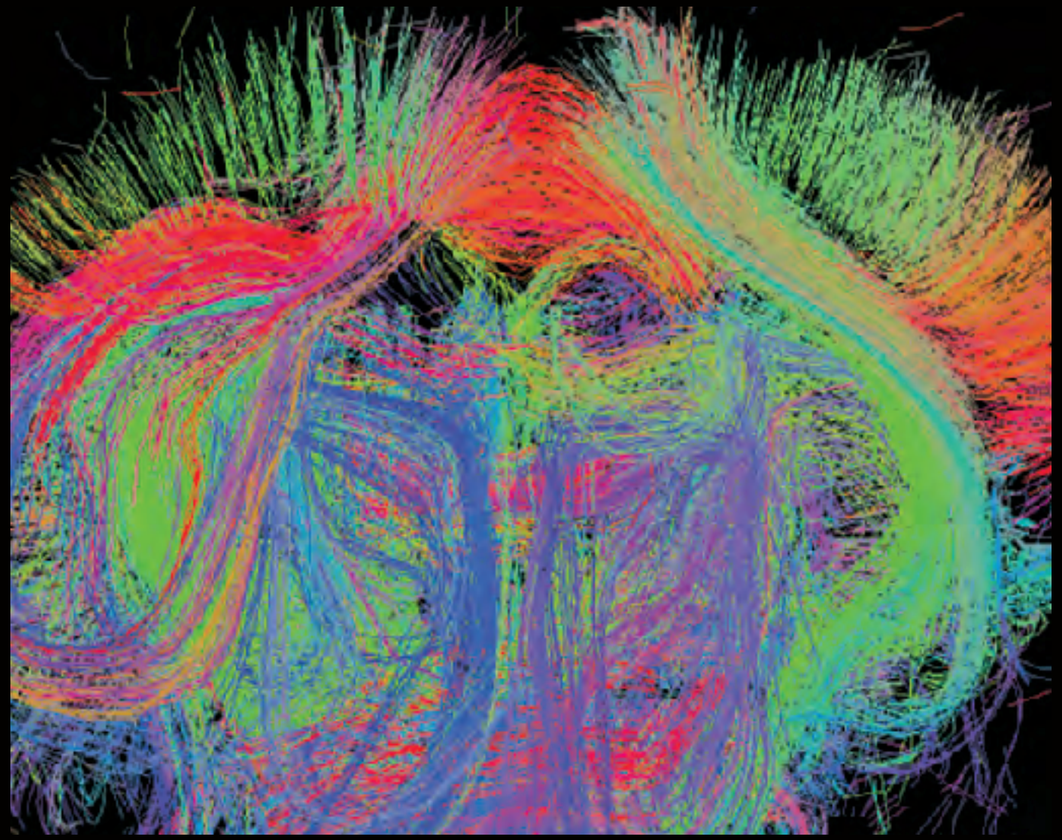
HHMI
HOWARD HUGHES MEDICAL INSTITUTE

4000 Jones Bridge Road
Chevy Chase, Maryland 20815-6789
www.hhmi.org

Brain Tease

On a typical brain scan, the tangle of neurons in a mouse brain is hard to decipher. A new technology lets researchers follow the paths of individual neurons with more ease—and it produces wildly colorful images. Called diffusion tensor imaging, or diffusion MRI, the method relies on the fact that water molecules will move more quickly along neurons extending downward than those growing sideways. The neurons in the resulting image, shown here in an 18-day-old mouse embryo, are color-coded based on direction of growth: red for left to right, green for back to front, and blue for top to bottom. The new images help researchers like Elizabeth Engle study how misplaced neurons can lead to eye disorders (see "Eyes Wide Open," page 18).



Emi Takahashi Oki, Ellen Grant, and Elizabeth Engle

HHMI BULLETIN • Howard Hughes Medical Institute • www.hhmi.org

vol. 24/no. 01

FEB. '11 | VOL. 24 • NO. 01

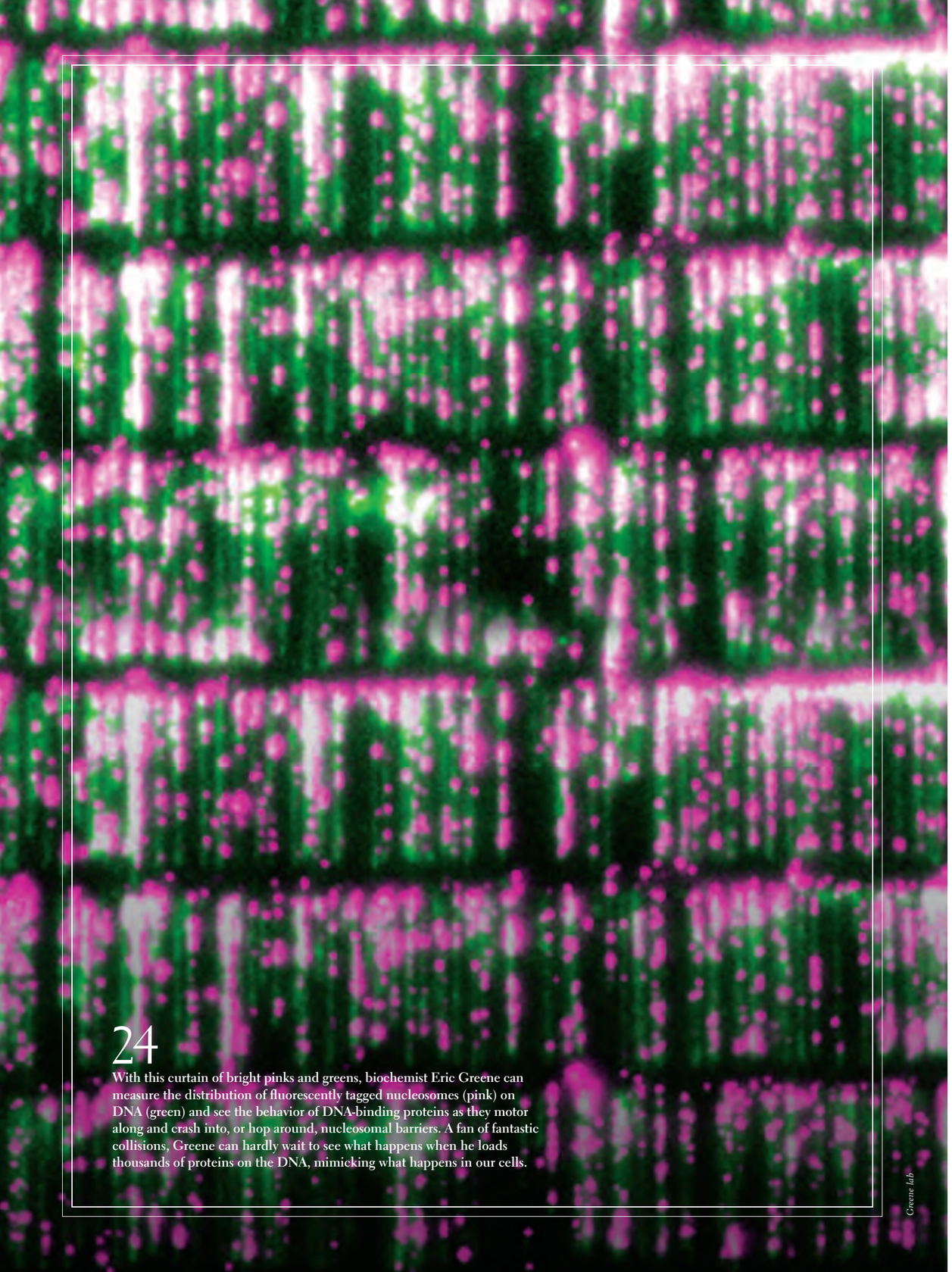
HHMI BULLETIN

Howard Hughes Medical Institute www.hhmi.org

IN THIS ISSUE
EYES ON ELIZABETH ENGLE
DNA CURTAINS
PROGRESS AGAINST ALS

NAVIGATING THE POSTDOCTORAL WILDERNESS

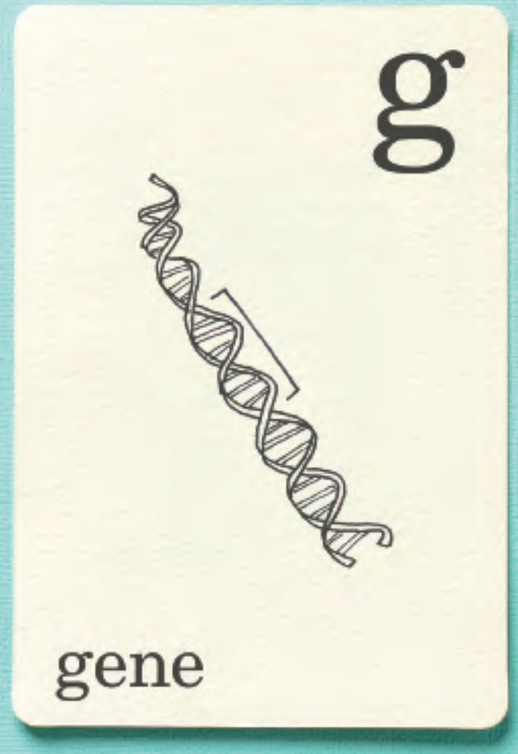
EXPERIENCED RESEARCHERS HAVE FIGURED OUT HOW TO FIND AND TRAIN THE MOST PROMISING POSTDOCS.



