A mutation in a gene for antibiotic pigments called phenazines makes bacterial colonies grow in this shriveled—but surprisingly symmetrical and predictable—pattern. Dianne Newman’s explanation: Phenazines usually act as snorkels, helping the deepest bacteria in a thick colony breathe without oxygen. Without these molecules, bacteria must arrange themselves in this unusual way so that each cell has direct access to oxygen in the air (see page 26).

Where’s My Snorkel?

ILLUMINATING BIOLOGY
A spectrum of fluorescent proteins won Roger Tsien a Nobel Prize. Now he’s shining his light on cancer cells and memory formation.
It was time to pack up and go home. Alfred Wallace was two thousand miles upriver from the Atlantic Ocean, on the Rio dos Uaupés tributary of the Amazon—farther than any European had ever gone. Since arriving in May 1848, he had spent nearly four years exploring and collecting, but had been laid up the last three months with yellow fever. He was too exhausted to go on. His younger brother, Herbert, who accompanied him up to the Rio Negro, had long before turned back. Unbeknownst to Wallace, Herbert was stricken with yellow fever and died before he could board a boat to England. Wallace had accumulated a large menagerie of live animals—monkeys, macaws, parrots, and a toucan—that he hoped to take all the way to the London Zoo. Their upkeep was killing him. Besides the animals, he also had a couple of years’ worth of specimens, both with him and stored downriver, that he had not yet been able to ship to England for sale. Wallace began to dream of green fields, neat gardens, bread and butter, and other comforts of home. On July 12, 1852, he boarded the brig Helen with thirty-four live animals, many boxes of specimens and notes, and set sail for England.

“I’m afraid the ship’s on fire; come and see what you think of it.” Just after breakfast, three weeks out of port and somewhere east of Bermuda, the captain of the Helen was concerned enough to visit Wallace in his cabin. And rightfully so—smoke was pouring out of the hold.

The crew tried, but could not douse the smoldering blaze. The captain ordered down the lifeboats. Wallace, still weak from his bout with yellow fever, looked on as if the scene was a feverish dream. It wasn’t. He reentered his hot, smoky cabin and salvaged a small tin box and threw in some drawings, some notes, and a diary. He grabbed a line to lower himself into a lifeboat, slipped, and seared his hands on the rope. His pain was compounded when his injured hands hit the salt water. Once in the lifeboat, he discovered it was leaking.

Wallace watched his animals perish, and then the Helen, along with all of his specimens.

From Into the Jungle: Great Adventures in the Search for Evolution, by Sean B. Carroll ©2009 Benjamin Cummings. Reprinted with permission of the author.
Luminosity
Roger Tsien won a Nobel Prize for designing a rainbow of proteins that shine as they do their work within a cell. Now he’s using his fine-tuned aesthetic to cure cancer and flag memory formation. [COVER STORY]

The Macro World of MicroRNA
Short stretches of “junk DNA” are surprisingly influential in preventing or limiting disease — so influential, they are now high on the agendas of many drug companies.

Between a Rock and a New Place
With a wide-angle view of the world and a commitment to follow her fascination, this scientist is making imaginative connections between rocks, bacteria, and medicine.

When Cells Grow Old
Just like a whole person, cells change as they age. Clues about what controls cellular aging are revealing puzzles and possibilities for combating a host of diseases.
Born near Milan, Italy, **RICCARDO VECCHIO** (cover and “Luminosity,” page 14) studied applied and fine arts at the University of Trier, Germany, and then spent a year at the European Institute of Design in Milan. In 1994, he received a Fulbright Scholarship to enroll in the master’s program at the School of Visual Arts (SVA), New York City. Since graduating, Vecchio has been a faculty member in the SVA illustration department. His work has been commissioned for a wide variety of magazines, books, and other media in the United States and abroad. (1)

Writer **CATHY SHUFRO** (“Rocket Man,” page 6) is drawn to scientists because of their intense engagement in their work. She recently returned from the Thailand–Burma border, where she wrote for Johns Hopkins Public Health about health workers who risk their lives to attend births in eastern Burma’s civil war zones. Shufro lives in Connecticut, where she teaches writing at Yale University. (2)

**JIM SCHNABEL** (“Found in Translation,” page 8), who has written features for Nature, New Scientist, and other publications, is embarking on his second career as a science writer. The first tailed off in the late 1990s when he fell in love with a certain beach in the tropical Pacific. Now, after ten years, two very bad typhoons, and a not-particularly-lucrative venture as a Third World real estate developer, he’s finally got paradise out of his system and is living and writing back in the United States. (3)

**JOÃO CANZIANI** (“Chasing Amyloid,” page 10) was born and raised in Lima, Perú; spent his adolescence in Vancouver, Canada; and is now based in Los Angeles. A fascination for different cultures has led him to travel whenever and wherever he can, always with camera in hand. Named by Photo District News as one of 30 emerging photographers to watch in 2005, Canziani frequently shoots for W magazine, Spin, and Travel & Leisure, among others. (4)
A Responsibility to Experiment

Nearly 10 years ago, just after the trustees of the Howard Hughes Medical Institute elected me as the Institute’s fourth president, I had the opportunity to meet with the staff in the auditorium at our headquarters in Chevy Chase. There was an air of anticipation in the room, one that echoed the excitement I felt on the cusp of my new responsibilities to HHMI.

As a long-time HHMI investigator, I had great appreciation for all that had been accomplished under the leadership of Purnell W. Choppin, the man I was about to succeed. I thought—certainly at the time—that I understood the opportunities ahead. Yet, the journey has been more surprising, fascinating, and demanding than I ever could have anticipated in May 1999. I know that my successor, Robert Tjian, can look forward to a similarly challenging and rewarding journey during his tenure.

So let’s step back briefly to that moment in the auditorium—the scene of so many investigator meetings, the backdrop for the Holiday Lectures on Science, a space now named in memory of HHMI’s legendary chief scientific officer, W. Maxwell Cowan. With the benefit of hindsight, the words with which I described HHMI’s impact on my own research could just as easily apply to the role of the Institute within the broader scientific community. As I said at the time, “Howard Hughes has allowed us to go into some new
directions that we otherwise wouldn’t have been able to. In fact, I think really it is more the other way around. I almost felt a responsibility to go in some new directions because we had the advantage of not having to rely on traditional sources of funding.”

The responsibility to experiment has been a recurring theme of the past decade of my leadership. But before I mention a few of the Institute’s recent experiments, I need to say that we experiment within the confines of a set of values—including freedom, flexibility, creativity, and integrity—that define HHMI’s science-based culture.

These values have never been more important. The public has begun to express deep concern about the loss of objectivity that can occur at the intersection of science and commerce. At HHMI, we have remained tough-minded about Institute policies that reinforce our independence from company-funded research. Also, during this event-filled decade, the nation has twice endured periods of great financial upheaval. With the government’s attention focused on issues related to national security, we have seen the impact of continued fiscal and political pressure on federal research agencies, particularly the National Institutes of Health (NIH). HHMI is hardly immune from these currents. Many of our investigators also conduct research that is supported by the NIH, and they are based at medical schools, research universities, and institutes that are best served by steady year-to-year resources. The Institute’s commitment to plan for long-term, consistent funding serves our scientists well.

Given the opportunity to examine our own programs and activities in the light of pressing national needs, I have worked with Peter Bruns to launch several new educational initiatives. The goal has been to expand opportunity in key areas: to promising college students who come from disadvantaged or minority backgrounds, to graduate students who want to study across disciplinary boundaries or to incorporate medicine into their Ph.D. studies, to physician-scientists at a variety of career stages who seek to combine research with clinical practice. On the biomedical research front, I first worked with vice presidents Gerry Rubin and David Clayton to expand the cadre of patient-oriented physician-scientists within the ranks of our investigators and later with Jack Dixon to open the investigator selection process to direct application from scientists. We have also taken steps to ensure that the results of our investigators’ discoveries will be broadly shared within the scientific community.

One of the Institute’s most visible experiments is the Janelia Farm Research Campus, which opened in 2006 along the Potomac River in Ashburn, Virginia. The notion of creating a freestanding campus with a distinct scientific culture arose in conversations I had with my colleagues Gerry Rubin and David Clayton in 1999. We touched on many subjects during those conversations, most notably the HHMI investigator program. That program seemed to be just the right size—a much larger program might have reduced quality and been difficult to manage—so we concluded that additional Institute resources should be invested in a novel direction.

According to our analysis, the research opportunities most challenged at U.S. academic institutions were in the area of interdisciplinary research—bringing physics, chemistry, computer science, and engineering to bear on problems of biology. By building a research facility without departmental barriers, we thought we could catalyze such collaboration. As we looked for a suitable piece of property, we refined the scientific challenges that an interdisciplinary campus might address. The initial goals of what is now the Janelia Farm Research Campus are to understand the neural circuits that enable complex behavior and to create new imaging and computational technologies. Already, the campus has become a hive of activity for the resident scientists as well as for our
investigators and their collaborators from around the world. Janelia Farm has added to—rather than subtracted from—the vibrant culture of HHMI’s investigator program and that, for me, is an important touchstone.

One area where HHMI charted an independent course concerns research involving human embryonic stem cells. HHMI enabled its investigators to work outside the constraints of a national policy that limited the stem cell lines that could be used and barred the development of new cell lines in federally funded research. We made our decision in consultation with outside advisors, including ethicists, and with the support of our Trustees. The care with which HHMI proceeded made the initiative no less bold. Our stem cell investigators, who aim to cure some of our most devastating diseases, have established new cell lines and have published important, sometimes surprising, findings on tissue development and regeneration.

Back in the 1980s, HHMI president Donald Fredrickson believed the Institute should locate its headquarters “inside the Beltway” so that it would be proximate to the NIH and the officials (elected and otherwise) who make important decisions about science policy in the United States. The decision to base HHMI in the Washington, D.C., area was prescient and has facilitated my interactions with the National Academy of Sciences, NIH, National Science Foundation, American Association for the Advancement of Science, and the numerous scientific societies headquartered in Washington. Often we find ourselves facing the same issues about biomedical research in the United States. These issues include the challenges early career scientists face and the cumulative effect of conservative funding decisions on innovation in America.

Last year, the Institute launched a new open-application competition to identify talented researchers at the very beginning of their independent careers, between years two and six of their first academic appointments. We’re betting that unfettered financial support to these Early Career Scientists, coupled with the new interactions they’ll forge as part of the HHMI community, will have a big impact on their ability to develop full-fledged research programs.

This issue of the HHMI Bulletin highlights another response by HHMI to the current research environment. Jack Dixon, our chief scientific officer, has led the creation of the Collaborative Innovation Awards. Using HHMI investigators as the nucleus, we challenged them to assemble teams of scientists to tackle transformational research projects that are too big or too risky for any single laboratory to handle. If a quarter of these efforts succeed, HHMI will have done something worthwhile. That’s the whole point: we don’t want to be assured that these teams of scientists will solve the problem; we do want to ensure that they have the means to explore big questions.

As I prepare to return to my laboratory at the University of Colorado at Boulder—to focus more on some scientific questions of my own—I am deeply conscious of the fact that the Institute’s successes of the past decade reflect the contributions made by HHMI employees around the country, the members of the Medical Advisory Board and other advisory groups, and, most particularly, the Trustees. Led by Hanna H. Gray, the Trustees have ensured that HHMI remains true to its mission as a medical research organization and lives up to the highest standards of excellence. Thank you for the privilege of leading this great organization.
Rocket Man

If you’re a serious surfer—and Steven F. Dowdy qualifies—you act nonchalant as you bob on your board, chatting with other surfers. You don’t let them see you scanning the ocean.

“You’re just looking for the dark little sliver on the horizon and wondering if anyone else has noticed,” says Dowdy, an HHMI investigator at the University of California, San Diego. “You want to get that wave.”

You paddle on your stomach toward the incoming set of swells and choose your wave. As it rises in front of you, you turn toward shore. The water stacks up beneath your board as the belly of the swell meets the reef. When the swell begins to go vertical, you stroke once, maybe twice, to stay with it. The wave crests, then rolls, and you feel your board accelerate.

“It’s an entirely different way of applying my brain,” says Dowdy, who largely devotes his intellect to inventing ways to transport cancer-fighting macromolecules across cell membranes. “You’re really focused. You do not want to get slammed on a coral reef or get hit on the head by a 20-foot wave.”

As your surfboard drops over the lip of the breaking wave, you stand. At the bottom of the wave, you turn your board parallel to the wall of water. The moving water grabs the surfboard fins and shoots you forward through the liquid tube. It’s like getting shot out of a rocket. Once you know you are free and clear, you’re just screaming with joy.

Dowdy has sought out that adrenaline rush all over the world— as far as New Zealand, Indonesia, and Europe. On a trip to Portugal, when his family hit a beach with lackluster waves, a local surfer, who turned out to be a chemist, showed them a better beach. Dowdy later hired him.

Most often, though, Dowdy surfs four blocks from home. He walks down to South Bird Rock in La Jolla, carrying one of nine custom surfboards under his arm. (He buys mostly blue boards, on the theory that his wife, computer scientist Lisa Dowdy, might not notice a new one when it shows up in the “board room,” their converted garage.) Dowdy often surfs with his son and daughter. (Lisa gave up surfing after a board broke her nose.) He celebrated Connor’s 2007 high school graduation with a surfing trip to Sumba Island, Indonesia, where the two also volunteered at a malaria clinic. In July, he plans another graduation trip to Sumba, this time with Kelsey, who is captain of her high school surfing team. Of surfing with his kids, Dowdy jokes, “I’m getting concerned, because they’re starting to take waves that are my waves.”

Raised on Naples Island in southern California, Dowdy first stood up on a surfboard when he was 7. By age 11, his parents let him leave the house at dawn to surf with his buddies before school. The boys would let their motorboat drift until they were far enough offshore to avoid waking the neighbors.
with the revving motor. The friends have been reuniting to surf every year since one of the group died suddenly at age 38.

Dowdy throws a summer beach party for his research team and neighboring labs, where novices line up for lessons. Granted, a beginner may only stay upright for a second, says Dowdy, but “once you get someone standing up, they’re just howling, having a blast.” —Cathy Shufro

WEB EXTRA: Visit the Bulletin online to see video footage of Steve Dowdy and his son surfing off Sumba Island.

Play On

Lacing up his fútbol shoes, for biochemist Fernando Goldbaum, is the first step in rebooting his brain. After that, he may look like he’s just kicking a soccer ball. But as he dribbles, passes, and shoots, he’s uncluttering his mind, the better to concentrate when he gets back to the lab.

“Soccer is very important for me,” says Goldbaum, an HHMI international research scholar at Leloir Institute in Buenos Aires. “Without any doubt, it clears my head.” That’s why, at least once a week, he and about a dozen researchers, fellows, and professors from the institute meet for an after-work match at a nearby indoor field.

Other sports offer fitness, competition, and social interaction, but they’re mere exercise. Fútbol (we call it soccer) is one of Goldbaum’s two lifelong passions—and a family tradition. “My father played soccer and was also a referee,” he says. “And I have an uncle who played professionally, a goalkeeper in the Argentinean leagues for 20 years.”

A devoted amateur player since childhood, Goldbaum has downsized from the full outdoor field to the smaller indoor pitch without regret. At age 48, “The main thing is that, for 90 minutes, you can keep playing with people 10 or 20 years younger than you are. On the big field, that’s almost impossible.”

Goldbaum’s second passion, of course, is science. As a small boy, “I used to make little experiments at home and at school.” He recalls masking the surface of a leaf with a disc of foil cut from the seal on a wine bottle. “You leave it on the leaf for a week, and when you remove it, you have a green leaf with a white circle where the chlorophyll has disappeared, due to the lack of sunlight. That was astonishing for me.”

Decades later, sunlight still fascinates. He and his team are developing vaccines based on their discovery that blue wavelengths in sunlight activate proteins that drive the spread of brucellosis, an infectious disease that costs the Brazilian and Argentinean cattle industry $100 million a year.

The peak moments come, Goldbaum says, when the rewards of the lab and the playing field converge. “One match I’m still proud of was during the first congress of the Protein Society’s Latin American chapter, in Angra dos Reis, Brazil. We organized a match between the Brazilian and the Argentinean researchers. Brazil produces some of the best players in the world, so you can imagine how my team felt when, at the end of the congress, they announced the final score: we beat Brazil, 6 to 3, on their home ground.”

