fellows supported by Hughes before the Institute’s official founding in 1953.

Thorn’s formal tenure at HHMI began in 1955, when he was appointed to the Institute’s medical advisory board. Over the ensuing decades, Thorn served the Institute as a member of the Executive Committee, President, Trustee, Chairman of the Trustees, and Chairman Emeritus. He completed his service officially in 1990, but continued as an adviser until 1998.

Thorn’s contributions to HHMI were so varied and his service so long and so vital to the success of the Institute that “it is impossible to measure their full impact,” said Purnell W. Choppin, the Institute’s president emeritus. “He played an incomparable role in shaping the Institute’s future with wisdom, dedication, and great good humor.”

Born in Buffalo, New York, on January 15, 1906, George Widmer Thorn was educated at the College of Wooster in Ohio and went on to receive an M.D. degree from the University at Buffalo School of Medicine in 1929. A world-renowned endocrinologist, Thorn served for three decades as physician-in-chief at Boston’s Peter Bent Brigham Hospital, a forerunner of Brigham and Women’s Hospital.

Thorn pioneered the use of cortisone for treating Addison’s disease and found a treatment for the disease using an extract of the adrenal cortex. Thorn’s work with the extract—and later on with synthetic cortisone and natural adrenal hormones—paved the way for modern treatment of Addison’s disease and also led to advancements in the treatment of hypertension, rheumatoid arthritis, and diabetes. Among other notable innovations, Thorn was instrumental in bringing the first kidney dialysis machine to the United States and in organizing the medical team that performed the world’s first successful organ transplant (kidney) in 1954.

Thorn’s outstanding medical service resulted in his being named as Hersey Professor of the Theory and Practice of Physic, which is this country’s oldest chair in medicine, at the Harvard Medical School. He was also founding editor and editor-in-chief of the textbook that is now Harrison’s Principles of Internal Medicine, a landmark medical resource.

Thorn authored more than 400 publications and taught at Ohio State University, Johns Hopkins and Harvard medical schools, and the Royal College of Physicians in Great Britain.

Thorn was an active tennis player well into his later years, and his interests extended beyond the bounds of science and medicine to include music—he paid for much of his medical school tuition by playing tenor banjo in a dance band—horticulture, and travel. His heightened desire for discovery led him to expeditions into the interior of active volcanoes.

At their meeting following Thorn’s death, the HHMI Trustees adopted a resolution honoring his life and myriad contributions to the Institute. The statement concludes: “His energy, perspicacity, and acumen have been essential to the Institute’s emergence as the nation’s leading scientific philanthropy, a legacy that will continue to inform and reshape the medical and scientific landscape in successive generations. We shall miss this remarkable man and colleague.”

Helen K. Copley

1922–2004

HHMI lost another of its founding Trustees with the death in August 2004 of Helen K. Copley.

Mrs. Copley was a charter Trustee of HHMI, appointed in 1984 by the Delaware Court of Chancery to oversee the Institute. She served as a Trustee for 11 years, providing wise counsel and thoughtful guidance during a period of sustained growth and transformation for the Institute.

A leading business executive, Copley was chairman and chief executive officer of The Copley Press, Inc., and publisher of the San Diego Union-Tribune for nearly three decades. She retired in 2001 and remained publisher emeritus and chairman emeritus of the company. Copley earned countless awards for her philanthropy and service in community and public affairs.
Until recently, researchers were so focused on fighting “bad” bacteria—such as those that cause cholera, typhoid, and other infectious diseases—that they ignored the more valuable bacteria we also carry around. But in the past few years, researchers have begun to better understand the value of “good” bacteria.

**Kinesins**

Cells employ a set of miniature tools: moving proteins that haul molecular cargo along cellular superhighways. Scientists have worked out high-tech methods for watching and learning how these molecular motors—named kinesins—move. In the process, they’ve gained unforeseen insight into some devastating human diseases.

**Carolyn Bertozzi**

In August 2004, HHMI investigator Carolyn R. Bertozzi set the world of chemistry abuzz. She and colleagues found a new way to study chemical reactions in living organisms, tagging sugars in a way that does not disrupt a cell’s biology. We’ll take a closer look at this researcher’s work.