24

With this curtain of bright pinks and greens, biochemist Eric Greene can measure the distribution of fluorescently tagged nucleosomes (pink) on DNA (green) and see the behavior of DNA-binding proteins as they motor along and crash into, or hop around, nucleosomal barriers. A fan of fantastic collisions, Greene can hardly wait to see what happens when he loads thousands of proteins on the DNA, mimicking what happens in our cells.

Greene lab



GRASPING THE FUNDAMENTALS

Before they started writing their book *Genetic Twists of Fate*, HHMI investigator Stan Fields and Mark Johnston wanted to know whether the general public had a good understanding of what a gene is. So, they took an informal poll of family and friends. The results surprised them.

None of the people we queried who weren't biologists came close to providing a definition of the gene that would pass muster in a high school biology classroom. Many associated the gene with physical traits or emotional characteristics, a reflection of the realization that genes come from our parents and grandparents, and the notion that they explain Johnny's big ears and Mary's quick temper. The gene was occasionally linked to concepts such as DNA or chromosomes, although those terms were fairly fuzzy to those more than two years out of tenth grade biology.

Most commonly, our requests to explain what a gene is were met with the same look that might surface in response to a question about what the World Bank does, or why tort reform is needed, or how tornados start. But unlike those technical details, which we can safely leave to economists, lawyers, and meteorologists, genes are too important to be relegated to the safely obscure. We fail to understand them at our peril, because they influence crucial aspects of our daily life: our health, our lifespan, our mood, our insurability, and our food supply, to name a few.

But before we deal with what *genes are, let's ask an even simpler question:* where *in your body are genes found? This was the query posed to Americans of varied ethnic and educational backgrounds by Angela D. Lanie, a scientist at the University of Michigan. Nearly a quarter of the respondents said genes are "in the brain"; about one eighth said "in the blood"; a few said in the reproductive system, or heart, or bones, or lymph nodes or various other locations.*

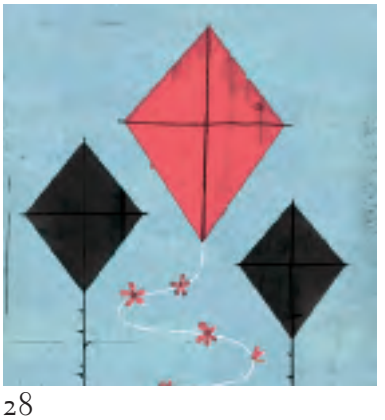
Only about one third of Lanie's respondents gave the correct answer: genes are present all over the body—everywhere, in virtually every cell. Genes are found in your skin and your stomach cells, your lung and your liver cells, your brain and your bone cells. They are in every part of your body. And not just in your body: they are in every cell of broccoli and beets, and chicken and cows, and apples and apricots.

VSA Partners

FEBRUARY '11 VOL. 24 · NO. 01

12

18



24

28

Web Only Content

- Explore Fred Alt's "family album" of postdoctoral trainees.
- Learn Louisa Stark's perspective on using Web-based tools to teach science.
- See how scientist-sculptor Bertalan Andrasfalvy brought his "Butterfly" to Janelia Farm.
- Watch the molecular effect of a genetic eye disorder on the brain and ocular nerves as revealed by Elizabeth Engle.
- Join us at www.hhmi.org/bulletin/feb2011.

Features

COVER STORY

THE NEXT GENERATION

12 Experienced researchers have figured out how to find and train the most promising postdocs.

EYES WIDE OPEN

18 Elizabeth Engle isn't afraid to learn—whole new fields, if necessary—to find the cause of a group of rare eye disorders.

BEHIND THE CURTAIN

24 Stringing molecules on threads of DNA gives a new view of their interactions.

HOPE FLOATS

28 With a new arsenal of robust models of ALS, drug development may move to the fast track.

Departments

PRESIDENT'S LETTER

03 TK

CENTRIFUGE

04 Harboring Seals

05 Curved Wings

06 Lost Mountain

UPFRONT

08 When Worlds Collide

10 Skin Sense

PERSPECTIVES AND OPINIONS

34 Crucibles of Dynamism

36 Q&A—Tell us something a postdoc said or did during an interview that made you sure he/she wasn't right for your lab.

CHRONICLE

SCIENCE EDUCATION

38 Road Warrior

INSTITUTE NEWS

40 2010 Holiday Lectures on Science—Viral Outbreak: The Science of Emerging Disease

41 SEA Reaches New Shores

41 HHMI Launches International Competition for Early Career Scientists

LAB BOOK

42 Forgetting Fear

43 Releasing the Brakes on Cell Fate

44 Warming Malaria

ASK A SCIENTIST

45 How do internal stresses cause the external appearance of humans to change with things such as wrinkles? Can appearance predict general health?

NOTA BENE

46 News of recent awards and other notable achievements

OBSERVATIONS

Grasping the Fundamentals

I have to admit, I was a skeptic. The iPad seemed like one more way to spend serious money on a nifty electronic device. But receiving one as a Christmas present (my husband broke his vow of no more gifts with an on-off button, sworn to after seeing other digital gifts gather dust on my desk) has made me a believer. The iPad is a marvelous machine.

Witness "Star Walk." Open this app on your iPad, point the lightweight tablet toward the sky, and behold—every constellation and major star in that part of the heavens is identified. There's Virgo, with bright Spica—the 15th brightest star in the nighttime sky, you are told. Swing the tablet to the right and the screen adjusts—there's Leo, with bright Regulus twinkling on his chest. I've learned more about the night sky in the last two weeks than I have in my first five decades!

Now that I have this window into myriad new worlds, it makes me even more proud and pleased to announce that the *HHMI Bulletin* is available to iPad users. Downloadable free from the iTunes store, the app is a new, interactive way to learn more about the cool science coming out of HHMI. We are having fun figuring out creative ways to take you deeper into the scientific discoveries the *Bulletin* reports.

With this new format, we're hoping to reach an audience not currently familiar with the *Bulletin*. We're eager to get our stories in the hands of what we think is a sizeable—and global—contingent of science-curious readers.

Given the iPad's potential as a learning tool, we also hope the *Bulletin* app finds its way into the classroom. In January, a *New York Times* article reported escalating orders for iPads by public schools across the country, including 2,000 in New York City alone. With upward of 5,400 educational apps available, of which nearly 1,000 are free, the tablet offers a way "to extend the four walls of the classroom," says one Long Island teacher.

Science educators know that hands-on is the best way to teach science. Maybe tablet computers like the iPad will turn out to be second best. Time will tell. Meanwhile, I'll be trawling the iTunes store for more brilliant apps like Star Walk—and for more science magazine apps like the *HHMI Bulletin*. I can't wait to see what I learn next.

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Telephone (301) 215.8855 • Fax (301) 215.8863 • www.hhmi.org

Paul Vetters

Seeding Plant Science

James Keglev

FOUR YEARS AGO JOANNE CHORY MADE A BOLD STATEMENT WITHIN the pages of the *HHMI Bulletin* that "the study of plant genomes might contribute more to human health and well-being than the study of any animal genome." As one of a handful of plant scientists within the HHMI community, the investigator at the Salk Institute for Biological Studies has spent many an hour explaining to quizzical colleagues how much they could learn from the mouse ear cress (*Arabidopsis thaliana*)—as distinct from the mammalian mouse (*Mus musculus*).

Chory's carefully cultivated seeds have now borne fruit. Earlier this fall, HHMI and the California-based Gordon and Betty Moore Foundation announced that we would hold a joint competition to identify up to 15 of this nation's most creative and talented plant scientists. When selected in 2011, they will join the Institute as investigators and also receive substantial grant support from the Moore Foundation over a five-year period. We think the creation of our joint program underscores the importance of investing in fundamental plant science and will encourage others in the United States to make analogous commitments. We also believe this core group will have an outsized impact on their fields, particularly in attracting a new generation of graduate students and postdoctoral researchers.

Why now? Plant research proved its value long ago—after all, study of the humble pea helped found the modern field of genetics—but one could argue there has never been a more important time in our history. Plant scientists have tremendous potential to help us understand—and possibly find solutions to—some of the most pressing concerns that face society: food production, human health, protection of the environment, identification of renewable energy sources. The 2009 National Research Council Report, "A New Biology for the 21st Century," provides a much-needed framework for discussing these important issues among policy makers and academic leaders. Other positive developments are on the horizon, including a plan for developing a competitive grants program within the National Institute of Food and Agriculture, part of the U.S. Department of Agriculture.