As a fan, he says, “I’ve always followed the Buenos Aires team called Racing Club. They went for many, many years without a championship. Finally, in November of 2001, they won the local cup for the first time in almost 40 years—exactly when I received my first HHMI fellowship. Now, that was a time for celebration.” —George Heidekat
Found in Translation

Bringing the autobiography of a Jewish scientist, who narrowly escaped the Holocaust, to a predominantly Shia Muslim readership is not as quixotic a project as it might seem, insists Pouya Jamshidi.

“Anyone anywhere who is interested in neuroscience will relate to Eric Kandel,” says Jamshidi, an Iranian-born college student who spent last summer in Kandel’s lab at Columbia University College of Physicians and Surgeons. “His contribution is one of a kind and really, to a great extent, his life story is also the story of neuroscience.”

Jamshidi immigrated to California with his parents in 2002 and is now a 26-year-old senior at the University of California, San Diego (UCSD). He calls his time in Kandel’s lab “a life-changing experience. I was surrounded by the most brilliant people, including Eric himself, who is a legend in neuroscience yet was very approachable and friendly.”

Reading Kandel’s book, In Search of Memory, Jamshidi sensed a connection. “Aside from having a tremendous intellect, he has a real passion for science. I like to think that in my own perhaps naïve way I have that passion too.” He decided to translate the book into Persian in the hope that others in Iran, Afghanistan, and the Persian-speaking diaspora will be inspired to choose a life in science.

Kandel, a longtime HHMI investigator who won the Nobel Prize in Physiology or Medicine in 2000, has genuine affection for Jamshidi—“just a wonderful human being”—and, despite concern that the translation venture could absorb too much of Jamshidi’s time, says that “it seems perfectly innocuous, so I won’t stop him.”

Kandel also admits to pleasant surprise that he and Jamshidi turned out to have so much in common: “He’s extremely engaged in the science and very serious. But he’s also very cultured. Unlike most kids of his generation, for example, he likes the music I like”—both are opera aficionados—“and not only that, he’s much more knowledgeable about it than I am.” (Before he emigrated, Jamshidi, at 20, was the assistant conductor of the Tehran Philharmonic Symphony Orchestra.)

Jamshidi, who has been working in a nerve growth factor lab at UCSD, spent the summer studying similar growth factors in Kandel’s signature animal model, the giant sea slug Aplysia californica, as part of HHMI’s Exceptional Research Opportunities Program. He has been invited to return to Kandel’s lab after he graduates. Once there, he plans to start the translation project and begin contacting academic publishers in Tehran, from whom he expects “a very positive reaction.”

Long term, he plans to apply to M.D./Ph.D. programs, with hopes of doing neuroscience within a neurosurgery practice at a teaching hospital. At UCSD’s Center for Neural Repair he’s been participating in animal surgeries to optimize nerve growth factor gene delivery; the techniques could one day lead to treatments for nerve injuries and even neurodegenerative diseases in humans. “In the future these kinds of therapies are probably going to be delivered by neurosurgeons,” notes lab director Mark Tuszynski.

Jamshidi feels the tug of pure neuroscience but finds the blend with a medical career a better fit with his passions. “I love doing surgeries but I also love studying the brain,” he says. —Jim Schnabel
He wrote the classic textbook on the properties of steam, ice, and liquid water in 1969, but David Eisenberg has always remembered his pediatrician father’s aspirations for him—to go into medicine. Moving closer to that dream, Eisenberg recently solved the structure of a disruptive protein called amyloid that accumulates in cells and appears to play a role in diabetes and several mind-robbing diseases, including Alzheimer’s. He’s training up-and-coming researchers in crystallography so they can make a difference as well, maybe even for patients. Dad would be proud.
Chasing Amyloid

Though it’s taken decades, solving the mystery behind the structure of this errant protein has opened a door to new therapies.

After solving the atomic structure of amyloid, David Eisenberg found the same zipper-like conformation in similar segments of 30 disease-related proteins. Now he’s devising new therapies.
SOLVING A PROTEIN’S STRUCTURE—THE THREE-DIMENSIONAL ARRANGEMENT and spacing of its atoms—is vital for understanding its behavior and is a major step in drug development. But coaxing balky proteins to form crystals for x-ray analysis can consume months or years of frustrating effort. Success requires doggedness and creativity; luck helps, too. David Eisenberg, an HHMI investigator at the University of California, Los Angeles, lives for such challenges. Using x-ray crystallography and computational methods, he has specialized in determining the structural details of how proteins bind to other proteins. Now, he is using that knowledge to develop patient therapies.

About 10 years ago, Eisenberg began to attack a particularly stubborn protein—amyloid. A tough, fibrous substance that can accumulate in cells like trash in a landfill, amyloid has been linked to at least two dozen disorders, from Alzheimer’s and Parkinson’s diseases to type 2 diabetes and “mad cow” disease. These proteins—in fact, almost any protein, apparently—can lose their intricate folded configurations and form fine hair-like amyloid “fibrils.”

Understanding the pathology of amyloid would be vastly aided by learning its atomic structure, but for decades this goal was elusive. Amyloid isn’t soluble in water, thereby confounding conventional analysis methods; and it has proved extremely resistant to crystallization, which would illuminate its chemical bonds.

As early as 1935, British scientist William Astbury, who studied the physics of textiles, fired x-rays at strips of poached egg white that he stretched to form amyloid-like fibers. With the x-rays scattering in a pattern that he called “cross-beta,” Astbury inferred that the protein was composed of “beta strands” (short stretches of amino acids) connected by hydrogen bonds to form flat and extended “beta sheets,” packed tightly against each other. It was a good start, but it provided only a rough picture. “Astbury’s analysis tells you that the protein is organized into beta sheets with certain spacing, but it doesn’t tell you where the atoms are,” explains Eisenberg.

In the 1990s, pathologists began making the connection between amyloid deposits in brain cells and several neurodegenerative disorders—most famously Alzheimer’s disease, in which plaques of beta-amyloid are scattered through the brain. It is still unclear whether the deposits directly kill nerve cells or are the end result of a toxic process during fibril formation.

An important lead came, Eisenberg says, when HHMI investigators Susan Lindquist (Massachusetts Institute of Technology) and Jonathan Weissman (University of California, San Francisco) independently showed that fibril formation could be initiated by a segment made up of about 100 amino acid residues at one end of the protein. Following up on this insight, Eisenberg and graduate student Melinda Balbirnie narrowed the segment down to just seven essential fibril-forming residues, publishing the discovery in 2001. “Melinda and I realized that it took only a tiny bit of the protein to form an amyloid fiber,” says Eisenberg. “To me, that was the paradigm paper.”

In the same paper, they reported that the seven-residue segment could also form crystals—a huge breakthrough. “We were thrilled,” he says, “but there was a big problem: the crystals were 50,000 times smaller than the ones we usually work with. They had beautiful faces and edges, but their width was typically around 1 micrometer.” No existing x-ray beam was narrow enough to probe these “microcrystals.”

Then, luck intervened. At a meeting in Grenoble, France, had just launched a new “microfocus” machine that fired a beam of x-rays only 4 micrometers wide—the size of the largest amyloid crystals. By teaming with European scientists, Eisenberg and graduate student Rebecca Nelson got permission to use the new beam line, and in a matter of weeks they had captured the elusive atomic structure.

Published in 2005, the research confirmed in fine detail that amyloid fibrils contain a cross-beta “spine” made of short amino acid chains along which beta sheets assemble, forming a lengthening fiber as more units are added. The surprise was that side chains emanating from the sheets interact closely with each other like the teeth of a zipper, locking the sheets tightly in place.

“That was the ‘aha!’ moment,” Eisenberg recalls. “That’s what causes molecules to form the fibers.” Moreover, this so-called “steric zipper” seals the interface between sheets so that water is excluded, explaining why amyloid is so persistent and insoluble in tissues.

This landmark finding was a jumping-off point for Eisenberg. In a 2007 Nature paper, he reported the discovery of similar cross-beta spines in amyloid-forming segments of 30 disease-related proteins, including the amyloid beta and tau proteins that make up the characteristic plaques and tangles in the brains of people with Alzheimer’s disease. Toxic fibrils formed by human islet amyloid polypeptide are found in the pancreas of most patients with type 2 diabetes, but their atomic structure wasn’t known until Eisenberg reported it in September 2008 in Protein Science.

While much remains to be learned about the role of amyloid fibers in disease, the information gained from their atomic structure has already led to work in Eisenberg’s lab on potential clinical applications. His group is designing molecular caps, for example, to attach to the ends of amyloid fibers. “It is conceivable that capping the end of a fiber might stop it from lengthening and help to break it down,” he explains.

The pursuit of amyloid and potential human therapies marks a departure for a scientist at age 69 whose body of work has leaned away from medicine (in 1969 he published the definitive book on the properties of ice and water). It’s a career move he thinks would have pleased his father.

“He was a doctor and always wanted me to go into medicine,” Eisenberg says. “I thought it was about time I did something that had medical applications.”

FOR MORE INFORMATION: To learn about Eisenberg’s class on x-ray crystallography, see “Crystal Clear” on page 52.
KLF6 as a tumor suppressor gene that is blocked in prostate cancer. The work was an outgrowth of research on the liver; Narla trained, as a medical student and then as an M.D./Ph.D. student, in the laboratory of Scott L. Friedman, a physician-scientist at New York’s Mount Sinai School of Medicine who studies liver disease.

With Friedman and colleagues, Narla, who launched his own lab at Mount Sinai in 2006, recently revealed that KLF6 also encodes a protein that drives cancer development and progression. On the basis of this discovery, they’ve proposed ways to predict a man’s risk of developing prostate cancer and whether the disease, once diagnosed, is likely to recur. In 2008, they designed what may one day be a new treatment for the most aggressive forms of the disease.

In 1998, Friedman was studying a class of cells (hepatic stellate cells) involved in liver healing. His lab group had found that when these cells reproduced, KLF6 was active. Although every cell in the body expressed KLF6, Friedman wanted to know the gene’s role in the liver. As an HHMI medical fellow, Narla engineered mice with overactive KLF6 in the liver.

The mice were unusually small—with small livers. At the time, “we thought, maybe this gene actually tells cells to stop growing,” Narla recalls. He tested the idea and discovered that KLF6 engages well-known cellular machinery for growth suppression—specifically, tumor suppression.

Narla scoured the literature and found that many patients’ prostate tumors have DNA damage in a region of the chromosome that includes KLF6. He dissected human prostate tumors and confirmed that most lacked one copy of KLF6 or had mutations in the gene. Moreover, when he activated KLF6 in cultured tumor cells, the cells grew more slowly.

In human and mouse tumors, he found four versions of KLF6 messenger RNA (mRNA). Cells read the gene like a choose-your-own-adventure story, skipping some parts and transcribing the others into mRNA.

Protein made from the shortest version, called KLF6-SV1, drives cellular changes that speed the spread of tumor cells in mice.

In 2005, the team studied the DNA of more than 3,400 men and found that some have a hereditary variation in KLF6 that changes how cells read the gene. As a result, they make more KLF6-SV1 and some that includes KLF6. He dissected human prostate tumors and confirmed that most lacked one copy of KLF6 or had mutations in the gene. Moreover, when he activated KLF6 in cultured tumor cells, the cells grew more slowly.

In human and mouse tumors, he found four versions of KLF6 messenger RNA (mRNA). Cells read the gene like a choose-your-own-adventure story, skipping some parts and transcribing the others into mRNA.

Protein made from the shortest version, called KLF6-SV1, drives cellular changes that speed the spread of tumor cells in mice.

In 2005, the team studied the DNA of more than 3,400 men and found that some have a hereditary variation in KLF6 that changes how cells read the gene. As a result, they make more KLF6-SV1 and...
less full-length mRNA. A man born with the variation is twice as likely to develop prostate cancer as someone without it. “He makes a little more KLF6-SV1, and over his lifetime that drives the process of cancer development forward,” Narla says.

Genetic testing for this variation may predict a man’s chance of developing prostate cancer.

The researchers also found, in 2008, that patients whose tumors had high concentrations of KLF6-SV1 had a greater chance of disease recurrence. This finding may lead to a test on a patient’s tumor biopsy that could inform treatment decisions.

Narla and colleagues also managed to reverse the effects of KLF6-SV1 in the lab. After inducing prostate-derived tumors in mice, they injected the tumors with small RNA segments, called siRNA, that attach specifically to KLF6-SV1 and prevent it from being translated into protein. The tumors regressed. These siRNA also killed prostate cancer cells in culture. The study was published in the Journal of Clinical Investigation in August 2008.

“It was really Goutham’s work that led us into an understanding of [the gene’s] role in cancer,” says Friedman, who adds that he’s gratified to see his student emerging as an independent scientist. The early career physician-scientist award, part of HHMI’s effort to support young scientists working to translate scientific discoveries into better treatments for patients, will provide five years of funding to Narla’s lab.

Narla believes he’s finally getting close to his goal of helping patients. “As a clinician I was struggling to find a way to apply this information to the patients we see every day with metastatic cancer. When we identified KLF6-SV1 and were able to actually target it and see tumors shrink, I was extraordinarily excited.”

—OLGA KUCHMENT
Roger Tsien won a Nobel Prize for designing a rainbow of proteins that shine as they do their work within a cell. Now he’s using his fine-tuned aesthetic to cure cancer and flag memory formation.
Roger Tsien is ready to move beyond his signature accomplishments. Specifically, the Nobel-prize winning biochemist, who devised a way to use fluorescence to watch proteins in action, wants to change how cancer surgery is done, among other things. But a shift away from glowing proteins is turning out to be harder than he expected. “Maybe it’s foolish,” says Tsien, an HHMI investigator at the University of California (UC), San Diego. It is rather late in his career, he admits, to delve into an unfamiliar clinical problem. The impact of his work makes it easy to see why he feels like he’s swimming against the tide.

“There’s been this great transformation in biology because of the tools that Roger developed,” says HHMI investigator Susan Taylor, a biochemist at UC San Diego who helped recruit Tsien to the school. They have provided “enormous extra information” about how cells function, she says.

The 2008 Nobel Prize in Chemistry, which Tsien shared with Osamu Shimomura and Martin Chalfie, recognized his work developing green fluorescent protein (GFP) markers to watch proteins in action. By manipulating genes from a glowing jellyfish and from corals, Tsien created a set of genes that produce colors ranging from violet to deep red. These genes allow scientists to tag any of the tens of thousands of proteins at work in the body to observe what they do, and, by marking multiple proteins, how they interact. GFP’s utility goes far beyond human biology as well; it’s been used to create bacteria that glow in the presence of arsenic, a significant problem in well water in Southeast Asia, and to identify explosives such as TNT.

Tsien isn’t leaving visualizing of otherwise invisible processes behind, but he’s set his sights on new targets: lighting up cancerous tumors for surgeons and watching memories form. His results on both fronts are promising. At a recent lecture at a Midwestern university, however, when he described his progress, the first question from the audience was about the work he’s been trying to leave behind: “When are you going to devise infrared fluorescent proteins?”

CELLULAR VISION
At a November science meeting at HHMI’s Janelia Farm Research Campus, Loren Looger wanted to set the stage for a discussion of his own research on sensors to study molecular
activity in the brain. So the Janelia Farm group leader described Tsien’s early work, which focused on calcium.

Calcium is an important cellular messenger that controls neurotransmitter release from neurons, governs muscle cell contraction, and plays a role in fertilization. When Tsien was a graduate student at the University of Cambridge, researchers could measure calcium levels in only a few extremely large cells that could tolerate being poked with a needle. During his graduate and postdoctoral studies, Tsien developed fluorescent dyes to measure calcium in most animal cells without having to inject them.

Later that evening, Tsien gave his own presentation, a last-minute addition to the agenda at HHMI’s request, to celebrate his Nobel win. Tsien himself was physically unassuming. Many of the slides were black and white bullet points put together in Janelia’s library that afternoon. The presentation’s color came from Tsien’s passion for his work, a sense of humor about himself, and an interest in others. He explained that he’d decided to speak primarily to the graduate students in the audience.

“I wish to explain how you can screw up and be screwed up and still, some day, accomplish something,” he began.

The first step? “Identify a big problem in biology, preferably one whose solution would assuage a personal sense of inadequacy,” he said. Tsien, who grew up in Livingston, New Jersey, comes from a family of high-achieving engineers—his father was a mechanical engineer and his mother’s brothers were engineering professors at the Massachusetts Institute of Technology (MIT). Tsien’s brother Richard, originally an electrical engineering major at MIT, is now a prominent neurobiologist at Stanford University and former member of HHMI’s scientific review board.