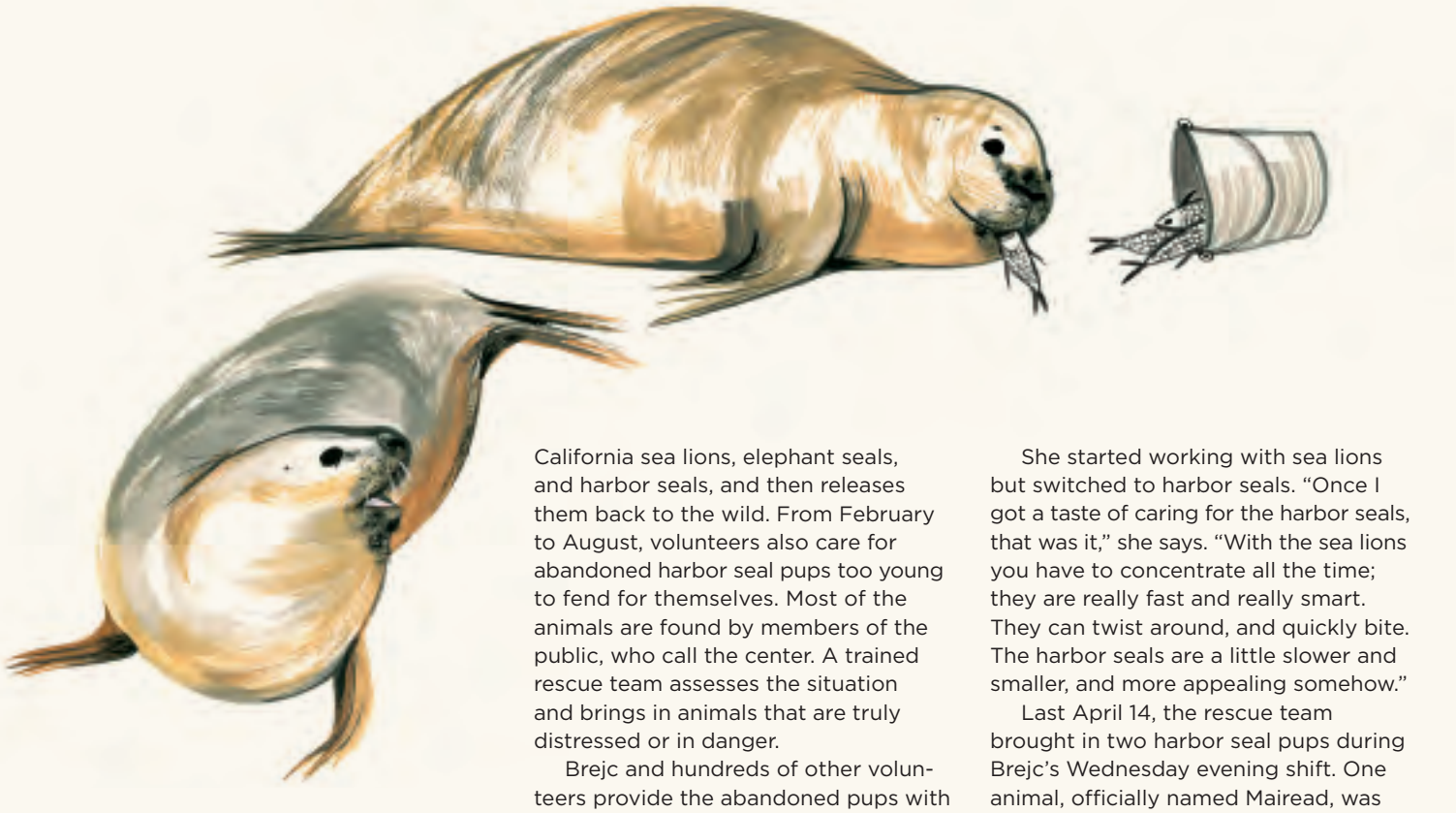
For too long, fundamental plant science has been something of an afterthought in the U.S.—where substantial resources are dedicated to applied agricultural research—and represents about 2 percent of overall life sciences spending by the federal government. A highly respected scientist like Chory may succeed in receiving grants through the National Institutes of Health (NIH), but she is an exception. At the turn of the millennium, for example, of some 24,000 scientists working with *Arabidopsis* as their model organism, fewer than five dozen received NIH research project grants.

The interagency National Plant Genome Initiative—funded through NIH, the National Science Foundation (NSF), the departments of Agriculture and Energy, and others—has generated useful tools and knowledge over the past decade. But the NSF, which supports many plant scientists, has had few dedicated programs in fundamental plant science. Elsewhere in this issue, Vicki L. Chandler, chief program officer for the Moore Foundation's science initiatives and a noted plant researcher in her own right, describes the challenges and opportunities that face her colleagues in the field.

> "Plant scientists have tremendous potential to help us understand—and possibly find solutions to—some of the most pressing concerns that face society."
>
> ROBERT TJIAN

The collaboration between HHMI and the Moore Foundation illustrates the extraordinary potential for targeted investment in plant science research because our organizations would not appear, at first glance, to be obvious partners. The Moore Foundation, which has long been committed to environmental conservation, has focused on supporting fundamental research in physical, life, and information sciences. Given HHMI's primary focus on biomedical research with the potential to improve human health, the Institute has historically viewed much of plant science research as outside its traditional scope. Just as the Moore Foundation sought to connect its environmental and scientific interests, HHMI began exploring potential new directions in plant science in a 2008 workshop that Chory helped organize. As a member of the Moore Foundation's scientific advisory board, I have seen first hand that our organizations share a commitment to supporting excellent science.

The result is something that ecologists might recognize: an example of facultative symbiosis that benefits both organizations and gives the scientists we support a greater chance to survive—if not flourish.



Harboring Seals

When Katjuša Brejc grew up in former Yugoslavia, she had pet parakeets. Later, when she moved to San Francisco, she knew she wanted to take care of animals, but she had to be picky. "Working with cats or dogs would be too dangerous," Brejc says. "I'd want to take them all home." So she signed on at the Marine Mammal Center in Marin County, California. There's no way she could take home a seal. But that doesn't mean she doesn't get attached.

"There are always a few animals that stand out," says Brejc, a research associate in HHMI investigator Barbara Meyer's laboratory at the University of California, Berkeley.

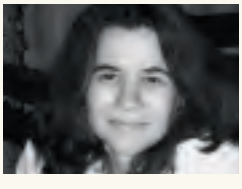
The Marine Mammal Center rehabilitates sick and injured animals, including California sea lions, elephant seals, and harbor seals, and then releases them back to the wild. From February to August, volunteers also care for abandoned harbor seal pups too young to fend for themselves. Most of the animals are found by members of the public, who call the center. A trained rescue team assesses the situation and brings in animals that are truly distressed or in danger.

Brejc and hundreds of other volunteers provide the abandoned pups with food and warmth. "When the harbor seal pups first come in—they are about a meter long—we feed them a fatty mixture of salmon oil and powdered milk through a tube," Brejc says. As the animals put on weight, they get solid food, fish. Volunteers teach the animals to eat fish by putting it directly in their mouths, a task that takes two volunteers: one to hold the animal and another to feed it. Eventually, the seals learn to catch fish on their own. "By the end of the season, we dump seven kilograms of fish in their pen and walk away," says Brejc, who's been working at the center for eight years.

She started working with sea lions but switched to harbor seals. "Once I got a taste of caring for the harbor seals, that was it," she says. "With the sea lions you have to concentrate all the time; they are really fast and really smart. They can twist around, and quickly bite. The harbor seals are a little slower and smaller, and more appealing somehow."

Last April 14, the rescue team brought in two harbor seal pups during Brejc's Wednesday evening shift. One animal, officially named Mairead, was just a day or two old and nearly pure white with big, doll-like eyes. Brejc and her crewmates nicknamed her Snow White. She weighed 17.6 pounds.

Brejc and the others kept regular tabs on Snow White as the season progressed, though they try to minimize their contact so the seals don't become too comfortable around humans. "At the end of the day, they are wild animals," Brejc admits. Two months later, on June 23, the team celebrated when Snow White was released in Bodega Bay at Scotty Creek, weighing 41.7 pounds and ready to fend for herself.
—Rabiya Tuma



"Working with cats or dogs would be too dangerous. I'd want to take them all home."

KATJUŠA BREJC

Illustration: Ping Zhu Photo: Courtesy of Katjuša Brejc

Andrew Cutraro

Curved Wings

"I always wanted to do something with my hands," says Bertalan Andrasfalvy, with a quick grin. That much is easy to believe. His long fingers seem perpetually busy—tracing the white granite edges of his butterfly sculpture at HHMI's Janelia Farm Research Campus, poking the air in philosophical emphasis, or pointing out the lab where he works with Janelia Farm group leader Jeffrey Magee.

Born in Hungary, Andrasfalvy is 40, with swift dark eyes, a chiseled jaw, and a casually athletic frame that stretches well over six feet. He exudes a quiet restlessness. He holds an M.D., a Ph.D., and two curricula vitae—one that lists eight peer-reviewed publications on neuronal physiology, and another that describes 20 sculptures, several of them commissioned and many gifts.

The pivot between art and science started early. When Andrasfalvy was 10 years old, his father gave him a woodcarving set. By age 20, he had completed his first stone sculpture—and was on the path to medical school. Ever since, he's spent weekends sculpting leftover granite or marble salvaged from graveyard and construction suppliers and weekdays doing research and crafting tools for scientists studying the brain. He recently developed, with Janelia Farm physicist Alipasha Vaziri, an optogenetic technique for precisely activating neurons.

"My philosophy is that there are only two things you can do to keep creative, like a child: art and science," Andrasfalvy says. "In these fields, you can follow your curiosity. How does this work? What does it look like? Everything else is a job."

Andrasfalvy has worked in academic research labs in Budapest, Tokyo, and New Orleans. In 2006, he arrived at Janelia Farm to work with Magee, who studies how neurons work. He also acts as a consultant to the physics group at Janelia. And as resident artist.

In 2008, he approached Janelia Farm director Gerry Rubin about creating a sculpture for the campus. Rubin agreed, and Andrasfalvy placed his order for raw stone: a massive 15x5-foot slab of white granite, a foot thick, from Vermont. Over a year of weekends, working in a studio owned by stone carver Malcolm Harlow, he took a diamond-disc saw to the slab, transforming it into a modern, milky white butterfly, with curved wings 7 feet tall and 3 feet wide.

A butterfly is fitting, he says, given HHMI founder Howard Hughes' love of flight. The smooth, rounded piece also softens the spare architecture and the metallic sculptures on campus. "Besides, with Rubin's developmental biology work inside, half the building is working on flies," jokes Andrasfalvy. "This is *such* a nicer insect."

Today, the butterfly sculpture sits high on the horizon, just outside the main building, watching over a cluster of willow oaks and feathery grasses. It rests on a giant rock, evoking a quiet energy, as though—with the right wind—it might take to the air.

—Kathryn Brown

WEB EXTRA: For a photo slideshow of Andrasfalvy's butterfly sculpture, visit www.hhmi.org/bulletin/feb2011.

Lost Mountain

In the final push to the 11,000-foot summit of Monte Perdido in the Spanish Pyrenees, climbers face a treacherous, half-mile-long incline of snow and ice that corkscrews like an Olympic luge track along a sheer rock drop-off.

The perilous slope is aptly named La Escupidera—the Spittoon. "It's very steep and tilted sideways, so if you slip you get spit down the cliff," explains Rafael Yuste, an HHMI investigator and avid mountaineer who negotiated the slope last July. By one local news account, some 60 people have fallen to their deaths here in the past 30 years.

There were times going up "when I didn't think I was going to make it—I thought 'this is going to be the end of Rafa,'" he recalls, still shivering at the memory. "I was dead tired; my heart was beating out of my chest and I thought, 'I'm going to fall from exhaustion or have a heart attack and slide off the cliff.'"

Traditionally, mountaineers allow two days to scale Monte Perdido (Lost Mountain), stopping overnight at a mountain hut before making the final ascent the next day. In the morning, they start out fresh and make the ascent when the snow is still packed hard, and their crampons—metal spikes attached to their boot soles—can get a firm grip.

During the ascent, the weather became threatening. Yuste, a neurobiologist who studies the microcircuitry of the brain's cortex at Columbia University, and his companions—a veteran group of 40-something professionals, mostly Spanish—decided to go up and back in a single day to avoid getting caught in the coming storm.

They reached the bottom of La Escupidera at 3 p.m. Two of the nine-member climbing party had already turned back. "We had been hiking uphill for more than seven hours, it was getting late and conditions were getting bad," recounts Yuste. "The snow turned soft and our crampons didn't work. It was so foggy we couldn't see the summit above us."

The remaining climbers inched upward, hugging the inner wall of the Spittoon as far from the cliff edge as possible.

"Each of us had an ice axe in one hand and a long pole in the other, and we had to plant them both in the snow to keep us from sliding, then take a step and repeat the process," Yuste says. "We had to stop and rest after every 10 steps."

This went on for about an hour until they reached the cold, windy summit, rewarded by a spectacular 100-mile view of the central Pyrenees. After a brief rest, they headed carefully back down the chute-like Spittoon.

As they descended, one of the party fell. The others watched anxiously as he slowly slid toward the edge of the cliff, repeatedly stabbing his axe into the soft snow. "He wasn't going very fast, but he couldn't stop himself," Yuste says. "Finally the axe held. He just lay there, gripping it with both hands."

When their leader went to the rescue, he, too, slipped and fell, slithering toward the cliff, chopping with his axe in vain. Fortunately, he came to a stop, slowly made his way to the other climber, and both rejoined the party.

Twelve hours after setting out, the adventurers returned to the mountain hut, "completely destroyed" by fatigue, but tremendously happy.

"It's hard to explain why we continue to do these crazy things," confesses Yuste. "To reach the peak is beautiful. We feel we have accomplished something really important. Having done it, other challenges in life seem attainable. As in science, you set yourself a high, apparently unattainable goal, and take small careful steps, without losing track of the summit and without being discouraged by the difficulties, until you get there." *—Richard Saltus*

WEB EXTRA: To see photos from Yuste's Monte Perdido climb, visit www.hhmi.org/bulletin/feb2011.

Ping Zhu

upfront

08 WHEN WORLDS COLLIDE
The right time and place led to a new RNAi-like pathway in bacteria.

10 SKIN SENSE
An early question about aging skin has revealed how cells code for their location.

WEB ONLY CONTENT

RIGHT BEFORE YOUR EYES
Revisiting an old technique provides researchers the breakthrough they need to couple protein sequence to function, thousands of variants at a time. Read the story at *www.hhmi.org/bulletin/feb2011*.

Our lives are driven by technology. It takes just a few keystrokes or a Skype account to reach out to scientific collaborators across the globe. Effortless long-distance communication makes it easy to forget about the resources down the hallway or across campus. Two teams of researchers, however, were able to dig deeper into their research questions by partnering with an investigator from another field, who happened to be in the same zip code. Find out what's going on across the quad. Invite somebody for a cup of coffee—face to face. Good for the soul and maybe even the research.

When Worlds Collide

The right time and place led to a new RNAi-like pathway in bacteria.

THOUGH THEIR LABS AT UNIVERSITY OF CALIFORNIA, BERKELEY, ARE only a few buildings apart, biochemist Jennifer Doudna and geobiologist Jill Banfield were strangers. But when Banfield needed expertise outside her scope, their worlds converged. ¶ Banfield studies microbes that grow in extreme environments. She needed a partner who knew about the gene-silencing process known as RNA interference (RNAi).

Doudna, an HHMI investigator who studies RNAi in eukaryotic cells, had solved the crystal structure of Dicer, a key enzyme in that process.

Realizing her luck at having a resource so close by, Banfield called Doudna to discuss what appeared to be a microbial gene-silencing strategy analogous to RNAi that was accomplished by highly repetitive DNA sequence elements known as CRISPRs—an acronym for clustered regularly interspaced short palindromic repeats.

Banfield and others suspected that microbes were using CRISPRs to fend off invaders. But nobody knew how CRISPRs could function as an immune system, and scientists were intrigued by what else the tiny bits of RNA derived from CRISPRs might be doing.

The two researchers met for coffee a few times in 2006 and explored the overlap between their worlds. Those conversations and the well-timed arrival of two new lab members led to Doudna's team capturing the first snapshot of a CRISPR enzyme in action. They reported in September 2010 that the CRISPR-associated enzyme Csy4 has the unusual property of recognizing, binding to, and cleaving RNA in a sequence- and structure-specific way.

"As often happens in science, there is a lot of serendipity involved," Doudna acknowledges.

The Timing was Right

Just as Doudna was becoming keen on the idea of CRISPRs, Blake Wiedenheft appeared. While earning his Ph.D. in Montana, he had become intrigued by the odd repetitive snippets of DNA he found in the microbes of Yellowstone National Park's boiling acid pools. Wiedenheft was convinced that there might be a system in play in these organisms similar to RNAi in eukaryotes. He wanted to pursue the question in Doudna's lab.

Soon after Wiedenheft joined the Doudna lab, graduate student Rachel Haurwitz arrived and became fascinated with Wiedenheft's project. "CRISPR in general is a pretty new field," says Haurwitz. "When I started my project, there had been a lot of computational groundwork done and it was really on the biologists to test the hypotheses."

In 2007, the first report surfaced on CRISPR-mediated acquired immunity. The Danish food production company Danisco needed a way to protect its yogurt-producing *Streptococcus thermophilus* cultures from bacteriophages, viruses that attack bacteria. Its scientists established the relationship between CRISPR and phage resistance. The immunity depended on the bacteria acquiring new DNA in the CRISPR region of their genome upon phage infection. The DNA sequences were identical to sequences in the infecting phages, and subsequently the bacteria were immune to infection with the same phages.

Armed with that new information, Haurwitz began her thesis project, asking: What enzyme in the CRISPR system is responsible for making the small bits of

Nick Tassone

RNA that are later used to target a phage, and how does it function?

The team worked with *Pseudomonas aeruginosa*, a human bacterial pathogen with a CRISPR that had not been implicated directly in defending against phage infection. As such, it offered an opportunity to study its enzymes and mechanisms and then compare the findings with what was already known from *Escherichia coli* and *Streptococcus*. Hints of CRISPR involvement in biofilm formation suggested that it could be a potential target for controlling the persistent *Pseudomonas* infections in the lungs of cystic fibrosis patients.

A Discriminating Enzyme

Haurwitz identified Csy4 as the protein responsible for cutting up the RNA transcribed from a CRISPR sequence into a segment specific to *Pseudomonas*. Then, Haurwitz and Martin Jinek, a structural biologist and postdoc in Doudna's lab, set out to learn how Csy4 works.

Haurwitz designed a mutated version of Csy4's RNA target that allowed the enzyme to bind but prevented cleavage of the RNA, thus immobilizing the enzyme in action. Haurwitz and Jinek devised a method to create individual crystals of the RNA/enzyme complex, so that its three-dimensional structure could be determined by x-ray crystallography.