Roger Tsien says that it was a given he’d end up in science, too, but his path to chemistry and biochemistry was in part shaped by his role as the youngest of three brothers in search of his own niche. “Part of the reason [I pursued] chemistry is that it was one of the things that Dad and my brothers didn’t really like,” he says. “Dad’s idea of taking care of the lawn—since he was an impatient mechanical engineer—was to get on his hands and knees and dig up the weeds. I was allergic to them and wanted to pour herbicide on them from a distance.”

Tsien explained that his work inventing calcium dyes at Cambridge was the fourth project within his dissertation; his first three efforts had run into dead ends. When he started work on the calcium indicator he didn’t tell his advisor, who surely would have vetoed the effort. The subterfuge paid off, and his career was launched. Today, researchers around the world rely on the dyes Tsien created to study calcium, pH, sodium, and other molecules.

At the Janelia lecture, he showed an image sequence called the Blush of Conception. When a sperm fertilizes an egg, it initiates a wave of changes inside the cell, including creating an intracellular wave of calcium. Tsien made those processes visible. As the sperm hit the egg, the calcium change was depicted as a wave of color—as if the egg were blushing—washing across it from the point of contact.

Mark Ellisman, a professor of neurosciences and bioengineering at UC San Diego, notes that each time Tsien develops a new tool, in the paper that introduces it he affirms its value by showing how it can be used to understand some substantive problem in biology. “As soon as that’s out, the world picks up on it and starts to use the technology on a large number of problems,” says Ellisman, who collaborates so closely with Tsien that the two sometimes hold joint lab meetings.

“He’s passionate about things that are useful to society,” Ellisman adds. “He’s got a very beautiful orientation to what’s important.”

BEYOND THE JELLYFISH
As a young faculty member at the University of California, Berkeley, Tsien’s personal insecurity drove his next big success. Surely, he thought, the prestigious biochemists and molecular biologists at Berkeley were looking down their noses at a scientist in the physiology department working on a “mere metal ion like
calcium.” So he decided to find a way to use a process called FRET to build indicators of the essential cellular messenger cyclic AMP (cAMP).

When two fluorescent dyes get close to each other, one steals energy from the other, changing the color it emits. The process—called fluorescence resonance energy transfer, or FRET—is particularly useful in watching cAMP split apart the subunits of protein kinases that depend on cAMP.

Tsien began collaborating with Susan Taylor at UC San Diego, the world’s expert on cAMP-dependent protein kinase. But sending samples by FedEx between Berkeley and San Diego wasn’t working, so Tsien moved south.

“Even after the move, it took a year of my people being physically in the lab adjacent to Susan’s for us to make the cAMP reporter work,” Tsien says. “It required laboriously growing bacteria, purifying two proteins, chemically labeling them in a test tube with two different colors, recombining and repurifying them, and then microinjecting them into the cells of interest”—and the cells had to be large enough to handle microinjection.

Tsien fretted over these limitations. There had to be an easier way. With transgenic technologies, he could insert foreign genes into a cell’s genome, making the cell create whatever protein the gene called for. Tsien searched for a gene that encoded a fluorescent protein he could monitor—a daunting challenge. He remembered reading a review of a protein called aequorin, which is produced by the Aequorea victoria jellyfish in the Puget Sound. “[The paper] said if you’re really careful, you can get rid of this awful contaminant, green fluorescent protein,” Tsien recalls. “In despair, I typed green fluorescent protein into Medline in early 1992.”

The rest, as they say, is history. He found that a scientist at the Woods Hole Oceanographic Institution, Douglas Prasher, had just cloned the gene for GFP. When Tsien contacted him, Prasher said his funding was ending and he was stopping work on it, but he agreed to send the DNA for GFP to Tsien. Another scientist, Martin Chalfie also contacted Prasher and received the DNA.

Would the gene only work in the jellyfish? If GFP needed to interact with other chemicals in jellyfish cells in order to glow, it wouldn’t solve Tsien’s problem; he needed a protein he could add to any cell. Chalfie quickly answered that question by creating transgenic bacteria and worms that gloved, showing GFP would glow in other organisms as well.

Tsien immediately recognized the potential of GFP, but he also saw its flaws—it gave a big peak in the UV spectrum where scientists didn’t want to illuminate; the visible blue-green peak was much smaller. And in its native state, five-sixths of it was in a form useless to researchers. He modified the gene, creating a series of highly effective fluorescent proteins in colors from blue to red.

And that Midwestern questioner will be happy to know Tsien’s lab has made progress toward producing an infrared fluorescent protein for viewing cells and organs inside living animals without having to open the animals. (The deep red color of blood obscures the current palette of fluorescent proteins.)

The Nobel Prize can only be split three ways, but Tsien has been adamant about recognizing the contribution of Prasher, who eventually left science and now lives in Huntsville, Alabama.

“If there’s anyone who’s underappreciated, it’s Douglas Prasher,” Tsien says. “If he hadn’t cloned the GFP gene, progress in the field would have been delayed indefinitely. I don’t know anyone else who was working on the cloning.” Tsien and Chalfie flew Prasher and his wife Virginia Eckenrode to Sweden for the Nobel ceremony.

MARKER FOR MEMORY

“Seeing things, that’s sort of buried in my psychic makeup,” says Tsien, explaining why his work is so focused on visualization. Today, he’s applying that inner aesthetic to new problems.

“How and where synapses adapt to create and preserve a memory is one of the most important problems in neurobiology right now,” says Michael Lin, a postdoctoral researcher in Tsien’s lab. When new memories form, unique neural connections, or synapses, grow in size. This process involves new protein production, so Lin and Tsien are working on ways to watch the proteins that are created during synapse growth.

To do this, Lin fuses three genes in sequence: an easy to visualize tag, a protease from hepatitis C virus, and a synaptic protein of interest. The hepatitis viral protease likes to cut off anything attached to its own ends, so, left alone, the triple protein splits itself into its three constituents, leaving the synaptic protein untagged. If Lin administers an anti-hepatitis C drug that cripples the protease, all the fusion protein molecules made after that point remain intact with their tags still attached.

“Seeing things, that’s sort of buried in my psychic makeup,” says Tsien, explaining why his work is so focused on visualization.
The work remains at an early stage, but Lin has used the system to create transgenic flies, labeling a protein that’s known to be a major structural component of synapses. Neither he nor Tsien thinks they’ve found the perfect marker for memory formation, but, says Lin, “We have a good enough candidate to begin work.”

Their “dream experiment” would be to find a master protein that is freshly made when a new synapse is born but is not being continuously refreshed in existing synapses. They would then create an animal with that protein labeled and train it in some task while giving it the anti-hepatitis C drug. All copies of the master protein formed from that point forward would be labeled. By observing where those proteins accumulate, they could see where nerve synapses had formed or expanded. From a practical standpoint this process is a bit more daunting than it sounds. They still don’t know all the proteins that help create synapses. Tsien hopes other scientists will take up the memory question as well, so that they can divvy up the many potential protein candidates.

GLOWING TUMORS
The effort that Tsien calls “foolish,” but is taking most of his energy, is his foray into cancer. It reflects a wish to do something about the disease that claimed the lives of his father and his Ph.D. supervisor.

When surgeons remove a tumor, they rely on their knowledge of how cancer tissues look and feel to remove as much as they can without taking out too much surrounding tissue. “The way we try to tell whether we have gotten it all is to take samples of what’s left and send them to the pathology lab,” says Quyen Nguyen, a head and neck surgeon at the UC San Diego School of Medicine who also works in Tsien’s lab. Examining tissue for cancer can take 10 or 20 minutes per sample, so that process can mean keeping a patient under anesthesia for an extra hour or longer.

Fluorescent proteins don’t help here, because their main advantage is that they can be delivered by gene transfer, which is fine for experimental animals but not human patients. Nguyen and Tsien have found a way to load tumors with synthetic nanoparticles that not only are visible by magnetic resonance imaging but also glow on the operating table, making the edge of the tumor visible to the surgeon. The technology holds promise as a way to detect tumors and improve the success of surgery and, possibly someday, as a way to deliver drug treatment.

(continued on page 56)
The Macro World of MicroRNA

Short stretches of “junk DNA” are surprisingly influential in preventing or limiting disease—so influential, they are now high on the agendas of many drug companies.

by Maya Pines

illustration by IC4 Design
One reason most of us don’t get cancer is that we are endowed with tumor suppressor genes. Until recently, scientists believed these genes operated through proteins that put the brakes on cell growth, thus preventing cancer’s development. Now scientists realize that some tumor suppressor genes do not produce proteins at all. Instead, they make tiny snippets of RNA, called “microRNAs.”

RNA, a nucleic acid, is still best known for its role as a messenger, relaying instructions from the DNA of genes to the parts of the cell that make proteins. But its many other talents keep surprising researchers. MicroRNAs, for instance—short stretches of 21 to 22 nucleotides, compared with the thousands of nucleotides that contain the recipe for making a protein—come from parts of the genome once considered worthless.

When the human genome was sequenced, only a small fraction of it—the “coding” part—seemed important. The coding DNA is first transcribed into RNA and then translated into proteins. Yet this part makes up only 1.2 percent of the 3 billion nucleotides in our genome. The rest was sometimes called “junk DNA” because it didn’t seem to do anything. As David P. Bartel, an HHMI investigator at the Massachusetts Institute of Technology (MIT) points out, however, “You know it’s not junk if evolution has retained it.” In fact, “noncoding” DNA is beginning to look more like a gold mine—a source of many kinds of potent RNA, including forms such as microRNAs (and the different but structurally related small interfering RNAs) that help regulate gene activity.

MicroRNAs influence nearly all aspects of health and disease—the “stemness” of stem cells, cancer, early development, diabetes, viral infections, schizophrenia, heart disease, aging, and Alzheimer’s disease, for example. Bartel calculates that more than half of human genes are regulated by microRNAs.

Underpinnings of Cancer

Cancer researchers in particular are excited by recently uncovered connections between microRNAs and major pathways of the disease. As many as 50 percent of all cancers involve a cellular pathway governed by the p53 gene, a tumor suppressor. When this gene is mutated and fails to produce normal protein, malignant cells can grow wildly. In 2007, five lab groups independently reported that a family of microRNAs called miR-34 sits in the middle of the p53 pathway.

One team, led by HHMI investigators Gregory J. Hannon and Scott W. Lowe at Cold Spring Harbor Laboratory in New York, found that when they switched on these microRNAs in mouse cells there was a rise in cell senescence (a kind of genetic death in which cells lose the ability to replicate—see “When Cells Grow Old,” page 32). Other teams showed that miR-34 could also promote apoptosis (cell death). Both of these responses protect the organism when a particular cell’s DNA is damaged through environmental exposures. By contrast, when researchers decreased the activity of miR-34 microRNAs, cancerous cells survived and proliferated. The scientists hope that before long these microRNAs can be medically delivered to living animals in a safe and efficient manner, to disrupt cancer pathways. Their success could be a step toward preventing or treating the disease in humans.

Tyler Jacks, a cancer researcher and HHMI investigator at MIT, is also looking into the therapeutic potential of microRNAs—particularly those of the let-7 family, which are extremely scarce in cells from mice with lung tumors. To find out whether increasing these microRNAs would reduce the development of cancer, he turned to a mouse model of lung cancer. He had already used such a model in 2005 in a landmark study of the relationship between microRNAs and cancer. (Led by HHMI investigator Todd R. Golub at the Dana-Farber Cancer Institute in Boston and H. Robert Horvitz at MIT, the study showed that microRNAs are expressed at a much lower level in various tumor cells than in normal tissue, but it was not clear why.)

This time Jacks chose mice whose lung tumors looked like those of humans with advanced, non-small cell lung cancer. When researchers in his lab activated let-7 microRNAs that they had delivered into the animals’ lungs, “this dramatically inhibited the tumors’ development,” says Jacks. Unfortunately, the tumors later became resistant to the microRNAs. His group is trying to analyze why that happened.

Other researchers want to use microRNAs as early markers of cancer. Muneesh Tewari and his colleagues at the Fred Hutchinson Cancer Research Center in Seattle showed that microRNAs are present in samples of plasma and serum “in a remarkably stable form.” Thus, it may be possible to monitor tumor-derived microRNAs, which are present in different amounts in healthy individuals and cancer patients, the group reported in the July 29, 2008, issue of Proceedings of the National Academy of Sciences.

Most promising, perhaps, are recent findings that raise the possibility of stopping a cancer’s metastasis—the spread to other organs, which ultimately kills the patient. Last year, HHMI investigator Joan Massagué and colleagues at Memorial Sloan-Kettering Cancer Center identified two microRNAs (miR-126 and miR-335) that were missing in the most aggressive mouse and human breast tumors. When they delivered these critical microRNAs to the breast cancer cells, the tumors lost the ability to spread. And on December 12, 2008, Arul M. Chinnaiyan, an HHMI investigator at the University of Michigan Medical School, reported in Science that his team had discovered that a microRNA called miR-101 must be active to prevent the spread of prostate cancer. When this microRNA is lost, an enzyme called EZH2 that promotes the spread of cancer cells springs into action.

Thus, “for treatment purposes,” Chinnaiyan says, “replacing miR-101 in solid tumors that have lost it could reduce...
their metastatic properties.” This procedure might apply not only to prostate cancer but also to breast, ovarian, and colon cancers as well as to certain forms of brain and lung cancers and leukemia. “The problem is the delivery issue,” he says. “Several drug companies are now working on ways to put specific microRNAs into ailing cells while avoiding healthy cells, where [the agents] might produce some damage.”

Many other diseases besides cancer have been linked to microRNAs. For example, at the Gladstone Institute of the University of California, San Francisco, Deepak Srivastava produced genetically engineered mice that lacked miR-1-2, a microRNA normally found in the animals’ heart cells. These mutants produced offspring with life-threatening holes in their hearts or fatal disruptions in their cardiac rhythms. Srivastava found that other microRNAs were deficient in mice with cardiac hypertrophy, a condition that can lead to heart failure.

In diabetes, however, scientists see the opposite problem: an overabundance of microRNAs. The goal in that case is to silence the microRNAs involved. For instance, researcher Markus Stoffel of the Swiss Federal Institute of Technology in Zurich is collaborating with an American drug company, Aplylam Pharmaceuticals, to develop what they call “antagomirs”—small fragments of RNA that can travel through the body and reduce the expression of certain microRNAs in specific organs. (HHMI investigators Phillip D. Zamore and Thomas Tuschl invented antagomirs in 2004. Zamore, Tuschl, and Bartel are among the founders of Aplylam.)

Idiosyncratic No Longer

No one realized that microRNAs would be so important when the first one was discovered, in 1993, in the microscopic worm Caenorhabditis elegans. Since his time as a postdoctoral fellow in Horvitz’s MIT lab, Victor Ambros had been trying to determine how the mutant gene lin-4 made worms develop abnormally. The gene controlled developmental timing, Ambros (now on the faculty of the University of Massachusetts Medical School) and Horvitz concluded. When lin-4’s function was missing, this timing was off and cells that were supposed to behave as if they belonged to older larvae got stuck at an earlier stage, repeating cell-fate programs that they should have expressed only once.

What lin-4 normally does is turn off another gene, lin-14, as Ambros realized after studying the interactions between lin-4 and lin-14 mutations. Gary Ruvkun, also a postdoc in Horvitz’ lab, cloned lin-14 and found that it encoded a protein which was expressed in juvenile worms. Try as they might, however, Ambros and his colleagues could not find any protein that corresponded to the lin-4 gene.
Then came the breakthrough in 1993, recalls Horvitz. “In his own lab, Victor made the unexpected and remarkable discovery that lin-4 encodes a tiny, 21-nucleotide RNA. He and Gary further showed that this RNA—later recognized as the first microRNA—is complementary in sequence to its target, lin-14.” This observation, Horvitz says, led them to establish “a common mechanism of microRNA control of gene function—that microRNAs act by preventing the translation of their messenger target or targets.”

“The biomedical world, however, remained indifferent,” he notes. Most biologists viewed these molecules as just “weird little idiosyncratic things, limited to developmental timing in worms,” says Joan A. Steitz, an HHMI investigator at Yale University who studies other kinds of small RNAs and is now turning her eye to microRNAs. But seven years later, Ruvkun’s lab cloned another mutant gene, let-7, which also encoded a 21-nucleotide RNA, and—most importantly—found that it is a very ancient gene, conserved in a wide range of animals from flies and sea urchins to humans. (It is the same let-7 microRNA that MIT’s Jacks now hopes to use against lung cancer.) That discovery made scientists take notice.