The team discovered that Csy4 recognized both the stem-loop, or hairpin, structure of the RNA molecule and the sequence of the RNA at the base of the stem (the double-stranded portion of the hairpin). "This exquisite combination of sequence- and shape-specific recognition allows the Csy4 protein to discriminate its target RNA from all the other cellular RNAs," says Jinek. The group published its results in the September 10, 2010, issue of *Science*.

"It has been quite a surprise to find that bacteria might be using a pathway similar to RNA interference but with a distinctly different set of enzymes," says Doudna. The rewards of venturing into this parallel universe, she says, are considerable. She can envision controlling microbial gene expression, gene silencing in eukaryotic cells without interfering with the cells' RNAi machinery, and developing diagnostic tools and therapeutics that rely on Csy4's ability to specifically target and destroy RNA. ■ –MITZI BAKER

Skin Sense

An early question about aging skin led to answers on how cells code for their location.

Howard Chang found that one long intervening noncoding RNA, called HOTAIR, *is important in breast cancer. Too much of it encourages a primary breast tumor to spread.*

Ramin Rahimian

EARLY IN HIS RESEARCH CAREER, HOWARD CHANG ASKED A SIMPLE QUESTION: what happens to skin cells as an organism ages? Using genetic technologies just emerging in 2000, Chang explored his question and revealed another complexity of skin—one that he's still puzzling over. ¶ Chang's curiosity also led him into a new realm of study: long noncoding RNAs. In the past year, the HHMI early career scientist at Stanford University debuted a technique for determining RNA structures, and now he's making novel links between a specific type of RNA and human health.

Chang is a dermatologist with M.D. and Ph.D. degrees. During his clinical residency in dermatology, he worked in the Stanford research lab of HHMI investigator Patrick O. Brown, where he learned about microarrays. Chang saw the technology as an opportunity to determine the genetic differences between young and old skin cells.

He collected skin samples from banks of foreskin tissue taken from newborn boys. From adults, he gathered biopsies of arm, scalp, and back skin. It was widely assumed that skin was skin—identical all over the body. Chang's research revealed, on the contrary, that differences in gene activity among the tissues stemmed not only from age but also location.

"I realized these cells have functional differences based on where they are," says Chang. "It's like a built-in address code."

Chang started frequenting the morgue, collecting skin samples from different body parts of cadavers. Using microarrays, he discovered that the differences in gene activity among skin cells could be traced to *Hox* genes, a large family of genes already known to control positioning of body parts during development.

In 2004, Chang started his own lab. His team devised a method to look at expression of the 39 *Hox* genes—whether each is turned on or off—and also at expression of the DNA flanking each gene.

In humans, more than 200 of these surrounding regions were actively expressed. The researchers found this puzzling, since these regions of DNA are transcribed into RNA but never go on to produce proteins. They're called noncoding RNAs. His lab began to focus on one called *HOTAIR*. The researchers hypothesized that blocking cells from making *HOTAIR* would affect the neighboring *Hox* gene. But instead, the expression of a *Hox* gene on a completely different chromosome changed.

"This raised the idea that these RNAs can have very profound effects all over the genome," says Chang. His lab is beginning to understand these far-reaching effects.

Chang compares *HOTAIR*—which is a large intervening noncoding RNA, or lincRNA—to a train. It's a long, stringy molecule that carries various enzymes to *Hox* genes to regulate their expression. Like train cars, *HOTAIR* sections are modular, specific to their cargo. If Chang can figure out which RNA structures carry which enzymes, he may be able to predict the functions of lincRNAs other than *HOTAIR*.

> "I realized these cells have functional differences based on where they are. It's like a built-in address code."
>
> HOWARD CHANG

But the challenge, he says, is the structures. The classic way to determine RNA structures, called reverse transcriptase PCR, is "tedious, a lot of work, and only successful on short pieces of RNA," he says. So his lab developed a way to separate lincRNAs into manageable pieces.

"If you have a chocolate bar with grooves along it, and you throw it on the ground, the pattern it breaks in will likely follow the grooves," says Chang. He uses chemicals to break the RNA at its thinnest and most exposed sections, like the grooves in the chocolate. Then his team sequences the fragments and pieces them together again. His lab used the technique, called parallel analysis of RNA structure, or PARS, to puzzle out structures of the full 3,000 RNAs in yeast cells. Their results appeared in *Nature* on September 2, 2010.

In the spirit of data sharing, they've created an iPhone application to allow others to access the structures (see Web Extra). Their own lab, of course, will use the data to find connections between the structures and functions of lincRNAs.

While this research sounds far removed from skin, Chang still practices dermatology, seeing patients once a week. It reminds him why *HOTAIR* and positional identity in skin cells are so important: they relate back to human disease.

"One of the biggest clues to a skin disease is where it occurs," he says. If a rash is on your hands and feet, that means something completely different than a rash that is only on your belly button." Understanding how cells code for their location using lincRNAs could explain why certain skin diseases affect only one area and maybe how to stop those diseases.

Recently, Chang's team found that *HOTAIR* is important in breast cancer. When breast cancer cells make too much *HOTAIR*, their address code—which should point to the breast—becomes jumbled, allowing the cancer to spread. The levels of *HOTAIR* in a primary breast tumor predict whether it will metastasize, the researchers concluded in an April 15, 2010, *Nature* article. Not something Chang expected to find when he asked a decade ago how skin cells age, he says, but no doubt a worthwhile finding. ■ –SARAH C.P. WILLIAMS

WEB EXTRA: For more about Chang's iPhone application, visit www.hhmi.org/bulletin/feb2011.

EXPERIENCED RESEARCHERS HAVE FIGURED OUT HOW TO FIND AND TRAIN THE MOST PROMISING POSTDOCS.

BY KENDALL POWELL · ILLUSTRATION BY MICAH LIDBERG

JOURNEYING ALONG AN UNCHARTED RIVER HAS PARALLELS TO EXPLORING SCIENCE, SAYS JIM BARDWELL, AN HHMI INVESTIGATOR AND AVID OUTDOORSMAN.

THE POSTDOCTORAL FELLOWSHIP, FOR EXAMPLE, IS LIKE SHOOTING A SERIES OF CHURNING RAPIDS WITH ONLY A PADDLE AND A HELMET FOR PROTECTION. IT'S A HIGH-PRESSURE SERIES OF CHALLENGES TO JOIN THE BEST LAB AFTER GRADUATE SCHOOL, EXECUTE AND PUBLISH STELLAR SCIENCE, AND THEN SECURE A FULL-TIME POSITION AFTERWARD.

POSTDOCS ARE A LAB'S ENGINES OF CREATIVITY BUT ALSO LABORING APPRENTICES. THEY WORK LONG HOURS FOR LIMITED PAY TO GAIN THE EXPERIENCE AND PUBLICATIONS THEY NEED TO EARN THEIR FIRST INDEPENDENT JOB—BE IT LAB HEAD, INDUSTRY RESEARCHER, OR GOVERNMENT SCIENTIST.

Bardwell understands the importance of having the right team—in the lab and on adventures. With just a fold-up canoe in his backpack, he and a friend hiked through the wilderness of Papua New Guinea scouting rivers in 1994. They chose the seemingly easier Ramu River over the imposing Jimi but midcourse had to escape before the Ramu spit them into a desolate part of the Pacific Ocean.

"Quite frequently, you have to redirect to find the path through the jungle, and science is often redirected quickly or dramatically, as well," says Bardwell, at University of Michigan. In both cases, the expedition team has to be knowledgeable, hard working, flexible, and fearless. "You can run into indications that your hypothesis is completely wrong. You don't want people to wimp out on you, curl up in a ball, and start whimpering. And believe me, it can happen both on expeditions and in the lab."

Choosing the right people can make or break a lab group. And although there's not one right way of going about it, experienced investigators are quick to say it's not rocket science. With further prodding, however, they begin to describe well-honed techniques for recruiting, selecting, and managing postdoctoral fellows.

The stakes for both postdocs and mentors are high. Today more than ever, postdocs need to find a lab with a strong pedigree. And

PART 1 OF 2 IN THE NEXT ISSUE, OUR SERIES CONTINUES WITH THE VIEW FROM THE POSTDOCS THEMSELVES. WHERE DO THINGS STAND FOR THEM TODAY? WHAT CAME OF THE BUSTLE OF ACTIVITY TO IMPROVE THE LIVES OF POSTDOCS IN THE EARLY 2000s? DOES THEIR FUTURE LOOK BRIGHT?

"THE EXPEDITION TEAM HAS TO BE KNOWLEDGEABLE, HARD WORKING, FLEXIBLE, AND FEARLESS."

JIM BARDWELL

the investigators who hire them must identify a gifted experimental scientist who is compatible with the rest of the lab's employees.

"Any researcher over 50 will say that the trainees who come out of their lab are more important than any other page on their CV," says HHMI scientific officer Ed McCleskey.

DIGGING FOR GOLD

Seasoned investigators scout for talent at scientific meetings, summer training courses, and when giving seminars at other universities.

"When I go to a meeting I try not to hang out with my buddies too much; I talk to a lot of younger people," says Karolin Luger, an HHMI investigator at Colorado State University in Ft. Collins. If she finds the right mix of innate curiosity and enthusiasm, she extends an invitation to apply to her lab.

Matchmaking is important too. A referral from a colleague who knows the lab's research and management style holds a lot of weight. Knowing firsthand a candidate's true training level—that they are guaranteed "good hands" at cell culture or crystallography—is invaluable.

Above all, though, these investigators are choosy from the outset, carefully selecting people who fit the lab's goals and personality. "You really have to get over the excitement that someone wants to work with you, and then dig deeper," says Luger.

When screening applicants, investigators rank different key elements first. For many, publications and productivity are most important.

"The number one thing I look at is their publications," says Michael Green, an HHMI investigator at University of Massachusetts Medical School in Worcester. "Can they spearhead the project from its inception to its conclusion, a publication?"

Bardwell also emphasizes publications. "If an applicant has succeeded in publishing, that's a good indication they will succeed as a postdoc." He looks for quality publications on creative studies rather than quantity.

Luger looks for people who will bring interesting techniques to her group and who will speak their minds. "Are these people going to go out on a limb and fight for their ideas? Do they have their own vision?"

She recently hired a postdoc who came from a "hard-core thermodynamics laboratory," as opposed to a crystallography lab like hers. His influence on the lab resulted in development of a key assay and changed the way her group thinks about chromatin and the energetics that govern its assembly. She knew his perspective would bring fresh ideas.

Frederick Alt, an HHMI investigator at Children's Hospital Boston, remembers being impressed by an applicant whose one-page cover letter projected where certain projects in Alt's lab might be headed and positioned her skills within the lab team. "Wow, she's really taken some time here," he recalls thinking. He hired her and she recently landed a faculty slot at National Jewish Health in Denver.

When probing applicant references, veteran investigators put little stock in letters of recommendation. "Letters of recommendation are very tricky because there is never a negative letter. I like to say they are 'interpretatively ambiguous,'" says Green. He prefers to e-mail references with specific questions.

Bardwell and Luger call references to get a faster, candid assessment. Bardwell also likes to call candidates to quickly gauge their enthusiasm and personality. "I like a lab that is fun, where people inspire each other to higher heights."

Jean-François Collet vividly remembers that call and how it sealed his decision to join Bardwell's group. Collet, then at

Peter Baker

JIM BARDWELL LOOKS FOR POSTDOCS WITH QUALITY PUBLICATIONS WHO WILL FIT HIS LAB'S GROUP DYNAMIC.

Catholic University of Louvain in Belgium, sent an e-mail application and two hours later the phone rang. Bardwell opened the conversation in Collet's native French, which impressed upon the young researcher that Bardwell was a world-class communicator.

"I had a young daughter and my wife would have to quit her job to move. He cared about that and wanted to make sure they would be happy in the States, in part because he knew that would make me more productive," recalls Collet.

He then did a unique calculation to confirm the match. For each of the protein folding labs he wanted to join, Collet divided the lab's recent publications by the number of people in the lab. Bardwell stood out with a higher ratio of papers to people. "I thought it was better to go to a smaller lab that publishes a lot, rather than a big lab that is like a lottery, where you don't know if you will be the lucky one."

Everybody's got an individual interviewing style. But they all have the candidate meet current lab members to check the fit. "The cohesiveness of your group is critical to having it hum along as a functional unit," says Stephen Elledge, an HHMI investigator at Harvard Medical School.

Karel Svoboda, a group leader at HHMI's Janelia Farm Research Campus, is very selective. He never hires a postdoc without two interviews, mostly as a way to gauge how much the candidate really wants to be in his lab. Several drop out and find another lab between the first and second conversation. He says playing hard to get pays off. In 13 years, he's trained roughly two dozen postdocs, 18 of whom are now in tenure-track positions.

"THE TRICK IS TO SURROUND YOURSELF WITH POSTDOCS WHO ARE SMARTER THAN YOU AND WITH WHOM YOU HAVE A GOOD PERSONAL RAPPORT."

RICHARD AXEL

Luger dines with candidates the first night for small talk, saving the science for the next morning. The more relaxed discussion gives her a better sense of a candidate's social and communication skills and how well the lab fits his or her long-term plans.

She also probes personalities with questions like, "What's the biggest challenge you had to overcome in your Ph.D.? How would you deal with a messy bench mate?" she says. "Let them talk," she advises. "I made the rookie mistake as a new PI to talk and talk to fill the void."

Nobel laureate and HHMI investigator Richard Axel at Columbia University doesn't waste his time sitting through applicant job talks or thesis seminars. He gets right to a lengthy one-on-one conversation to probe the candidate's grasp of science and whether the two will click. "The trick is to surround yourself with postdocs who are smarter than you and with whom you have a good personal rapport," says Axel. Several of his former postdocs have become icons in neuroscience, including HHMI investigators David Anderson, Linda Buck, Catherine Dulac, Leslie Vosshall, and HHMI alumnus Richard Scheller.

A KEY PART OF TRAINING POSTDOCS, ACCORDING TO FRED ALT, IS PREPARING THEM TO EVENTUALLY RUN THEIR OWN LABS.

Jeff Barnett-Winsby

A SUBTLE GUIDE

Axel calls postdocs the "pillar" of his laboratory, providing the intellectual and experimental firepower for the group. He keeps his door open and reserves several hours a week for each fellow to discuss science, letting them talk through ideas, speculate, and hypothesize. "[This approach] forces them to develop their scientific imagination and scientific

KAREL SVOBODA PUTS CANDIDATES THROUGH TWO INTERVIEWS TO GET A CLEAR SENSE OF THEIR INTEREST IN HIS LAB. KAROLIN LUGER TRIES TO LEARN AS MUCH ABOUT THEIR PERSONALITY AND ABILITY TO DEAL WITH CONFLICT AS THEIR SCIENTIFIC EXPERTISE.

approach," he says. "I tend to be intimately familiar with their data but not overbearing in my influence. I like data—it's my friend. I like to pet it, touch it, and embrace it. That's where I can be of help to them. They're smarter than me, but I have much more experience than they do—I'm an old man!"

Alt follows a lesson he learned from his own postdoctoral days in David Baltimore's lab, then at MIT. He holds a lab meeting every week where all of his 15 or so lab members present their data and progress. "It gets you used to talking on your feet, in front of a regular crowd," he says. "It really helps postdocs going out for seminars and interviewing for jobs."

Most postdocs are ready to test their own scientific wings, Alt says, so a light-handed, subtle approach to mentoring works.

Joanne Chory agrees. "I joke with my lab manager that postdocs are here to come in and do a project," says Chory, an HHMI investigator at the Salk Institute for Biological Sciences in La Jolla. "They might wreck the lab, but you just have to let them." After the initial start-up, Chory is a "sink or swim" manager. "I came from that background and I like it when my postdocs work based on their own motivations."

But what if a postdoc isn't performing to expectations? Addressing a lack of motivation or someone's spinning wheels can be "the toughest thing we do," Chory adds. Conflict management isn't usually part of a scientist's upbringing. She does not like to fire postdocs (nor is that an easy option in many academic settings). Instead, she tries to identify the source of the problem, talking to the postdoc about what might work better. "You cannot make someone work harder by force. It's not worth the energy," she notes.

Luger tries to root out problems sooner rather than later. "I've learned to keep good notes on my conversations. It becomes really handy to remind someone of what we discussed." A written record also becomes invaluable if it does come to letting someone go. "Don't be too squeamish if you see there's a conflict coming. Be proactive and get a dialogue started early." It's not always a "stay or go" decision, either. Postdocs have been known to shift career goals midstream, so it may just be a matter of realigning a project to match the new focus.

Ultimately, says Svoboda, postdocs are adults who must decide for themselves how hard to work. But, he's also frank about time management. "Success as a postdoc demands a certain kind of investment of time and effort. It's like compound interest—put in 10 percent more and the payoff is a lot more over time."

VENTURING OUT ON THEIR OWN

"Getting postdocs well-prepared to run their own lab is a major part of the training, as is trying to enhance their success in my lab," says Alt. "Those two things go together and make it a self-reinforcing system." Clearly, he's tapped into a formula for success—he has trained more than 100 postdoctoral fellows and graduate students and estimates that more than half of his former postdocs are now tenured faculty members (see Alt lab alumni album at www.hhmi.org/bulletin/feb2011).

That's a good track record. In a 2010 *Science Careers* survey of about 3,500 former and current postdocs, 61 percent of respondents expected to land a tenure-track position, but only 37 percent actually did.

Bardwell sees more postdocs choosing nontraditional posts. He's had former postdocs who took jobs in industry overseeing large groups of 30 scientists as well as one who became an assistant professor in Saudi Arabia.

"I try to walk in their shoes rather than pretend they have the same aspirations I have," he says. He plans to encourage the colleague in Saudi Arabia to stay in contact since he's likely to be more isolated from the broader scientific community.

(continued on page 48)

Svoboda: Andrew Cutraro Luger: Matt Slaby

Eyes Wide Open

Elizabeth Engle isn't afraid to learn—whole new fields, if necessary—to find the cause of a group of rare eye disorders.

BY SARAH C.P. WILLIAMS · PHOTOGRAPHY BY JEFF BARNETT-WINSBY

The toddler's eyes were frozen in an uncontrollable stare at the floor and his eyelids drooped over the whites of his eyes.

To look up at neurology resident Elizabeth Engle, he had to tilt his head way back. A conversation with the boy's mother revealed that this defect ran in the family, but they knew nothing about what caused it—or even if it had a name.