Shortly afterward, in 2001, a blockbuster series of three articles was published in Science. In these articles, three separate labs headed by Ambros, Tuschl (then at the Max Planck Institute in Göttingen, Germany, and now an HHMI investigator at the Rockefeller University), and Bartel announced the existence of “a large class of tiny noncoding RNAs” with potentially broad regulatory functions in animals. This finding started a deluge of other newly identified microRNAs.

“We keep discovering more of them,” says Bartel, who estimates that humans have at least 500 different microRNAs, which regulate thousands of genes. “Back in 2001, we were happy when we had sequenced 300 small RNAs, 55 of which were unique microRNAs. Now, with high-throughput methods, we can sequence 5 million small RNAs at a time, from humans or any animal we choose. This gives us the ability to find microRNAs that we’d missed earlier.” Those they found early on are highly conserved through evolution, according to Bartel. “As we dig deeper, we get those that are expressed at lower levels and are less likely to be conserved in other animals,” he says.

Nevertheless, in a paper published October 30, 2008, in Nature, Bartel and colleagues at MIT; the University of California, Berkeley; and the University of...
“We keep discovering more of them,” says Bartel, who estimates that humans have at least 500 different microRNAs, which regulate thousands of genes.

Queensland showed the influence of microRNAs throughout history. “MicroRNAs have been available to regulate and shape gene expression as far back as we can go in animal evolution—they might even predate animals,” he says. “They might have helped to usher in the era of multicellular animal life.”

Exploring Biological Mechanisms
To find out what microRNAs do biologically, scientists have been knocking out the known microRNA genes in worms. In December 2007, Horvitz, Ambros, Bartel, and their colleagues reported in PLoS Genetics on a new collection of engineered worms in which 95 of the 115 microRNAs that had been clearly identified in C. elegans were knocked out.

“To our surprise, the worms in general seemed perfectly okay,” Horvitz says. The researchers looked for worms with abnormal behavior or anatomy but, apart from those with deletions in lin-4, let-7, or lsy-6 (identified earlier by Oliver Hobert, an HHMI investigator at Columbia University), they could find only one—a worm that was constipated. This worm’s “abnormally long defection cycle” was caused by the deletion of two microRNA genes, miR-240 and miR-786, both of which are conserved in other species.

“The microRNAs are in families,” Horvitz explains. “If you knock out the entire family, defects can appear,” but knock out just one microRNA and in most cases the worms look normal. To see a change, he says, it takes not just single or even double knockouts, but at least triple, and perhaps six or seven knockouts in a single animal. “We can do this in the worm, but imagine how difficult it would be to do it in a mouse!”

It takes much longer—about a year, as opposed to a week—and costs a great deal more to produce a mouse with a specific gene deletion. Nevertheless, the Sanger Institute in Cambridge, England, is creating a library of knockouts of each of the 500 microRNAs identified in the mouse genome so far. This resource will serve as a counterpart to the National Institutes of Health Knockout Mouse Project for protein-encoding genes. Eventually, stem cell lines of each mouse in each library will be available to scientists who want to use these rodents as models for human diseases.

Meanwhile two studies—one led by Matthias Selbach and Nikolaus Rajewsky of the Max Delbrück Center for Molecular Medicine in Berlin, Germany, and the other by Bartel and Steven Gygi of Harvard Medical School—have measured how proteins change after a cell encounters a specific microRNA. Using a new technique called SILAC (an updated version of mass spectrometry), they examined several thousand proteins and concluded, in the September 4, 2008, issue of Nature, that a single microRNA can repress the production of hundreds of proteins—but for each protein this repression is relatively mild.

Yet even small changes in protein expression can make a huge difference, says Phillip Zamore, an HHMI investigator at the University of Massachusetts Medical School. Zamore is comparing highly selective microRNAs with those that control many genes at once. He points out that animals in which researchers have knocked out a gene—such as the fruit flies he studies in his own lab—are well fed and live in temperature-controlled environments. But what if a fly that is less than perfect is put in a normal environment? “In real life, flies experience noonday sun and cold nights. And their proteins are distributed in gradients that change with temperature,” he says. “Something may look like a small defect in the lab, but you know what would happen in the wild? The fly would die.”

Everything depends, of course, on the microRNAs’ targets. MicroRNAs cannot act until they have paired with complementary nucleotides on a cell’s messenger RNA. Once paired, they generally shorten the lifetime of the messenger RNA, or block or weaken its instructions, thus reducing the output of protein. Now, one big push in microRNA research is to find an efficient method of zeroing in on their targets.

Several groups have developed computer programs that predict which genes are the most likely targets of each microRNA. To evaluate these programs and find targets that might be missed by the programs, researchers are also developing new methods for identifying targets.

At Stanford University, for instance, HHMI investigator Patrick O. Brown is using the kind of microarray chips he pioneered 14 years ago to examine large quantities of messenger RNA, looking for targets. Brown’s results show that a program developed by Bartel and colleagues does a relatively good job of predicting the targets of the microRNAs he examined. He suggests that many segments of messenger RNA are ready to be “tuned” by specific microRNAs, as needed, in a “continuous scale of regulation.”

And former HHMI investigator Jennifer A. Doudna (now at Genentech) is attacking the problem from a different angle. Her UC Berkeley lab is looking at what allows a specific microRNA to target a specific messenger RNA in terms of both nucleotide sequence and structure. “We want to harness microRNAs and manipulate them for use in therapy,” she says. “Potentially, it could have a large impact on the way drug companies develop microRNAs.”
Between a rock and a new place

With a wide-angle view of the world and a commitment to follow her fascination, this scientist is making imaginative connections between rocks, bacteria, and medicine.

by Sarah C.P. Williams
photography by Leah Fasten
Dianne K. Newman grew up embracing two cultures. Born to American parents in Argentina (her father was a diplomat) she took to the transitions with ease: speaking English at home and fluent Spanish when she stepped outside in her adoptive cities throughout Argentina, Venezuela, and Panama. She learned early to let her passions guide her, and she has a gift for convincing others that her ideas are worth pursuing. With these talents, Newman, an HHMI investigator at the Massachusetts Institute of Technology (MIT), has become a scientific diplomat: an ambassador between the once-disparate fields of geology and microbiology.

She has mastered the languages, techniques, and cultures of both fields to get to the crux of her work: using modern bacteria to understand how the first bacteria on earth could have survived.

The only way to extrapolate how—and when—early bacteria thrived is to study the relics they left behind: rocks. So Newman is probing how bacteria interact with minerals. She’s discovered how some bacteria produce energy from arsenic and iron and how they change their environments in the process. She thinks some of the oldest bacteria—those that existed before the atmosphere resembled its current, oxygen-dominated state—relied heavily on iron to drive their metabolisms. Today, the remnants of a long-ago iron-rich environment are visible as striking red hands on ancient canyon walls, and Newman’s work is relevant for explaining those patterns.

“Choosing bacteria that can be worked with genetically really set her apart from her peers in the geosciences,” says her former teacher Thomas Silhavy, a Princeton University microbiologist. “She realized that genetic analysis opens all the doors for molecular sciences. You could argue that she created the field of geomicrobiology.”

Newman’s work has crossed over into modern medicine and ecology as well, helping explain how modern bacteria thrive in unusual settings—from the mucus-filled lungs of cystic fibrosis patients to arsenic-contaminated streams. Learning what keeps the bacteria alive could lead to novel ways to thwart their growth.

FOLLOW THE FUN

As an undergraduate at Stanford University, Newman never guessed she’d end up as a microbiologist. She majored in German studies and took a lot of environmental and materials science classes. “I loved everything, that was my problem,” she says. “I was an advising nightmare.”

She believed in doing what she loved, she says. If one day that was German and the next engineering, why not? That’s still how she conducts research, following the ever-winding path of her interests.

But as graduation loomed, Newman had to nail down something to do next. She chose environmental engineering—a combination of some of the things that had fascinated her most at Stanford—and began a graduate program at MIT.

With no real engineering experience, she had to push to find a professor who would take her on. “Even though I said ‘No thanks’ six or seven times, she really wanted to work with me,” says François Morel, who studies how microorganisms interact with metals—a topic that intrigued Newman. Her persistence paid off and Morel finally became her advisor.

In Morel’s lab though, Newman says she didn’t have the patience for the first project he gave her: studying how phytoplankton respond to silver. Beakers for these experiments needed to be washed repeatedly in acid baths to rid them of metal contamination. “I realized that multiple acid-washing would probably drive me crazy after a while,” says Newman, “so I asked if I could switch.”

Around that time, a senior student in the lab, who had gone all over Boston collecting bacteria that could metabolize high concentrations of arsenic, cleared off her desk. “She gave me one of her bottles of bacteria that was destined to be thrown away,” says Newman. “And it was this really bright yellow color, and the only thing I knew how to do—from my materials science background—was to figure out what compound was making it yellow.”

That’s how Newman stumbled upon the molecules that allow bacteria to metabolize metals. These colorful compounds guided her budding career. But the real pivot point came when Morel moved his lab from MIT to Princeton. Though Newman had planned on only getting a master’s degree and then pursuing patent law, she decided to accompany him and finish her Ph.D., for the fun of it. “I was doing things because I enjoyed them and I was lucky because the rest just took care of itself,” Newman says.

She started seeking out courses in genetics and quickly saw its potential for studying bacterial processes. Newman’s teacher of one short course, “Advanced Bacterial Genetics” at Cold Spring Harbor, was Bonnie Bassler, now an HHMI investigator at Princeton. Bassler says Newman caught her attention with her incredible energy.

“She came with little background in genetics but she knew she wanted to learn it. She was incredibly hard working, and unflappable. After long days of working and studying, everyone else would be too exhausted to move, and she’d bound out the door to go on a jog.”
The 36-year old Newman exudes not only energy, but confidence. She could easily be mistaken for a graduate student: small with dark, untamed hair and glasses perched on her nose, she’s dressed casually in brown and orange earth tones, except for a purple plastic watch. But she owns her lab like no graduate student could. At a recent lab meeting, mid-sentence talking about geology, she leans her chair back and rests her clog-adorned feet on the conference table. She’s perfectly at home here, leading a lab.

Her one-year old son Ronen occasionally makes the lab his home too. A pile of stuffed animals and a rubber duck sit on Newman’s office floor next to a thick textbook entitled “Principles and Applications of Aquatic Chemistry.” Although married to inorganic chemist Jonas Peters, she says science talk is rare at home—especially now, with the baby dominating their time. “Once in a blue moon we’ll talk about science,” she says. “Whenever I have a grant application that requires complex chemical structures, he double checks to make sure I’ve drawn the bonds right. Sometimes he just draws them for me.”

GEOLoGY GOES GENETIC
That summer at Cold Spring Harbor changed the direction of Newman’s research.

“I realized this huge power in genetics that could solve all these problems I was considering,” she recalls. Once at Princeton, she started characterizing the arsenic-metabolizing bacterium, which she named Desulfotomaculum auripigmentum for its gold-colored pigments. At that point, she couldn’t genetically modify the microorganism, but she held on to that goal for later.

Though she worked in a geosciences lab, Newman frequented molecular biology labs to absorb techniques and advice, says Princeton’s Silhavy, who had Newman as a
Iron is noteworthy because it was much more biologically available in the ancient, oxygen-depleted earth than it is today. Most bacteria need oxygen to survive because chemical reactions that give bacteria energy produce excess electrons. Oxygen, an electron acceptor, can collect these spare electrons. Other compounds—like iron—can accept electrons too but the mechanism was unknown before Newman started studying iron-metabolizing bacteria.

Newman had an inkling that the bacteria produced molecules that acted outside the cell as electron shuttles—carrying electrons from the cell and dumping them on iron. Her suspicions were right—she discovered evidence for such an electron shuttle by screening mutants that couldn’t metabolize iron. With that finding, Newman suddenly had a plethora of options for her future.

“Herman Newman arrived in my lab in January, and after she had been there less than a month, she came into my office and told me that she had three job interviews,” says Kolter. “This is how impressive she was. Nobody else at the time was thinking of combining environmental microbiology and genetics. She paved the way for a new field.” In 1998, Newman landed a postdoc position in the microbiology lab of Roberto Kolter at Harvard Medical School. “Her background really caught my eye,” says Kolter, who prides himself in having a diverse team. “I’d never had anyone with a degree in German studies apply to my lab.” She proposed to study bacteria that metabolize not arsenic, as she’d studied with Morel, but iron.

“I must say that I had never thought of studying this,” says Kolter. “When she first brought it up I thought ‘What’s the big deal? Why is this important?’ but after a few minutes with her I was convinced this was the project she should do.”

Iron is noteworthy because it was much more biologically available in the ancient, oxygen-depleted earth than it is today. Most bacteria need oxygen to survive because chemical reactions that give bacteria energy produce excess electrons. Oxygen, an electron acceptor, can collect these spare electrons. Other compounds—like iron—can accept electrons too but the mechanism was unknown before Newman started studying iron-metabolizing bacteria.

Newman had an inkling that the bacteria produced molecules that acted outside the cell as electron shuttles—carrying electrons from the cell and dumping them on iron. Her suspicions were right—she discovered evidence for such an electron shuttle by screening mutants that couldn’t metabolize iron. With that finding, Newman suddenly had a plethora of options for her future.

“She arrived in my lab in January, and after she had been there less than a month, she came into my office and told me that she had three job interviews,” says Kolter. “This is how impressive she was. Nobody else at the time was thinking of combining environmental microbiology and genetics. She paved the way for a new field.”

Newman stayed in Kolter’s lab for two years, though, learning everything she could about genetics before accepting a job at the California Institute of Technology—a joint appointment in geological and planetary sciences and biology.

ANCIENT RECORD, MODERN LUNGS
Today, oxygen makes up a fifth of the atmosphere of the earth and most life forms rely on it. But 3.8 billion years ago—when the earliest evidence of life is recorded in ancient rocks—oxygen was scarce. Nailing down exactly when, and how, the atmosphere transitioned to oxygen requires probing the fossil record for the appearance of microorganisms that produced oxygen. “Dinosaur footprints only go back a short ways,” says Newman. “If you want to understand the evolution of life on earth billions of years before that, you need to understand microbiology.”

Typically, geologists have used molecules called 2-methylhopanoids as markers of oxygen-generating life. They’ve been found in rocks that some claim are 2.7 billion years old. But other geochemical measurements do not find evidence for appreciable oxygen on the earth at that time. At Caltech, and now at MIT, where she moved her lab in 2007, Newman has probed this discrepancy by investigating the role of 2-methylhopanoids in modern bacteria. Her lab has found that the ability of modern bacteria to make these compounds is not related to their ability to generate oxygen. Although more work needs to be done before ruling out 2-methylhopanoids as oxygen markers, Newman says the “case doesn’t look very good.”

The biggest focus in the Newman lab now is one that started as a side project: how the bacteria Pseudomonas aeruginosa...
metabolizes iron. *P. aeruginosa* produces colorful phenazines—compounds Newman identified in her search for molecules that shuttle electrons to iron. But *P. aeruginosa* isn’t relevant only to the ancient earth—it’s the bacteria that most often infect the lungs of people with cystic fibrosis.

The minuscule cellular brushes that normally sweep the lungs clear of mucus are missing in people with the disease. Their lungs build up layers of mucus, a fertile place for bacteria to thrive. These thick layers of mucus have low concentrations of oxygen, resembling the oxygen-deprived atmosphere of the planet billions of years ago.

“Pseudomonads colonize the lungs just like they colonize other surfaces,” says Newman. “They aggregate into multicellular communities that become increasingly resistant to antibiotics with time.”

Since Newman suspects that phenazines are critical to *P. aeruginosa*’s survival in these deep layers without oxygen, she hopes that blocking phenazine cycling might be a way to combat lung infections.

**A RAINBOW OF BACTERIA**
Thanks to the bright colors of phenazines, it’s easy to see whether bacteria are producing them. Newman’s postdoctoral fellow Lars Dietrich shows the drastic effect of oxygen on phenazine production by pulling two beakers of murky bacterial soup off a shaking platform that keeps the liquids constantly churned and exposed to oxygen. One beaker, filled with unmodified *P. aeruginosa*, is the color of green Kool-Aid (other natural *P. aeruginosa* strains produce blue, orange, red, and yellow phenazines). The other beaker, full of almost transparent fluid, is chock full of bacteria that were modified so they can’t produce phenazines. Dietrich sets the beakers on his lab bench and immediately the green in the first sample begins to fade.

“Since it’s not being shaken around to get lots of oxygen, it’s using up the phenazines,” he explains. “Having phenazines is like having a snorkel to breathe underwater.” The bacteria are dumping extra electrons onto the phenazines, which carry the electrons out of the cells.