Engle didn't know either, so she referred the boy for a battery of neurology and ophthalmology tests. A short note back from the consulting ophthalmologist stung: "Why are you putting the child through all these tests? He has a well-described syndrome that's been in the ophthalmological literature since the 1800s. Congenital fibrosis syndrome."

Embarrassed that she hadn't recognized the syndrome, Engle—now an HHMI patient-oriented researcher—went straight to the literature. True, the disease was well described, but it wasn't well understood. She was determined to look at it with fresh eyes.

Engle saw the case as a chance to put fledgling genetic technologies to the test and track down the gene that caused the disorder. She didn't consider it a problem that she had no idea how to do the required experiments, or that she knew nothing about the eye disorder; she viewed it as a learning opportunity. That mindset has characterized Engle's career.

"Elizabeth had a fire in her belly to really do research," says Alan Beggs, an early mentor of Engle's.

That 1992 encounter with the young boy at Children's Hospital Boston led pediatrician and neurologist Engle to become a scientist who follows the trail of her research wherever it takes her: genetics, developmental biology, neuroscience, cell signaling. She's discovered how a class of rare eye disorders, including the one affecting the boy, are caused by mishaps in the development of the nervous system, and she's linked specific gene mutations to seven of the disorders.

At each fork in the road, she chooses the gutsiest way forward, the path she hasn't traveled before. The course of her career has been as complex and circuitous as that of the developing neurons she studies. Now, she's closer than ever to her final destination: complete understanding of the disorders.

The Brain's Intricacies

Science wasn't always forefront in Engle's mind. She grew up in Columbus, Ohio, devoting her spare time to playing the cello. Then, in junior high school, she met a teacher who took students on annual treks to the Bahamas to study marine biology.

"My parents are both academics, and I learned early on that they would support my interests if they viewed them as educational," says Engle, "and I thought this was the perfect way to go on a cool trip." She got more than just a tan, it turned out. She got hooked on marine biology and returned to the Bahamas throughout high school. "I could spend hours snorkeling over the Andros Barrier Reef watching fish dart among coral, or floating in a mangrove ecosystem watching crabs move about," she says.

But Engle's interests evolved as a freshman at Vermont's Middlebury College when she enrolled in a genetics course because she couldn't get into her desired geology class. She found herself addicted to the testable hypotheses that genetics presented. "I loved to ask questions when I knew there was a real foundation for an answer and an organized way of getting there," she says. As her interests in the cello and marine biology faded, Engle was introduced to genetic research by a mentor at Middlebury and then debated her next step. She was interested in both research and patient care. She chose to go to medical school, figuring that would leave both doors open.

At the Johns Hopkins University School of Medicine, Engle's first anatomy class sparked another new interest: the brain. "I hadn't thought much about how the brain was put together prior to med school," she says. "To suddenly look at this puzzle that is the brain, with its precise tracts and complicated anatomy, was so fascinating."

With her overflowing interests, Engle chose to do two residencies—one in pediatrics at Hopkins and a second in neurology at Children's Hospital Boston. She spent a gap year between them in a fellowship in neuropathology—that is, autopsies of the brain—at Boston's Massachusetts General Hospital.

"I had this need to know what a diseased organ looks like, feels like, smells like," Engle says. "To really get to know what a disease is, I felt it was necessary to look at it grossly and microscopically, and to put my hands on it." Though she's no longer elbow

deep in body parts, Engle has an innate need to understand what makes the body tick.

Under the tutelage of E.P. Richardson, whom Engle calls "one of the great fathers of neuropathology," she learned her way around a pathology lab. She spent her days looking at brains, cutting them up, preparing samples, and looking at them under a two-headed microscope with Richardson. She grew to know, and love, the intricacies of the brain. It's an experience that few neurologists get now, Engle says, since MRI technology has become the go-to way to understand neuroanatomy and diagnose neurological diseases.

Family Studies

Three years later, in the last year of her neurology residency, Engle was forging ahead with her plan to learn genetics by studying the toddler's disorder, specifically called congenital fibrosis of the extraocular muscles (CFEOM) type 1. For help, she approached Beggs—a colleague at Children's Hospital studying muscular dystrophy with former HHMI investigator Louis Kunkel.

"She literally walked up to me in the quad one day with this proposal," says Beggs. "I was quite taken aback."

In muscular dystrophy, the eye muscles are spared from the progressive muscle weakening that affects the rest of the body. So Engle presented her idea to study CFEOM type 1 to Beggs and Kunkel as a project that could answer some of their questions about the uniqueness of the eye muscles. They agreed to take her on.

Engle and Beggs drove to the toddler's home in New Hampshire and spent an afternoon sitting in the family's kitchen meeting relatives affected by the disorder. Engle collected blood samples, Beggs labeled the tubes, and they both observed the signs of CFEOM. They would do this many more times over the next three years. As she'd hoped to do, Engle learned the ins and outs of running a genetics study—everything from enrolling patients to using the novel technologies of the 1990s to zero in on the genetic mistake causing a disorder.

"The time that we worked together was marked by a very steep learning curve for Elizabeth," says Beggs. "And she learned things much faster than anyone I've ever worked with."

Only a few years later, in 1994, Engle's search for the gene causing CFEOM type 1 led her to the center of chromosome 12—one of the 23 chromosome pairs in which every human cell

ELIZABETH ENGLE WAITED IN STUDY PARTICIPANTS' DRIVEWAYS UNTIL MIDNIGHT TO ENROLL THEM IN HER GENETICS STUDIES OF EYE DISORDERS. SHE DIDN'T WANT TO MISS ANY OPPORTUNITY TO GET CLOSER TO A CULPRIT GENE.

"We did what I felt would be most exciting and meaningful in the long haul. Just follow the trail wherever it leads us."

ELIZABETH ENGLE

packages its genetic information. Frustratingly, at that time the centers of chromosomes were notoriously hard to decode, packed with repetitive strings of DNA. But the Human Genome Project was under way, and Engle knew it wouldn't be long until other scientists published a complete genetic map of chromosome 12. So she made a calculated move: she stepped back to wait for the map of the chromosome before finishing her gene hunt.

Her lab spent the next several years building up what Engle calls their "treasure chest" of rare eye disorders—finding families, defining their disorder, and then determining which ones fell into groups. "I admit I am a tenacious individual," says Engle. "I would travel all over the country finding families. I would wait in someone's driveway until midnight when they got home so I could enroll them in our study, because maybe they'd be the person that would get us that much closer to a gene."

By 2003, Engle had pinpointed the mutations that cause CFEOM type 1, as well as CFEOM type 2, which she'd discovered in families in the Middle East. Both mutated genes suggested that the root cause of CFEOM disorders was in the development of neurons, not in the stiffening of the eye muscles (as was previously thought and suggested by the "fibrosis" in CFEOM). Engle was intrigued by exactly how the genetic mutations caused the developmental problems. But she knew little about this area of biology.

Her lab had the choice of continuing to identify genetic causes of eye disorders, and then pass the genes along to other groups to study, or delving into the molecular details of the disorders themselves. She chose the harder path, heading straight to developmental biology textbooks. "We did what I felt would be most exciting and meaningful in the long haul," Engle says, "just follow the trail wherever it leads us."

That same year a graduate student in neurobiology approached Engle, interested in working in her lab. Max Tischfield arrived at just the right time and was a key player in the lab's evolution over the next seven years, bringing his own expertise in developmental neuroscience to the then largely genetically oriented lab. He led the effort to study the latest set of gene mutations that the Engle lab had discovered—work that drew the lab into studying the role of microtubules in nervous system development.

Tischfield, now a postdoctoral fellow in the lab of HHMI investigator Jeremy Nathans at Hopkins, says, "I'm very proud of where Elizabeth's lab is today. When I joined, the lab was very much clinicians, trying to classify these diseases. Now, there are experimental neuroscientists, cell biologists, geneticists. That was the natural transition that the lab was headed for, and I'm very glad I got to be there for that."

Correcting Genetic Mistakes

Neurons are born amidst a concoction of chemical signals in a developing embryo's brain. Each cell's location in the brain is genetically programmed. Moreover, each neuron must stretch its long axon out to a specific location in the body—be it a big toe, a liver, or an eye muscle. To get there, each neuron follows chemical road signs, which interact with unique sets of surface proteins on each axon's tip. For the axon to lengthen, stiff structural filaments—called microtubules—arrange themselves in the right direction inside the neuron. Microtubules also become the highways that carry materials and messages between the axon and the neuron's control center, back in the brain.

Engle's lab has discovered that when it comes to the neurons that lead to the eye muscles, mistakes at any step of this process can lead to disorders. Some disorders are caused by a mutation in genes that give these neurons their identities. Others develop from mistakes in the proteins on the tips of axons. In these cases, the axons may veer off in the wrong direction and never reach the eye. Microtubules that don't form properly cause another set of

disorders, and malfunctioning molecular motors that carry cargo along the microtubules cause yet another, thwarting the relay of messages from the brain (see Web extra). Engle's team has linked mutations to seven complex eye movement disorders, including CFEOM types 1, 2, and 3; horizontal gaze palsy; and Duane's retraction syndrome.

"Wiring the brain to the periphery requires remarkable and complex genetic programs," Engle says, "and as you might imagine, these developmental programs can go wrong." Humans likely have variability in the exact projections of their axons to muscles, she hypothesizes, but in most cases, these errors probably go undetected. "A crooked smile is viewed as charming. A small error in wiring to the huge quadriceps muscle may make you less of an athlete, but wouldn't be very noticeable."