Phenazines also affect whether *P. aeruginosa* can form biofilms—colonies of bacteria encased in a slimy goop of extra-cellular matrix. Bacteria banded together into biofilms exist on unbrushed teeth, on slimy rocks, and in the lungs of cystic fibrosis patients. Since bacteria in the deepest layers of the biofilms don’t have access to oxygen, they need phenazines to breathe. In the bacterial strain that Dietrich has blocked from making phenazines, the colonies form not smooth films but curly, bumpy, surprisingly predictable shapes that expose every bacterium to oxygen. It’s another finding that links back to cystic fibrosis: if the bacteria without phenazines can’t form normal biofilms when oxygen is limited, they can’t survive in mucus-filled lungs as easily.

Morel says Newman’s diverse background is what allows her to make these imaginative connections. “Her background gives her a lot of different things to think about when she looks at a problem,” he says. Ask Newman how she’s come to make such creative connections in her work, and she brings it back to her parents: “I have to credit them as people who always appreciated new things and encouraged my wide-ranging interests.” On weekends they drove her to high school debate tournaments, and helped her track down materials for science fair projects that emitted horrible noises from the basement, which she says they tolerated happily.

The way she charts her own path is a lesson that resonates strongly in her students. Tanja Bosak, one of Newman’s first students at Caltech and now an assistant professor at MIT, says she tries to model parts of her own lab after Newman’s.

“She let me really develop my own project the way I wanted but was always there to support me and answer questions,” explains Bosak. “She taught me that you can have fun and do science. And that it’s really important to run a lab the way you want—you don’t have to try to be something you’re not.”

Whether it’s the quick switch from English to Spanish to order a sandwich from a Latino cashier at an MIT café, her ability to bridge geology and microbiology, or juggling her work life and home life, Newman moves with ease across several worlds.

“She’s gone from being a student, when I first met her, to the head of a huge lab and a leader in her field,” says Bassler. “But to me she seems exactly the same as that day she walked into Cold Spring Harbor. Her science has certainly grown and changed, but in her spirit, she’s one of the brightest lights in the bacterial community. Her modesty and enthusiasm and talent are always a breath of fresh air.”

As for the future, Newman hesitates to predict where her next research projects will take her, beyond the obvious continuation of her foray into cystic fibrosis. “My hope is that in the longer term, my research will take me in directions I can’t even imagine now,” she says, “because that’s what makes this so fun.”
Just like a whole person, cells change as they age. Clues about what controls cellular aging are revealing puzzles and possibilities for a host of diseases.
Sometimes the evidence of aging hits a little too close to home.

HHMI investigator George Daley, of Harvard Medical School, saw signs of his own mortality as he peered into a Petri dish. Daley has been studying how to coax adult cells into stem cells, using lab members’ cells—and his own.

Adding just four genes can turn adult cells back into embryonic-like cells, able to develop into any cell type in the body, according to Daley’s studies. In culture dishes, cells from a younger postdoctoral fellow in Daley’s group were “youthful and vigorous,” he says; many of them morphed into stem cells. But Daley’s cells were stubborn, refusing to reverse their clocks. It seems as a person ages, cells get increasingly stuck in their ways.

Daley isn’t taking it too personally. “I’m deficient in a lot of things, and reprogramming seems to be one of them,” he says. He plans to use the observation to understand how to reprogram cells most efficiently.

His finding points out an important concept: cells might not sprout gray hair, get achy joints, or forget where they put their car keys, but they do age. Several HHMI researchers are just beginning to learn what happens to cells as they grow old, and they’re making connections between those changes and cancer, deficiencies in wound healing, and other problems that increase in likelihood as a person ages.

A HELP OR A HINDRANCE?
The first inkling that cells might grow old came in the early 1960s, when gerontologist Leonard Hayflick was playing with cells in culture. The conventional wisdom at the time held that cells in a culture dish could split an indefinite number of times. But Hayflick found that after 50 or so divisions—now called the Hayflick limit—cells stopped dividing. The cells turned into zombies. They didn’t die but remained in a kind of hibernation, a state known as cellular senescence.

Over the following decades, researchers elucidated the environmental conditions that push cells to senesce and defined many of the genes and molecules that control the process. Several signals can put cells to sleep. Accumulating DNA mutations can send cells into senescence. Fraying chromosomes also serve as a trigger. Protective caps of DNA called telomeres keep chromosomes from unraveling, but each time a cell divides, its telomeres get a little shorter. When telomeres get too short, chromosomes can break or fuse with other chromosomes, shuffling the genome and possibly causing cancer. A third signal also links to cancer. In 1997, HHMI investigator Scott Lowe of Cold Spring Harbor Laboratory, New York, and his colleagues found that activating a cancer-causing gene forces cultured cells into senescence.

The cancer connection seems counterintuitive. Typically, cancer-causing genes spur cells to divide uncontrollably, not grind to a halt. But Lowe’s result supports the idea that senescence is a cancer-prevention tactic. Cells must accumulate multiple mutations before turning into full-blown cancer. When a cancer gene turns on, senescence might help cells shut down before additional mutations push the cell to become cancerous. However, shutting down cells to fight cancer could hamper health in other ways. For instance, senescence might deplete the pools of cells that replace damaged ones and keep liver, bone, blood, and other tissues working properly. An accumulation of malfunctioning cells and a loss of new ones to replace them could underlie some of the effects of aging. So is senescence good or bad for the body?

In a sense, cancer and senescence are opposite sides of the same coin. To remain robust, tissues rely on dividing cells for replenishment; yet, left unchecked, cell division leads to cancer. Thus, it might seem a Faustian bargain to guard against cancer now at the expense of decrepit tissues later.

Evolution should favor the anti-cancer mechanism, Lowe says. Genes that improve survival early in life should help ensure that an organism reproduces, and thus the genes tend to be passed down to the next generation. That’s true even if the same
genes compromise health late in life. “We didn’t evolve during a period when we lived 80 years,” says Lowe. “The system isn’t built to last that long.”

Some studies on the tumor suppressor gene p53 have supported this idea of a trade-off between cancer and aging. The p53 gene acts as a master controller of anti-cancer mechanisms, including senescence, particularly when DNA incurs damage or telomeres erode. The gene also governs other processes such as a regulated cell death pathway called apoptosis. Several groups of researchers have shown that producing certain forms of p53 dramatically reduces cancer in mice, but those animals age more rapidly than normal.

SENDING SIGNALS
For decades, cellular senescence was thought to be an oddity seen only in the lab. Few studies provided any evidence that senescence influenced aging or cancer in a living creature. “[Researchers in the] cancer biology field felt it was a cell culture artifact,” says Lowe. “Senescence hadn’t been observed in tissues.”

But starting in the mid 1990s, researchers discovered ways to track senescent cells in living tissues, not just in cell culture. For instance, senescent cells produce an enzyme called beta galactosidase. When bathed in a particular sugar compound, cells with this enzyme turn blue, providing a way to spot senescent cells. Since then, researchers have used this method to show that tissues from people, as well as from animals such as rodents and monkeys, carried blue cells. And, “the older you got the more blue cells you had,” says Lowe. Other markers exist, and although none of the markers is exclusive to senescent cells—they tag other things too—these studies have provided robust evidence that senescence is important beyond the culture dish.

Recent research supports the notion that senescence helps divert cells when they start down the path toward cancer. Four studies in 2005 revealed that senescent cells accumulate in precancerous growths such as moles, and quieting senescence pathways suffices to trigger melanoma and prostate cancer. “Premalignant tumors are chock full of senescent cells,” says Lowe. The studies’ findings settled the controversy about whether senescence works to prevent cancer in people, he adds.

Senescent cells might seem to be introverted wallflowers, but new work suggests that they are rather social, communicating and influencing their neighbors. HHMI investigator Michael George Daley (top) aims to efficiently reprogram cells, with help from lessons learned on cellular senescence. Michael Green (bottom) is using senescence-inducing proteins to stop cancer cells from dividing.
Green, of the University of Massachusetts Medical School in Worcester, made the discovery after looking deeper into how senescence prevents melanoma from taking hold. A cancer-causing mutation in a gene called BRAF occurs frequently in melanoma; most benign mole cells also carry the mutation. Like other oncogenes, BRAF spurs cells in culture to senesce. Green wondered what spurred cells carrying BRAF to senesce rather than grow into tumors.

He and his colleagues identified a set of genes that appear to hold mole cells in senescence; when they shut down any of these genes, the cells grew like crazy. Green was surprised to discover that one of the genes makes a protein called IGFBP7 that gets discharged out of the cell. “We were expecting it to be a purely intracellular event,” he says. The team reported its findings in the journal *Cell* in February 2008. Mole cells didn’t need to make this protein themselves to stay quiet. Applying IGFBP7 to the outside of mole cells was enough to keep cells in senescence. The findings suggest that, by spewing IGFBP7, one cell could help its neighbors from turning cancerous.

At about that time, other groups also showed that other types of senescent cells discharge proteins that stall cell division. The results of all the studies are striking, says Lowe. “Senescent cells aren’t just sitting there.”

Melanoma cells don’t make IGFBP7, says Green, but his studies suggest that they still respond to the molecule. Bathing cancer cells in IGFBP7 prodded them to die. Green is exploring the idea of using IGFBP7 as an anti-cancer drug. He will collaborate with scientists at the National Cancer Institute to conduct preclinical studies that will lay the foundation for clinical trials.

**FENDING OFF FIBROSIS**

With mounting evidence that senescence is a cancer countermeasure, Lowe and his team wondered if senescence played a part in another disorder: liver disease. Previously, researchers had noticed senescent cells in diseased livers, and Lowe wondered whether they were harmful or beneficial.

Hepatitis infection, alcohol abuse, and fatty liver disease can bring on cirrhosis, a permanent scarring that undermines the liver’s normal function. A buildup of connective tissue, known as fibrosis, presages cirrhosis. When a liver is damaged, particular liver cells called stellate cells churn out extracellular matrix, the protein structure that forms fibrous tissue. If the cause of liver damage disappears, the stellate cells help other cells grow, repair, and replenish the liver. But a chronic drinking problem or untreated infection puts a liver under constant stress. Under these conditions, fibrosis spreads, cirrhosis sets in, and, ultimately, the liver fails.

Lowe’s recent studies suggest that senescence helps put a damper on fibrosis. His team injected mice with an agent that damaged their livers, spurring fibrosis. In the animals’ livers, senescent stellate cells peppered fibrotic regions, the team reported in August 2008 in *Cell*. In animals that can’t undergo cellular senescence because they lack p53, fibrosis was more pervasive. Lowe posits that senescence hampers fibrosis by preventing stellate cells from growing too heartily and manufacturing a flood of extracellular matrix.

Fibrosis is part of the normal response to repair an injury, and unless a liver suffers continuous insults, fibrosis eventually dissipates. Lowe also found that senescence not only prevents fibrosis, it also helps clear fibrosis once it starts. In normal animals, fibrosis subsided and eventually disappeared once the liver-damaging injections stopped. But in animals without p53—and therefore deficient in senescence—fibrosis persisted.

Researchers have known for a while that senescent cells exude sets of molecules that deplete extracellular matrix. His findings suggest one possible reason for this behavior. “It fits nicely with this puzzling gene expression pattern that’s been around for some time,” he says.
We didn’t evolve during a period when we lived 80 years. The system isn’t built to last that long.

Scott Lowe

Lowe adds that cirrhosis puts patients at increased risk for liver cancer, and he’s trying to understand why, in light of the new findings. Opposing Green’s IGFBP7 finding, previous studies have found that senescent cells produce molecules that spur cancer, rather than prevent it. Senescence might be built to cope with an immediate insult, says Lowe, even if, over the long haul, quieting cells creates havoc. The body “can’t stop everything,” says Lowe.

Once an obscure artifact of cell culture, cellular aging is emerging as a key force that shapes tissue health. And those who want to help patients are taking note. Harvard’s Daley, for instance, wants to understand how to make stem cells and use them to repair malfunctioning tissues. But he’s proceeding with caution. “Reprogramming is, in a sense, reversing senescence,” he says. Researchers must face the “sobering possibility” that doing so will accelerate cancer. Still, Daley is optimistic that “we can harness the process in a way that’s productive.” Hopefully, he won’t have to age much more before the answers become clear.

As the role of senescence in health and disease gains prominence, researchers new to the phenomenon are beginning to look for connections to their own work. HHMI investigator Joan Steitz of Yale University has been fascinated by how small RNA molecules control the activity of genes. Now, she’s starting to look at how this capability ties to senescence.

One particular type of small RNA molecules, called microRNAs, control how much protein a cell makes from a messenger RNA (see “The Macro World of MicroRNA,” page 20). In an actively growing and dividing cell, microRNAs dampen the amount of protein the cell makes. But microRNAs do the opposite and amplify protein production when cells are in a state called quiescence, Steitz’s group has found. Like senescent cells, quiescent cells don’t divide. But, unlike senescent cells, they can reactivate and start growing and dividing again.

“We want to know why microRNAs do one thing when cells are rapidly proliferating and another thing when they withdraw from the cell cycle,” says Steitz. She is now collaborating with Yale colleague Daniel DiMaio to understand how microRNAs work in senescent cells. “Would the same kind of controls be going on in senescent cells?” she asks. “Would it be a different protein that is associated? We don’t know.” In addition to the activity of genes, senescence might tie to the activities of mitochondria. These structures generate power in cells, and David Chan at the California Institute of Technology, who was named an HHMI investigator in spring 2008, scrutinizes how mitochondria merge and divide. He’s curious how this dance influences energy production and how missteps might trigger illness. Mitochondria accumulate mutations in their DNA over a lifetime. As the glitches pile up, they can cause mitochondria to produce less energy or work less efficiently. It’s particularly troublesome for the brain and muscles, heavy users of energy. A blackout in cellular energy production may also contribute to gray hair, weak bones, and other age-related changes. Chan has found that the many mitochondria in a cell split and join, allowing them to mix and redistribute copies of mitochondrial DNA, which means any particular cell is unlikely to remain stuck with a large number of faulty mitochondria. “Fusion is potentially a protective mechanism,” says Chan. Chan suggests that a connection between mitochondrial dynamics and cellular aging could exist. Old cells may have deficiencies in mitochondrial fusion that help mutations build up, he says. Faults in this process could be particularly important in neurodegenerative diseases. Evidence already links problems in mitochondria to Alzheimer’s disease, Parkinson’s disease, and Lou Gehrig’s disease (amyotrophic lateral sclerosis). Recent studies even suggest that a gene involved in mitochondrial dynamics is crippled in some inherited forms of Parkinson’s disease. Chan plans to dig deeper into these disease connections. With this new scrutiny, cellular aging could step farther into the spotlight as a key component of many diseases.—J.D.
IMPACT FACTOR

ASK STUDENTS WHAT KEEPS THEM IN SCIENCE—THEY JUST MIGHT TELL YOU.
What draws students to science? David Lopatto aims to find out. Since joining the faculty at Grinnell College, this professor of psychology has designed surveys to learn how undergraduate students respond to summer and classroom research experiences. His findings reveal best practices for creating programs that sustain students’ interest in science.

The number of undergraduate science students doing summer research has grown steadily for nearly 20 years. And from what their faculty mentors tell us, most of them are enjoying themselves as they are learning about the research process. But for me, these anecdotes aren’t enough. I want to know how and why research experiences enhance education and attract students to science. What’s more, how can we optimize these programs in ways that maximize learning and sustain a student’s interest in a science career?

About 10 years ago, I began developing research tools to investigate these questions systematically. The tool I use now, developed in 2003 with HHMI support, is called the Summer Undergraduate Research Experiences (SURE) survey. This detailed questionnaire allows me to quantify student responses to a variety of topics, such as learning gains in specific areas, the impact of summer research on future academic plans, and the influence of mentors. During each of the last two years, more than 2,000 undergraduate students have responded, representing more than 60 colleges and universities.

In part, their answers validate what we knew already from anecdotal reports. Students in undergraduate research report that they learn how to do science, clarify their career plans, and are very satisfied with their experiences. More than 90 percent rate their experiences positively, and most claim they would have another research experience if they could.

I’ve also seen something unexpected in the data: the experience for so many students is defined more by personal development than by professional growth. Most students give high marks for professional factors, such as learning laboratory techniques and how to analyze data. But for those who report a successful undergraduate experience, attaining personal benchmarks — such as enhanced tolerance for obstacles, a readiness for more demanding research, and gaining a better understanding of how scientists really work — is particularly important.

When science educators ask about best practices for summer undergraduate research programs, I emphasize the need to promote personal as well as professional goals. Cumulative data from the SURE survey reveal specific measures that optimize student experiences. For instance, seminars given by visiting scientists help students clarify career paths; the more they hear professionals talk about themselves and what they do, the more they understand what a science career is really like. Ethics seminars are also important; students need to know about the broader implications of conducting research. Organized social activities, such as picnics and trips to the ball park, can help students feel like they’re part of a learning community. Above all, mentoring by the research supervisor has the most dramatic effect on the student experience.

We’ve found that a good way to turn students off of science is to give them a bad mentor. But good mentors — who are supportive, aware of personal goals, and listen as much as they talk — can be a powerful factor in making the research experience a positive one.

Finally, we’ve found that students who close the experience with a poster session, a research paper, or some other final production hone key skills — such as science writing and oral presentation — that serve them well in the future. The take-home message is that a research experience isn’t like a summer job. Whether these experiences are HHMI-funded or not, they involve continuous interaction between students, who learn to work and think independently, and a faculty member who provides structure and oversight.

A similar philosophy applies in the classroom. A related survey instrument — the Classroom Undergraduate Research Experience, or CURE — found that “research-like” classes produce response patterns similar to those seen with SURE. Successful research-like courses aren’t organized like a summer job. Whether these experiences are HHMI-funded or not, they involve continuous interaction between students, who learn to work and think independently, and a faculty member who provides structure and oversight.

In short, the capacity to excel in science is tied closely to personal development. Students who make their own discoveries feel valuable in research, and that sense of worth generalizes across their character in many positive ways. We often hear that what this country needs in terms of science and technology is more innovation, invention, and transformation. If we want our students to advance those goals, then the research programs we design should encourage self-authorship, confidence, and creative thinking.

INTERVIEW BY CHARLES SCHMIDT. David Lopatto is also a professor of natural science and mathematics at Grinnell College.
Helen Hobbs, who thrives in the fast pace and intensity of medicine, did not settle easily into the more measured world of research. Now chief of human genetics at the University of Texas Southwestern Medical Center at Dallas, her desire for better treatments drives her to pore over susceptibility factors to learn who develops disease and who does not—and why.

What affects a person’s disease susceptibility?
The expression of a person’s genes over time, in the context of environmental factors such as exercise, diet, aging, and stress, produces susceptibility or resistance to certain diseases. The term phenotype is used to capture the physical and biochemical characteristics that result from this interplay of genes and the environment. Phenotype in humans includes not only overt effects, like a heart attack or stroke, but the more subtle changes we uncover through blood tests and imaging studies.

How are you using phenotype analysis in your research?
We’ve used sophisticated imaging together with more conventional medical testing to develop very precise phenotypes in a large study population. In the Dallas Heart Study, we enrolled 3,500 individuals—African American, Hispanic, and Caucasian—between 30 and 65 years of age, and obtained a series of phenotypes for each participant, including imaging studies to visualize the heart, blood vessels, liver, and other tissues and blood tests to measure the levels of many different proteins and lipids.

What have you found?
Our work with a gene called PCSK9 is the most significant contribution of the Dallas Heart Study. We discovered that variant forms of PCSK9 are associated with lower levels of low-density lipoproteins (LDL), the “bad” cholesterol. We found that one out of every 50 African Americans has a PCSK9 variant associated with a 28 percent reduction in LDL; Caucasians with a different variant experience a 15 percent reduction in LDL.

We assessed these PCSK9 variations in more than 12,000 samples collected in the Atherosclerosis Risk in Communities Study across 15 years. The findings were unequivocal: low LDL is remarkably cardioprotective. The African Americans with the PCSK9 mutations had an 88 percent decrease in heart disease risk—and this included many individuals with hypertension or diabetes, or those who smoked. The Caucasians with the other PCSK9 sequence variation had a 50 percent disease risk reduction. These are much greater reductions in coronary heart disease than we see when we lower plasma LDL to similar levels with a statin drug in adults.

We published those results in 2006. It now seems reasonable to consider lowering LDL during early adulthood in individuals who have other risk factors. The NIH is considering a trial to test this, and pharmaceutical companies are looking at PCSK9 as a drug target.

In this case, genotype seems to be especially important?
These results show how a genotype can provide information that is not captured in phenotype. The PCSK9 genotypes we identified tell us about the level of LDL in the blood over a lifetime rather than at one moment in time. Individuals with mutations in PCSK9 have lower levels of LDL starting at birth so the arteries feeding the heart are exposed to cumulatively fewer LDL particles over time. We have known for years that high levels of LDL are sufficient to cause heart disease, even in the absence of other risk factors. These new data tell us that the reverse is also true; low levels of LDL starting early in life provide protection from heart disease, even in those individuals who have other risk factors.

How do mutations in PCSK9 cause lower levels of LDL in the blood?
PCSK9 expression causes degradation of LDL receptors, which are proteins on the surfaces of cells that bind and remove LDL from blood. Mutations that interfere with the synthesis of PCSK9 result in an increase in the number of LDL receptors, which leads to lower levels of LDL in the blood.

Is there more to come?
Soon it will be possible to sequence entire genomes of study subjects. A major challenge will be to link the sequence differences identified in genes to traits and diseases. We will need large collections of very carefully phenotyped individuals of different ancestries. The Dallas Heart Study was designed specifically to address this need.

Very few population studies include large numbers of individuals whose ancestors are not from Europe. Genomes differ depending on ancestry. For example, we showed that Hispanics tend to deposit more fat in the liver, which can lead to liver injury and, ultimately, liver failure. In September 2008, we reported that sequence variations in a gene of unknown function called PNPLA3 are responsible for a large fraction of the differences in liver fat between Hispanics and other groups. Now we are exploring whether those sequence variations in PNPLA3 predispose individuals to liver injury after exposure to alcohol, drugs, or infection.

Interview by Richard Currey. HHMI investigator Helen H. Hobbs is director of the Dallas Heart Study.
Q&A

If you could trade places with someone for a day, who would it be?

Scientists are a curious bunch, and how better to satisfy curiosity about the world than glimpse things from someone else’s perspective? Four HHMI scientists offer their picks on how they’d spend a day in another person’s shoes.

— EDITED BY SARAH C.P. WILLIAMS

Leslie A. Leinwand
HHMI PROFESSOR
UNIVERSITY OF COLORADO AT BOULDER

“My top pick is Alice Waters, the chef and co-owner of Chez Panisse in Berkeley, California. Alice Waters champions the use of local, just-picked ingredients and appears to have a generous spirit. My first meal at Chez Panisse was a revelation; like nothing else I had ever eaten. Her food appears simple, but has layers of complex tastes and textures. Since I can’t actually be her for a day, I’d sure like to be able to spend a day with her in the kitchen.”

Alejandro Sánchez Alvarado
HHMI INVESTIGATOR
UNIVERSITY OF UTAH SCHOOL OF MEDICINE

“I’d love to trade places for a day with either of my two children. As a father and a scientist, I am often struck by the genuine, unencumbered, and fresh ways my children see and question the world around them. From the mundane—Where do dreams come from?—to the mysterious—What’s outside of outer space? My children’s natural interrogation of their world is always a fresh reminder of why I fell in love with science in the first place.”

Elaine Fuchs
HHMI INVESTIGATOR
THE ROCKEFELLER UNIVERSITY

“It would have to be the Flemish painter Pieter Bruegel on the day he finished The Triumph of Death, which now hangs in the Prado museum in Madrid. My introduction to Bruegel came when I was 6 years old and became fascinated by a reproduction of one of his paintings in my uncle’s home. As I got older and learned to appreciate art, I became passionate about seeing Bruegel’s paintings whenever and wherever I had the chance. My favorite is the masterpiece in the Prado, although The Beggars and The Fall of Icarus are also right up there. What a creative genius Bruegel was, whose pulse was on sixteenth century Europe in a unique and extraordinary way!”

David J. Anderson
HHMI INVESTIGATOR
CALIFORNIA INSTITUTE OF TECHNOLOGY

“Jonathan Miller, the British humorist, neurologist, writer, and theater director. There’s nobody else I know who has so successfully combined an interest in science/medicine and the performing arts. During a day in Miller’s shoes, I’d meet lots of interesting, talented people in fields I would normally never encounter. And I’d exercise the right half of my brain, the part that tends to atrophy during a career in science.”
The fluid shapes of Trypanosoma brucei shown here become distorted when treated with one immunosuppressant (see page 49). The parasite, which causes African sleeping sickness, spends part of its life cycle in the tsetse fly before infecting mammals.
Fire Ant!

MISSISSIPPI HIGH SCHOOL STUDENTS ARE GETTING UP CLOSE AND PERSONAL WITH THIS PERVERSIVE PEST—ALL IN THE NAME OF BIOLOGY.

KATHY MCKONE KNEW SHE WAS FALLING BEHIND. BY 2005, THE veteran high school biology teacher in rural Bogue Chitto, Mississippi, couldn’t recognize many biotechnology topics covered in the newest textbooks. Even the products listed for sale in teacher catalogues—everything from thermal cyclers to amplification primers—were unfamiliar.

“It was all foreign to me,” McKone says.

But not for long. McKone took action and, using the Internet, found teacher workshops in molecular biology that brought her to the campuses of some of the nation’s premier universities, including Princeton, Harvard, and Cornell. Her newfound excitement and knowledge are having a big impact in Bogue Chitto, a crossroads community an hour south of Jackson that consists of a post office, two country stores, two gas stations, and a few churches. Now they are spreading elsewhere in the state.

McKone is one of five Mississippi high school teachers collaborating with scientists from the University of Mississippi Medical Center in Jackson and the Marine Biological Laboratory (MBL) in Massachusetts to develop a biology curriculum centered on a pervasive Southern pest—the fire ant *Solenopsis invicta*. HHMI has supported development of the curriculum, and Millsaps College in Jackson is also part of the team.

“Most everybody in the South has been stung by a fire ant,” McKone says. As a child, McKone was often a victim as she played outside or worked in the family garden. And it hasn’t stopped now that she’s an adult: she was stung around eight times earlier this school year when she stood too close to a fire ant mound outside her K–12 school.

With apologies to William Shakespeare—who invoked “a muse of fire” in seeking inspiration for *Henry V*—the Muse of Fire project draws its inspiration from a venomous creature whose sting has disrupted many a family picnic or football game. It leaves its victims with a distinctive white blister and, on occasion, a life-threatening allergic reaction. Swarms have been known to kill livestock and invade nursing homes.

“The fire ant is a unique point of interest, especially among children,” explains Rob Rockhold, a scientist and administrator at the University of Mississippi Medical Center who has studied fire ant venom and conjured the Muse of Fire name from his college literature classes. “It also became obvious to us that fire ants just opened up a world of scientific disciplines.”

Rockhold, who oversees the fire ant project with colleague Donna Sullivan and MBL scientist William Reznikoff, says the Muse of Fire project speaks directly to students and will enable them to learn about subjects ranging from molecular biology and environmental science to toxicology and human health.

Teams of scientists and teachers met last summer in Jackson to develop the coursework and a variety of experiments. Then each of the five high school teachers tested one part of the curriculum in a class to make sure the lessons moved students beyond fire ants to wider biology concepts. Some teachers took their students to fields filled with tall grass to observe how fire ants affect other insects in their local environment. Others devised experiments that allowed students to understand how fire ants prey on their victims—in this case, crickets—by using venom substitutes to determine the concentration of a fatal dose.

This past fall, McKone incorporated elements from Muse of Fire in Bogue Chitto’s first biomedical research course. One September afternoon, she led seven seniors to a sandy area just outside the cafeteria that serves the school of 630 students. Although they searched for fire ants, their real quarry that day was a type of bacteria called *Wolbachia*, which lives inside the ant. Their goal? To determine whether Mississippi fire ants are infected with *Wolbachia* and, if so, the prevalence of the bacteria.

*Wolbachia* are complicated critters. They infect spiders and many insects, influencing their reproductive behavior in a variety...
of ways. For example, the bacteria can cause all an insect’s offspring to develop as females or allow only infected insects to reproduce. The bacteria live inside insect cells and are passed from generation to generation through the eggs. Wolbachia may also play a complex role in human diseases such as river blindness.

“The interesting thing is that one can use fire ants and other insects as investigative tools to find out if only some of them are infected with Wolbachia and why,” says Reznikoff, who helped McKone design the Wolbachia portion of the fire ants project.

Back in McKone’s large, bright classroom, the seven students huddle in three groups, smashing ants with the sealed end of a pipette tip. They make sure to crush the ant’s abdomen, where Wolbachia are typically found. The next step comes straight from a molecular biology textbook: finding the bacterial DNA (if it is present) inside the fire ant cells. Using polymerase chain reaction machines borrowed from Florida A&M University—the same thermal cyclers that so puzzled McKone in 2005—the students go through the step-by-step process of extracting the DNA samples.

McKone occasionally stops to quiz the students on what they’re doing, making sure they know what each chemical does or why they are heating up the sample. “They love it. They like understanding why.”

Getting her students interested in fire ants has been easy, McKone says. She doesn’t have to push them to read research papers or ask questions. They are motivated to learn because they are already interested in fire ants. “I didn’t know there were so many types of ants,” says student Jerry Fry. “I thought an ant was just an ant.”

So far, four ants from Bogue Chitto have tested positive for the Wolbachia parasite, all from the mound outside the cafeteria. Ants from another mound at the school and from two mounds off campus had no evidence of the parasite. When the students get the full Wolbachia DNA sequence back from MBL, McKone hopes it will tell them how these fire ants are related to those elsewhere or provide some insight into how the bacteria might manipulate fire ants’ reproduction.

This summer, McKone, her fellow teachers, and the scientists will assess what they have learned and then finalize the five-part curriculum. They plan to make it available to teachers wherever fire ants are found.

Rockhold hopes the students’ Wolbachia research can eventually be combined with that from other students throughout the South to help scientists find out what makes fire ants infected with the bacteria different from those not infected. “If there are enough folks doing the work at different sites, it might ultimately become a comprehensive and meaningful addition to the science,” he says.

Meanwhile, McKone is happy her students have learned about molecular biology using a subject close to home. Perhaps it will inspire them to become researchers—and find a solution to a local problem, she says. “They would love for somebody—and I would love for them to be a part of it—to find a way to control fire ants.”

—ANDREA WIDENER

2008 Holiday Lectures: Making Your Mind

HHMI investigators Eric Kandel (left) and Thomas Jessell (second from left) headlined the 2008 Holiday Lectures on Science, an annual December seminar that welcomes Washington, D.C.-area high school students to the Institute’s headquarters in Chevy Chase, Maryland. This year’s lectures delved into the complexities of the mind, memory, and movement. For fun, the students got to dabble in the ancient, now debunked, art of phrenology (far right). To learn more, visit www.hhmi.org/biointeractive./
A Call for Collaboration
PILOT PROGRAM FROM HHMI AWARDS $40 MILLION TO EIGHT MULTIDISCIPLINARY TEAMS.

Until four years ago, Danny Reinberg’s research comfortably revolved around molecules and cells, just what Reinberg—a biochemist and HHMI investigator at New York University—had been trained to study. But then, at a scientific meeting, colleague Shelley Berger of the Wistar Institute mentioned her recent fascination with ant behavior, after observing leafcutter ants in Costa Rica. She suggested that ants might be a perfect organism for studying gene expression and behavior.

That encounter led to a unique, multidisciplinary collaboration that calls on the expertise of everyone involved. It’s one of eight such collaborative projects that HHMI is funding as part of a $40 million pilot program.

The Collaborative Innovation Awards represent the first time the Institute will provide funding for specific research projects rather than funds for investigators to take their science in any number of directions. The new funds are intended to encourage HHMI investigators to join with scientists outside HHMI to undertake projects that are new and so large in scope that they require a team covering a range of fields. In Reinberg’s case, that means collaborating with Berger as well as Juergen Liebig of Arizona State University, a leader in studying the complicated social dynamics of insect societies. The team hopes to explain how gene expression—rather than gene sequence—is passed to generations of ants, affecting their behavior, social roles, and aging.

HHMI’s vice-president and chief scientific officer, Jack Dixon, says the newly funded projects represent an important step for the Institute. “This award permits our investigators to assemble the team of experts they need to attack these complex scientific problems,” he says. “We were looking for projects that could represent breakthroughs—those that could really change the way we think.”

At the Jackson Laboratory, in Bar Harbor, Maine, HHMI investigator Simon John has spent more than a decade using mouse models to study glaucoma—a major cause of vision loss and blindness. Glaucoma researchers desperately need new tools for measuring intraocular pressure, according to John. High intraocular pressure, which damages nerve cells in the eye, is a common cause of glaucoma.

With the help of a Collaborative Innovation Award, John will work with two Purdue University engineers to develop the world’s
first ultraminiature pressure-sensing device that can be implanted in the eyes of mice that have—or are at risk of developing—glaucoma.

“This program requires the investigators to step outside their normal comfort zone and expertise,” says John. “We thought our plan was a good match.”

Only slightly thicker than a human hair, and capable of transmitting the data it collects to the researchers via a tiny wireless antenna, the sensor could have other applications, says John—monitoring blood pressure or cerebrospinal fluid, for example.

HHMI investigator Catherine Dulac, of Harvard University, will use the new funding to lead a team of neuroscience experts in studying the impact of gene imprinting—a phenomenon in which only a single gene copy is expressed rather than both chromosomal copies—on behavior and brain development. She’s collaborating with three other Harvard researchers who specialize in how neurons make decisions, the role of genetics in neurological disorders, and the evolutionary role of imprinting in the embryo. By some estimates, the team could find 600 or more genes controlled by imprinting, but Dulac doesn’t really know what they’ll find.

“I have been thinking for many years about the mechanisms of gene regulation—such as imprinting—and the coevolution of neuronal function and behavior,” she says. “This has triggered the interest of several colleagues with very different fields of expertise, who can now brainstorm and test key hypotheses together.”

HHMI plans to evaluate the progress of the eight collaborative projects selected for this pilot phase and expects to expand the program in coming years. Philip Perlman, a senior scientific officer at HHMI who oversees the program, stresses that such funding is valuable because scientists often hesitate to undertake large collaborations, which can be seen as detracting from a lab’s primary focus. “Many research groups find it difficult to allocate resources to allow one or more members to devote years to a project that could yield important results but may never directly further the lab’s own mission,” says Perlman.

Reinberg, who now has to make room in his lab for boxes of ants frozen in liquid nitrogen and shipped from Arizona, loves his new collaboration because it allows him to study new areas—like neuroscience. “I have been working in cells for more than 20 years,” he says. “And now I have the opportunity to work on whole organisms and move into neurobiology. This project has opened the door for my next 20 years of science.”

—SARAH C.P. WILLIAMS

OTHER COLLABORATIVE INNOVATION AWARD PROJECTS

DOUGLAS REES, of the California Institute of Technology, is leading a team of three experts in structural biology and protein chemistry to develop a novel way to solve the three-dimensional structures of proteins embedded in membranes. Such proteins are vital to cells—they act as gatekeepers between compartments and between the cell and its surroundings—but they are notoriously hard to work with.

PETER WALTER, at the University of California, San Francisco, an expert in protein folding, will enlist five collaborators in San Francisco and Chile to probe whether it’s possible to target drugs to different steps of the pathway that guards cells against misfolded proteins.

SUSAN LINDQUIST, at the Whitehead Institute for Biomedical Research, wants to find strategies to target the biological mechanisms that break down in Parkinson's disease and other neurodegenerative disorders. Her long-term goal is to develop personalized treatments for patients. Partners include stem cell expert Rudolf Jaenisch and colleagues at the University of Alabama, Boston University, and Purdue University.

HUDA ZOGHBI has a plan for speeding up drug discovery. Her team, at Baylor College of Medicine and the University of Minnesota, is working on a rapid way to identify hundreds of genes involved in clearing disabled proteins from the brain—a process that goes awry in Parkinson’s disease. “If we can figure out the neurobiology for one of these diseases, it can then be applied to many of the other neurodegenerative disorders,” says Zoghbi.

XIAOWEI ZHUANG, whose Harvard University lab develops imaging techniques, is taking a closer look at the brain. By collaborating with creators of some of the most powerful and innovative methods for imaging, data analysis, and sample preparation, Zhuang seeks to map out all the neural connections in mammalian brains.
Probing Pigs
A PORCINE MODEL OF CYSTIC FIBROSIS HELPS RESEARCHERS STUDY THE DISEASE.

A new line of genetically altered piglets offers scientists a novel testing ground for the causes, progression, and treatments of cystic fibrosis. In the past, researchers relied on mice to study the disease, which in humans is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The mutation causes pancreatic failure, lung infections, and liver disease, among other problems. But mice with the flawed gene don’t show humanlike symptoms of the disease, says HHMI investigator Michael J. Welsh.

“Mice just don’t develop cystic fibrosis,” he says. “There are 20 theories as to why, but what it all comes down to is that if these animals don’t get the symptoms, you can’t use them to study the progression of the disease; you can’t use them to test whether something treats the disease.”

So Welsh, at the University of Iowa, and collaborators turned to pigs—closer to human in terms of anatomy, physiology, biochemistry, size, lifespan, and genetics. They engineered pig cells with mutations in CFTR to make piglets with one bad copy of the gene, and then mated those pigs to produce offspring with both copies of CFTR mutated. These piglets show many of the hallmarks of human cystic fibrosis. The results appear in the September 26, 2008, issue of Science.

Affected humans and piglets are born healthy but develop increasing symptoms as they age. Having the pig model will allow researchers to study how cystic fibrosis progresses over time, says Welsh. “This is a disease that affects a whole organism and now we can study it in a whole organism.”

By comparing the affected pigs to mice with the same mutations, researchers may also be able to figure out why mice don’t get symptoms of cystic fibrosis. “We can look at what’s different in mice and pigs that causes the mutation to have such different effects,” says Welsh. “Is it gene differences? Physiological differences? Anatomical? Maybe the answer will lead us to a therapy.”

IN BRIEF

IT TAKES TWO
The symptoms of most Listeria monocytogenes infections are often mild—a fever or stomachache at worst. But in pregnant women, the bacteria can be deadly to growing fetuses. Scientists long assumed that Listeria posed this heightened risk to a fetus because of a pregnant woman’s weakened immune system. Several years ago, however, researchers found evidence that the bacteria actively target the human placenta. Now, HHMI international research scholar Pascale Cossart and colleagues at the Pasteur Institute have pinpointed two proteins involved in this targeted attack.

The team already knew that when Listeria infect the intestine, the organisms rely on an interaction between internalin (InIA), a protein on the bacterial surface, and E-cadherin, a receptor on human epithelial cells. To see whether this held true in the placenta, the team infected pieces of human placenta with the bacteria. They discovered that not only InIA was involved, but also a related bacterial protein, InIB.

Moreover, the researchers found that in pregnant gerbils, Listeria that lacked InIA, InIB, or both, couldn’t infect placental tissue. And in mice—where the proteins do not normally play a role in placental infections—that were engineered to express human E-cadherin, the placenta was susceptible to the bacteria.

Cossart and her colleagues conclude in the October 23, 2008, issue of Nature that for Listeria to infect human placenta—and therefore risk harming a fetus—the bacteria must express both InIA and InIB, and that the pregnant woman’s epithelial cells must express E-cadherin. Understanding this interaction could lead to ways to combat Listeria.

LEARNING HOW NOT TO BE AFRAID
Like people able to keep their inner calm during stressful times, mice can be conditioned to feel safe in normally anxiety-causing situations. Researchers led by HHMI investigator Eric Kandel of Columbia University have probed the brain chemistry associated with this “learned safety.”

The researchers first taught mice fear in an area of the hippocampus. The mice performed similarly to mice treated with fluoxetine (Prozac). Antidepressants such as fluoxetine mediate part of the action by regulating the production of new neurons in adulthood in an area of the hippocampus. The scientists therefore next looked at how learned safety affects the production of new neurons in adulthood. The conditioned mice had more new cells in this area. Using radiation to blunt the birth of neurons here diminished the antidepressant effects of the conditioning.

In their paper published October 9, 2008, in Neuron, the team also reported that learned safety ramped up expression of a factor involved in neuron growth and differentiation, and affected key components of the brain’s dopamine system.

A MOUNTAIN OF CANCER MUTATIONS
The most detailed genetic survey yet of human tumors has revealed a multitude of...
Sleep-Inducing Monsters

A DRUG FOR TRANSPLANT PATIENTS ALSO KILLS THE PARASITES THAT CAUSE AFRICAN SLEEPING SICKNESS BY GROSSLY DISTORTING THEIR SHAPE AND BLOCKING CELL DIVISION.

A drug that helps transplant patients fight tissue rejection may lead to new therapies for African sleeping sickness. The disease is fatal if left untreated, and current therapies have severe side effects.

HHMI international research scholar Miguel Navarro of the Institute of Parasitology and Biomedicine at the Spanish National Research Council in Granada, Spain, and colleagues have found that a drug for transplant patients may also kill parasites that cause African sleeping sickness. The drug is rapamycin, a drug that helps transplant patients fight tissue rejection, and the parasites are Trypanosoma brucei, the parasite that causes African sleeping sickness. These parasites contain proteins similar to those that rapamycin inhibits in humans.

Studying how T. brucei evades the immune system, Navarro’s group realized that four genes of the TOR family enable the parasite to make evasive maneuvers. The genes are called TOR because their protein products are “targets of rapamycin.” The researchers wanted to see if rapamycin could inhibit these TOR proteins. To their surprise, it did more than that. Rapamycin-treated parasites enlarged, sprouting multiple nuclei and other cell parts, and then they died. “They look so weird and grotesque we call them monsters,” Navarro says.

The drug doesn’t cause the same cell deformities in the human immune system. In humans and all other previously studied organisms, rapamycin slows the rate of cell growth. In T. brucei, however, the drug inhibits cell division, the team discovered. Their results appeared in the September 23, 2008, issue of *Proceedings of the National Academy of Sciences*.

IN BRIEF

broken, missing, and overactive genes—some of which were previously unknown. HHMI investigator Bert Vogelstein and colleagues at the Johns Hopkins Kimmel Cancer Center, in collaboration with researchers elsewhere, sequenced 20,661 genes in cells from 24 patients with pancreatic cancer and 22 patients with glioblastoma—the most common form of brain tumors.

The team identified hundreds of gene mutations associated with the cancers. They also found numerous cases where the tumor cells had too many or too few copies of a gene. The typical pancreatic cancer, they discovered, contains 63 genetic alterations and the typical brain tumor contains 60.

The researchers say that the results—which appear in two reports published September 26, 2008, in *Science*—indicate that a small number of commonly mutated genes (“mountains”) and a much larger number of rarer gene changes (“hills”) cause the cancers.

“If you have 100 patients, you have 100 different diseases,” says Vogelstein. The number and variability of tumors poses a challenge to developing drugs. “It’s suggesting that maybe we shouldn’t be focusing so much on the individual genes that are mutated,” Vogelstein says. Instead, we should be thinking about the functional pathways in which these genes operate.”

MOLeCULAR MOTOr FOR MEMORY IDENTIFIED

Building new memories takes raw molecular materials and a cellular vehicle to truck the goods where they’re needed within a cell. When new memories are stored, the junctions between neurons in the brain are rapidly reconfigured and rebuilt so they can respond more readily in the future.

Researchers led by Michael Ehlers, an HHMI investigator at Duke University Medical Center, have identified the motor protein that carri es new receptors and other molecules to the synapse when they’re needed.

Scientists had already established that certain myosin proteins haul cargo in the cell. Ehlers’ team showed that one specific myosin—myosin Vb—resides with micron-sized dendritic spines of neurons, the sites that respond to memory signals. When neurons receive signals that trigger long-term potentiation—the repeated signaling at a synapse that enhances nerve cell connections during the creation of a memory—myosin Vb attaches itself to cellular containers full of the necessary materials, including new neurotransmitter receptors.

Using fluorescent tracers, the team was able to observe the myosin Vb motor proteins actually carrying the cargo to the tips of the neurons that had been stimulated. When the researchers shut down myosin Vb, the cellular steps of memory formation were blocked, they reported in *Cell* on October 31, 2008.

Scientists suspect that breakdowns in this transport process may contribute to deficits in learning and memory, so understanding their molecular basis could lead to treatments for disorders such as addiction and Alzheimer’s disease.

Cancer’s KEEPER

Although many cancer cells remain at their site of origin, some escape to lodge in distant organs, a process called metastasis. These metastases cause most cancer-related deaths. Scientists have found a systematic way to identify genes that might keep cancers from spreading and in the process have identified a gene that suppresses melanoma metastasis in mice.

The team, led by HHMI investigator Michael Green, confined mouse melanoma cells in a matrix that mimics the cells’ natural surroundings in the body and then administered RNA fragments to silence genes one at a time. They identi-
Avoiding Rupture

GENETIC CLUES COULD PROVIDE ADVANCE WARNING OF BRAIN ANEURYSM RUPTURE.

A ruptured brain aneurysm can kill with the speed and surprise of an assassin’s bullet: before rupture, most intact brain aneurysms—distended areas in blood vessel walls—do not cause symptoms. A lucky patient will have an intense headache before the aneurysm bursts; this warning can alert doctors to check for an aneurysm using a brain scan so that they can correct the problem surgically.

Now, an international study has found three genetic clues to help anticipate and prevent brain aneurysm rupture.

HHMI investigator Richard P. Lifton and Murat Gunel, of the Yale School of Medicine, led an international team in the first genomewide search for common genetic markers for brain aneurysms. They uncovered three genetic variations that together can increase the risk of the condition threefold—by about the same amount as known nongenetic factors such as high blood pressure, smoking, and age. The results were published in the December 2008 issue of Nature Genetics.

The study compared the genomes of more than 10,000 people—2,100 with intact or ruptured brain aneurysms and 8,000 controls. Specifically, the scientists examined over 300,000 single nucleotide polymorphisms (SNPs)—areas in the genome known to vary in the human population. They were looking for any SNPs more commonly found in individuals with aneurysms.

The subjects were Finnish, Dutch, and Japanese. These countries’ populations are relatively genetically homogeneous, which helped the researchers distinguish only the relevant genetic patterns.

The three genetic markers the scientists found will increase understanding of the biological causes of brain aneurysms and help doctors to identify people at risk. Two variants lie near genes that function in the formation and maintenance of blood vessels. The third, which had previously been linked to brain aneurysms, lies near a gene associated with arterial diseases.

“We ought to be able to identify more variants that contribute as we study more patients,” Lifton says. Both lifestyle and genetic factors would then help doctors identify individuals who should receive regular brain scans.

IN BRIEF

Malarial liver infection had already been linked to the organ’s filtering of lipids. To learn more, a team led by HHMI international research scholar Maria M. Mota studied how removing different lipoprotein receptors from liver cells affects infection. When cells’ SR-Bi production (or function) was blocked, infection levels fell 50–70 percent. Besides allowing parasites’ membranes.

The results were published in the September 2008 issue of Cell Host & Microbe. Now Mota, at the University of Lisbon, Portugal, and collaborators are working to design better SR-Bi inhibitors; brief treatment may be able to stop infection without significantly impairing liver cell function. The team is also searching for other Plasmodium doorways.

LOST GENE SPELLS NEURODEGENERATION

Losing one working copy of a specific gene causes major symptoms of human neurodegenerative disease in mice, researchers have found.

The team, including HHMI international research scholar Freda Miller, had previously discovered that the p73 gene keeps neurons alive in growing mice. But its function in adult mice was unclear, because most animals that lack the p73 protein die young.

“We had a number of hints that p73 might be doing something throughout the lifetime of the animal, but we hadn’t suspected that it had anything to do with neurodegeneration,” Miller says.

Her team found that while mice with one good copy of p73 develop normally, they lose neurons—and cognitive skills—as they age. Their brains develop tangles of the nervous-system protein tau, as seen in Alzheimer’s disease. The researchers reported their results on September 11, 2008, in Neuron.

Other studies have found that some Alzheimer’s patients have just one functional copy of p73. Miller, at the Toronto Hospital for Sick Children, and colleagues are now testing the link between p73 and neurodegenerative disease in human populations.
The skin contains millions of nerves that respond to a myriad of sensations, including touch, temperature, and pain. The number of nerves that cover the skin varies from one part of the body to another. Areas that need to discriminate fine sensations have more nerves than areas that do not require this ability. Your hands and fingers, for example, have many more nerves than your back, because hands and fingers need to do more intricate tasks, such as writing or threading a needle. You feel more pain from injections to the more sensitive parts of your skin because there are more nerves reacting to the “insult.”

You can test how many nerves are in an area of skin by closing your eyes and having someone pretend to write on that area with a finger. Most people instantly know the letters drawn on their hand but have a harder time figuring out a message written on their back.

To minimize pain, injections are typically given in the shoulder, buttock, or thigh. These areas are like the back in that they have fewer nerve endings than other parts of the body but are convenient and safe to inject. Rolling up your sleeve to expose your shoulder is much easier than taking off your shirt to expose your back.

More importantly, needles stuck in these areas are less likely to injure internal structures. Unlike the back, the shoulder, buttock, and thigh are relatively distant from vital organs.

Attempts have been made to reduce the pain felt during injections. A commercially available option is a gel patch containing lidocaine, a fast-acting numbing medication. Applied to the skin, the patch numbs the nerves. This patch is sometimes used for especially anxious children to minimize or eliminate the pain from needles.

Researchers are also working on an experimental system called transdermal injection, which eliminates the need for needles. Just as Star Trek’s Enterprise crew members received injections via “hyposprays,” transdermal injections force medications into the bloodstream by pushing them through the tiny pores of the skin. However, most drugs are too big to push through the skin, so researchers have more work to do. In the meantime, we will have to bear with the brief pain that occurs when we receive a medically necessary injection.

ANSWER RESEARCHED BY PETER JUN, M.D., a former HHMI medical student fellow, now a diagnostic radiology resident at the University of California, San Francisco.
**Crystal Clear** A master practitioner initiates young scientists into the arcane craft of crystallography.

A CRYSTAL IS A THING OF BEAUTY, BUT TO A PROTEIN scientist it is that and much more. The crystal form of a material embodies its essence—the unique framework of atoms and chemical bonds that determines the molecule’s function and how it interacts with other molecules.

But growing the crystal from a protein involves an uncommon blend of science, art, and tenacity—qualities that HHMI investigator and protein biochemist David Eisenberg strives to convey in his course on x-ray crystallography at the University of California, Los Angeles.

“At any of several steps, you can get stuck for months at a time,” says Howard Chang, a student in the course. “All you can do is continue to screen the hundreds or thousands of different conditions that influence crystallization in hopes that one will work. Even under optimum conditions, a crystal can take years to grow.”

But the payoff is huge, Chang adds, both in scientific terms and as a kind of pure wonder. “Seeing the molecules in the greatest detail possible, it’s beautiful how these intricate atomic-level ‘machines’ evolved by nature operate.”

Atoms are too small to see with visible-light microscopes. Instead, scientists infer the positions and orientations of atoms indirectly by using x-ray diffraction. They fire x-ray beams into the crystal, which are scattered in different directions by the atoms and captured on a detector surface. An experienced researcher can then devise a structural model of the protein from the resulting pattern. Knowing the structure gives insights, for example, into how amyloid proteins cause neurological disease (see “Chasing Amyloid,” page 10). And structure-based drugs, such as the protease inhibitors created to treat HIV/AIDS, are becoming more common.

Eisenberg, whose career has witnessed—and contributed to—many milestones of protein crystallography over the last three decades, puts a great deal of thought, personal experience, and enthusiasm into his course, which is taken by about 20 researchers—mostly graduate students—each year.

“We feel that x-ray crystallography is an extremely important tool for young scientists to acquire—it is no longer the province of specialists,” says Eisenberg, who is director of UCLA’s Institute for Genomics & Proteomics. The multi-instructor course includes the history of x-ray diffraction techniques, their theoretical underpinnings, hands-on practice in crystallizing a protein, “shooting” it with x-rays, and interpreting the data.

First-year graduate students Chang, 25, and Anni Zhao, 23, who recently joined the Eisenberg lab, started with little knowledge of the field. The learning curve was steep, but both were impressed by the instructors’ creativity and skill in conveying the needed information. For example, to appreciate the various kinds of symmetry crystals can adopt, students begin by identifying symmetrical elements in the mathematically inspired artwork of M.C. Escher.

In the sequence of instruction, the first part explains x-ray crystallography as well as nuclear magnetic resonance (NMR) and electron microscopy—techniques that can be applied to atomic structure. “We teach them how to read a paper, how to know if a structure is reliable, and what assumptions have been made,” Eisenberg says.

Next come two five-week segments delving deeper into the actual mechanics of x-ray and NMR crystallography—collecting and reducing data and then determining the structure from the data. Along with the lectures, students practice what they’ve been learning with a set of laboratory problems. “Because it’s such a complex concept to teach, it really helps that Dr. Eisenberg is able to bring in six instructors who specialize in different areas,” says Zhao.
Finally, it’s time to put it all together: the students are given a protein from which to grow crystals, freeze them, mount them in the x-ray apparatus, gather the data, and interpret the structure. “To get a good crystal that gives you a nice diffraction pattern is a big deal,” says Zhao. “The initial formation is difficult: there are many combinations of factors—concentration, pH, temperature, salts, buffers—and the possibilities go up exponentially.”

Zhao praises Eisenberg for his supportiveness in helping students weather the ups and downs. “He does not push you for results, but when you need direction or help, he is always available and extremely helpful,” she says. “People can go into his office very frustrated and walk out the door more motivated than ever.”

In practice, much of the tedious trial-and-error pipetting is now done with automated equipment, Eisenberg says, but going through the process manually is part of the learning experience. After completing the course, students are well prepared to join a lab group that relies on x-ray crystallography for structure analysis.

“Some of our students go on to become real experts in the field—they far surpass me in mastering the software and the hardware,” he says. “Others just use it for their research as biochemists. What I like about our training system is that it provides a basis for both of those careers.”

—RICHARD SALTUS
BRUCE ALBERTS, the HHMI undergraduate program director at the University of California, San Francisco, won the 2008 Carl Brändén Award from the Protein Society. The award is given annually to an outstanding protein scientist who has also made exceptional contributions in the areas of education and/or service to the sciences.

DAVID BAKER, an HHMI investigator at the University of Washington, JONATHAN S. WEISSMAN, an HHMI investigator at the University of California, San Francisco, and Martin Gruebele of the University of Illinois at Urbana-Champaign, were awarded the 2008 Raymond and Beverly Sackler International Prize in Biophysics. This annual award, administered by Tel Aviv University, recognizes the seminal contributions of the three researchers to studies of protein folding.

A total of ten HHMI investigators and five HHMI professors were named fellows of the American Association for the Advancement of Science. The investigators are MICHAEL J. BEVAN, University of Washington School of Medicine; DAVID E. CLAPHAM, Boston Children’s Hospital; JENNIFER DOUDNA, University of California, Berkeley; DOUGLAS E. KOSHLAND, Carnegie Institution for Science; BARBARA J. MEYER, University of California, Berkeley; JEREMY NATHANS, Johns Hopkins University School of Medicine; NIPAM H. PATEL, University of California, Berkeley; NORBERT PERRIMON, Harvard Medical School; SUSAN S. TAYLOR, University of California, San Diego; and LI-HUEI TSAI, Massachusetts Institute of Technology. The professors are JO HANDELSMAN, University of Wisconsin-Madison; DIANE K. O’DOWD, University of California, Irvine; BALDOMERO OLIVERA, University of Utah; REBECCA RICHARDS-KORTUM, Rice University; and GRAHAM C. WALKER, Massachusetts Institute of Technology.

Also named were DANIEL J. KLIONSKY, HHMI undergraduate program director at the University of Michigan; and JOAN RUDERMAN of Harvard University, a member of the HHMI medical advisory board.

Two HHMI investigators—MICHAEL B. ELOWITZ of the California Institute of Technology and JONATHAN K. PRITCHARD of the University of Chicago—were named to Discover magazine’s “20 Best Brains Under 40” list, which recognizes young innovators breaking ground in their fields. Elowitz studies how genetically identical cells have variability in their biological components, and Pritchard studies how organisms adapt to their environments over short periods of time.

Rutgers University and the Robert Wood Johnson Medical School awarded HHMI investigator STANLEY FIELDS, of the University of Washington School of Medicine, with the 2008 Paul Janssen Prize in Advanced Biotechnology and Medicine. Fields has developed technologies that use simple genetic strategies in yeast to analyze protein functions.

JO HANDELSMAN, an HHMI professor at the University of Wisconsin-Madison, received the 2009 American Society for Microbiology’s Carski Foundation Distinguished Undergraduate Teaching Award. This annual award, which has been supported by the Carski Foundation since 1968, honors an educator for outstanding teaching of microbiology to undergraduate students.

HHMI investigator OLIVER HOBERT of the Columbia University College of Physicians
and Surgeons received the 2008 Harland Winfield Mossman Award in Developmental Biology from the American Association of Anatomists. The award is given annually to an investigator in the early stages of his or her career for contributions to the field of developmental biology. Hobert studies how neural diversity and left-right asymmetries are generated in the nervous system.

HHMI-supported undergraduate CAITLIN E. MULLARKEY of Swarthmore College and JARRAD M. AGUIRRE, who worked in the lab of HHMI investigator Li-Huei Tsai at the Massachusetts Institute of Technology through the 2008 HHMI Exceptional Research Opportunities Program, were two of 32 U.S. students to be awarded a Rhodes Scholarship to study at Oxford University next year. A senior at Swarthmore, Mullarkey has been studying a gene implicated in glaucoma and glioblastoma. She intends to work in an HIV/AIDS lab at Oxford. Aguirre is a senior at Yale University majoring in molecular, cellular, and developmental biology. He will study medical anthropology at Oxford.

HHMI international research scholar RAÚL A. PADRÓN of the Venezuelan Institute for Scientific Research won the 2008 Science and Technology National Prize of Venezuela, the top scientific recognition in the country. Padrón studies how thick filaments of striated muscle are activated during muscle contraction.

RAFAEL RADI, an HHMI international research scholar at the Universidad de la República in Uruguay, was elected to the Latin American Academy of Sciences. The Academy has 222 members across Latin America and the Caribbean.

The 2008 Katz Prize in Cardiovascular Medicine was awarded to HHMI investigator CHRISTINE E. SEIDMAN, of Brigham and Women’s Hospital, and Jonathan Seidman, of Harvard Medical School. The award, presented by the Columbia University Medical Center, recognizes outstanding contributions to cardiovascular research. The Seidman research team has detected many genetic causes for heart diseases, including hypertrophic cardiomyopathy—a condition that thickens the heart muscle and often causes arrhythmias and heart failure.

LOUISA A. STARK, the HHMI pre-college program director at the University of Utah School of Medicine, received the 2008 Award for Excellence in Human Genetics Education from the American Society of Human Genetics. The award recognizes the work of Stark, and two colleagues, on the Genetic Science Learning Center, started with HHMI funds in 1994 to educate students, teachers, and the public about genetics.

JOAN A. STEITZ, an HHMI investigator at the Yale School of Medicine, received the 2008 New York Academy of Medicine Medal for Distinguished Contributions in Biomedical Science. She also received a Connecticut Women’s Hall of Fame Award. Steitz is interested in the roles played by small RNA–protein complexes in vertebrate cells.

HHMI investigator PETER WALTER, of the University of California, San Francisco, received the 2009 Stein and Moore Award from the Protein Society, which recognizes his contributions to the study of proteins. Walter has discovered how the endoplasmic reticulum of a cell senses when proteins are misfolded and sends them to be degraded.

Two HHMI-supported Davidson College undergraduates, PALLAVI PENUMETCHA and KRISTI MUSCALINO, were on a team that won a gold medal at the 2008 International Genetically Engineered Machine competition (iGEM), an undergraduate synthetic-biology competition with 84 teams from 21 countries. The Davidson team designed, modeled, and constructed a bacterial computer.
These nanoparticles are the molecular equivalents of self-adhesive name tags that come with a nonstick backing paper. As long as the “backing paper” is covering the “adhesive,” the molecules don’t stick to cells. Malignant tumor cells secrete specific proteases that they use to chew their way through normal tissues to reach the blood and distant organs. The “backing paper” is carefully designed to fall off when chewed by the tumor cells’ proteases, thus exposing the “adhesive” and coating the tumor cells with the nanoparticles.

The nanoparticles carry fluorescent and magnetic tags to make the tumors visible. Eventually, they may also carry drugs to kill any cancer cells left over after surgery. One obstacle is that other enzymes in a normal liver can also cut off the “backing paper” as currently designed, so that the liver picks up a lot of the nanoparticles. That doesn’t matter for surgery, but it could be a problem for delivering cell-killing drugs.

In transgenic mice with an especially aggressive form of breast cancer, surgical removal of the tumor offers only a 10 percent chance of tumor-free survival. But when Nguyen and Tsien made them glow, tumors were visually distinguishable from healthy tissue; tumor removal was more complete, and tumor-free survival quadrupled to 40 percent.

“[Tsien] can visualize, almost anthropomorphize, molecules in a very astute way, so that things work,” explains Nguyen. “He really has a sense of how these molecules interact.”

Their results, while not yet published, appear to bear out Nguyen’s praise. In addition to helping the mice live longer, they’ve tested the technique on biopsied human breast cancer tissue (results they plan to publish with the mouse studies); the cancer cells take up the marker. But, at least for now, mice are the only animals to benefit on the operating table. (As a side project, Tsien and Nguyen are working on a way to make nerves visible during surgery to help surgeons avoid damaging them.)

Between the lab and human trials, Tsien says, “there’s something called the valley of death, the gap between promising results obtainable with research funding versus the much more expensive studies necessary to convince companies to invest. Right now I’m looking at that valley of death, which is particularly wide during economic gloom.” He expects that clinical trials to test the safety and effectiveness of the technique in humans will cost millions of dollars.

But the potential payoff is enough to motivate this leap into new territory. “Some of the biggest touted therapies cost hundreds of thousands of dollars per patient treated, and they buy you a few extra months and then you die anyway,” he says. If, for even a few patients who need surgery, he can help surgeons cut out all the cancer, he could buy those patients extra years.

What’s Important
That kind of motivation is what Tsien thinks should drive scientific inquiry. At Janelia Farm, fresh from notice of his Nobel, Tsien admonished the students that if they wanted a successful career in science, they shouldn’t be “unduly motivated or impressed” by prizes.

What they should do, he said, is explore research questions that give some form of day-to-day pleasure. They’ll need that interest to sustain them through the periods between big discoveries or the times when things just aren’t working, when they find themselves staring at their own valley of death.

He acknowledged, however, that are there are some perks to a Nobel win. “Writing for samples is easier now,” he smiled. “People who used to ignore my e-mails are more responsive.”

CORRECTION: In a November 2008 article on funding for the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, the Bulletin incorrectly stated that MBL has been offering courses since 1982. The correct date is 1892. The Bulletin regrets the error.
It was time to pack up and go home. Alfred Wallace was two thousand miles upriver from the Atlantic Ocean, on the Rio dos Uaupés tributary of the Amazon—farther than any European had ever gone. Since arriving in May 1848, he had spent nearly four years exploring and collecting, but had been laid up the last three months with yellow fever. He was too exhausted to go on. His younger brother, Herbert, who accompanied him up to the Rio Negro, had long before turned back. Unbeknownst to Wallace, Herbert was stricken with yellow fever and died before he could board a boat to England. Wallace had accumulated a large menagerie of live animals—monkeys, macaws, parrots, and a toucan—that he hoped to take all the way to the London Zoo. Their upkeep was killing him. Besides the animals, he also had a couple of years’ worth of specimens, both with him and stored downriver, that he had not yet been able to ship to England for sale.

Wallace began to dream of green fields, neat gardens, bread and butter, and other comforts of home. On July 12, 1852, he boarded the brig Helen with thirty-four live animals, many boxes of specimens and notes, and set sail for England.

“I’m afraid the ship’s on fire; come and see what you think of it.” Just after breakfast, three weeks out of port and somewhere east of Bermuda, the captain of the Helen was concerned enough to visit Wallace in his cabin. And rightfully so—smoke was pouring out of the hold.

The crew tried, but could not douse the smoldering blaze. The captain ordered down the lifeboats. Wallace, still weak from his bout with yellow fever, looked on as if the scene was a feverish dream. It wasn’t. He reentered his hot, smoky cabin and salvaged a small tin box and threw in some drawings, some notes, and a diary. He grabbed a line to lower himself into a lifeboat, slipped, and seared his hands on the rope. His pain was compounded when his injured hands hit the salt water. Once in the lifeboat, he discovered it was leaking.

Wallace watched his animals perish, and then the Helen, along with all of his specimens.

Where’s My Snorkel?

A mutation in a gene for antibiotic pigments called phenazines makes bacterial colonies grow in this shriveled—but surprisingly symmetrical and predictable—pattern. Dianne Newman’s explanation: Phenazines usually act as snorkels, helping the deepest bacteria in a thick colony breathe without oxygen. Without these molecules, bacteria must arrange themselves in this unusual way so that each cell has direct access to oxygen in the air (see page 26).

ILLUMINATING BIOLOGY

A spectrum of fluorescent proteins won Roger Tsien a Nobel Prize. Now he’s shining his light on cancer cells and memory formation.