But eye movements must be precisely controlled. Your left and right eye must point in the same direction, focus on the same object, and move at the same time for ideal vision. We use our eyes to communicate, express feelings, and explore our world. Complex eye movement disorders turn out to be sensitive indicators of wiring gone wrong.

Back to the Patients

Since 2008, Engle's been running a monthly clinic with David Hunter, chief of ophthalmology at Children's Hospital and vice chair of ophthalmology at Harvard. Hunter is a specialist in surgeries that, in some cases, can correct complex eye movement disorders. Patients come from around the world, and the two doctors pair up to consult with them—taking on only a couple of patients in an afternoon so they have ample time to explore every aspect of each case. Using the genetic information that Engle has uncovered gives Hunter a better idea of what symptoms to ask the patients about and what to expect when he goes into surgery.

Cases of the disorders vary greatly in severity. Some disorders affect both eyes and are often accompanied by other developmental disabilities; others rarely affect anything more than one eye. Some cause drastic paralysis of particular eye muscles, while others can be corrected with glasses or patches.

"We're at the stage now where we see these patients knowing what the mutation is, and so when I do surgery I at least know that going in," says Hunter. He can often tweak the placement of an eye muscle so that the eye's default position is forward, rather than a fixed gaze downward or to the side.

"A lot of people didn't think it was a good idea for her to study such rare disorders," Hunter says about Engle. "It's just a testament to her persistence that it didn't matter to her what people said. It wasn't about trying to make a big discovery, it was about trying to help these patients."

But bringing the science back to the patients is especially tricky in the case of CFEOM, Engle is the first to admit. "Translating genetic research back to the patient is often challenging," she says, "but for these complex eye movement disorders it is particularly so. These neurons are born and extend their axons at around 4 to 6 weeks of human gestation; the developmental errors occur before a woman may know she's pregnant."

Engle and Hunter's clinic offers diagnostics and genetic counseling for those more severely affected who want to prevent the disorder in their children. But scientists aren't yet at the point where they can interfere with early developmental processes in the brain once the disorder is inherited.

There are still plenty of questions about CFEOM and other complex eye movement disorders that Engle hopes to answer. She is using her findings thus far as a springboard to jump off in new directions, enabled in part by HHMI funding which began in 2008. Many of the genes that are mutated in these disorders are in every neuron in the brain, and in some cases, other organs as well. So why aren't other parts of the brain and body affected? What makes these neurons vulnerable to some disorders and spared in others? And though Engle has identified the gist of how some of the mutated genes cause a disorder, there are still molecular caveats to work out. With a powerful new microscope that can zoom down to single molecules, for example, she's hoping to understand the effect that microtubule mutations have in the neurons leading to the eye. Is the microtubule too stiff or not stiff enough? Does cargo fall off the microtubules as it tries to move along? Does it stall in place? Where these questions will lead her next is anyone's guess.

Engle is incredibly devoted to her work and very intense—in a good way—says Beggs, and if anyone can answer these questions, she can.

"If reviewers of one of her papers come back and request some additional work that will take six months, a lot of authors might just try submitting it to another journal," says Beggs. "But she'll start whole new collaborations and learn new methods to address one of these questions. She's so precise in doing the best science that she can." ■

Behind the Curtain

Stringing molecules on threads of DNA gives a new view of their interactions.

by Sarah C.P. Williams · *illustration by Laura Carlin*

Eric Greene smashes molecules together. He precisely arranges proteins on strands of DNA and sends one zipping toward another, headed for an impact. Sometimes, a weaker molecule falls off the DNA immediately. Other times, in a feat of acrobatics, one protein hurdles another. Recently, Greene saw a protein push another one backward when the two collided.

How proteins behave in such a crash test gives scientists data about their structural integrity, how they attach to DNA, and how they behave in a cell—where molecular fender-benders are likely a commonplace occurrence in the jam-packed nucleus.

Greene, an HHMI early career scientist at Columbia University, is doing these kinds of experiments on an entirely new scale. Rather than looking at the behavior of just one molecule at a time, he lines up hundreds of DNA strands and watches hundreds or thousands of proteins as they interact with the DNA. The aligned molecules of DNA with bright dots of fluorescent protein look like glittering, beaded curtains—leading Greene to dub them "DNA curtains." But these flashing curtains provide more than stunning images. They give researchers powerful insights into how DNA-bound proteins interact with the DNA and with each other.

Corralling Lipids

As a postdoc at the National Institutes of Health in the late 1990s, Greene learned how to watch the dynamics of DNA-bound proteins under the microscope. The ability to see the physical wrangling at such a small scale fascinated him. But limitations of the technique—which involved essentially dumping proteins into a vat of DNA and watching one at a time—became frustrating.

"I'd spend hours doing tedious manipulations," says Greene, "and at the end of the day have only one or two data points. This really limited what we could do. It often took weeks or months just to figure out if an experiment was even working." Moreover, in many instances the proteins would stick to the microscope slide, and this nonspecific sticking was a huge problem in the field. Greene struggled to find a surface that worked for all his experiments.

By the time he started his own lab at Columbia, Greene had a plan. He realized that one surface was natural to all proteins: the lipid bilayer, which surrounds every living cell. Greene figured that a bilayer would provide an inert environment to prevent proteins from sticking to a glass slide and also a way to tether DNA in place.

The crux of his method lies in the ability to corral lipids into separate areas of a glass slide. He scratches miniscule lines into the glass, which keep lipids in separate areas—they can't move across the blemishes. He then encloses special lipids, tagged with a molecule called biotin, in one linear area. Unmarked lipids cover the rest of the glass slide. Specially engineered strings of DNA attach only to the biotin-marked lipids, so the ends of the DNA are arranged in a line. Finally, Greene turns on a flow of liquid across the surface, sweeping all the strands in the same direction, forming a curtain of aligned DNA.

The first time Greene tried this technique, he used a razor blade to scratch the glass—a rudimentary trial, but a successful one. Now, his lab uses electron-beam lithography, a technology developed for manufacturing electronic circuits, to make precisely controlled barriers on the glass that can be used to define the organization of thousands of DNA molecules.

Crash Test Dummies

In principle, the technique behind DNA curtains can be used to study any kind of linear molecule. A scientist could tether RNA, or long filaments that act as cellular highways, to the lipids. But Greene's passion is understanding how proteins interact with DNA. "These relatively simple interactions can drive what an organism looks like, what an organism does, or how it evolves," says Greene. "It's always fascinated me."

Some of the most important protein complexes on DNA are the nucleosomes, the packaging units that consist of DNA wrapped tightly around a protein core. Once Greene established that his curtains worked, he wanted to know whether they would still work for DNA wrapped around nucleosomes.

Greene and his team added nucleosomes to DNA before lining up the strands. When the strands were aligned, the scientists saw that nucleosomes are static once they've attached to DNA. Moreover, by recording the positions of thousands of nucleosomes at one time, Greene's lab had a powerful case for understanding where on DNA nucleosomes attach. The results appeared in the October 2009 issue of *Nature Structural & Molecular Biology*. The experiment was the first proof of concept that the technique worked on DNA associated with nucleosomes, says Greene.

For Greene, the next natural question was what happens when proteins moving along the DNA run into nucleosomes. To study proteins that move randomly along DNA, Greene adjusted his DNA curtains to avoid having a constant flow. Instead, he attached not one but both ends of the DNA to the glass slide. He added nucleosomes first, and then two types of DNA repair factors—which constantly peruse DNA strands for mistakes. He tagged each type of protein complex with a different fluorescent color.

By following the proteins along thousands of DNA strands, Greene and his colleagues found that the two behave differently when they encounter nucleosomes. One hops off the DNA and reattaches on the other side of the nucleosome; the other protein stops, unable to continue. Inside a living cell, Greene's team hypothesizes, another protein couples with the stalled protein to help it cross the barrier. The work, published in the August 2010 issue of *Nature Structural & Molecular Biology*, would have been exceedingly difficult if the experiments had to be done by looking at one molecule at a time, says Greene. You would never know if what you were seeing on any given strand was the norm.

Eric Greene found a way to ask what happens when one protein rams into another. "These types of collisions happen all the time in our cells," he says. "But we have a very limited understanding of how they are resolved."

Elizabeth Weinberg

Greene got back to collisions in his most recent work, published online November 24, 2010, in *Nature*. He sent a fast-moving molecular motor protein toward other stationary proteins bound to the DNA. "This paper embodies all of the excitement I felt when I was a graduate student first thinking about these problems," says Greene. "In essence, this boils down to a very simple question: what happens when one protein rams into another? These types of collisions happen all of the time in our cells, but we have a very limited understanding of how they are resolved."

His lab chose a protein called RecBCD, which processes the ends of broken DNA strands in bacteria and can travel at 1,500 base pairs per second. The lab then picked four different proteins as potential roadblocks. When RecBCD encountered the first of the four—one of the tightest known DNA binding proteins—RecBCD rammed into the stationary protein and pushed it thousands of base pairs before finally kicking it off the DNA altogether.

"It's like running a car into a flower," says Greene. "The car feels nothing and keeps going, while the flower takes the brunt of the punishment." After this remarkable show of strength, it wasn't surprising that RecBCD did similar damage to the other three proteins that Greene's lab put in its way.

Power in Numbers

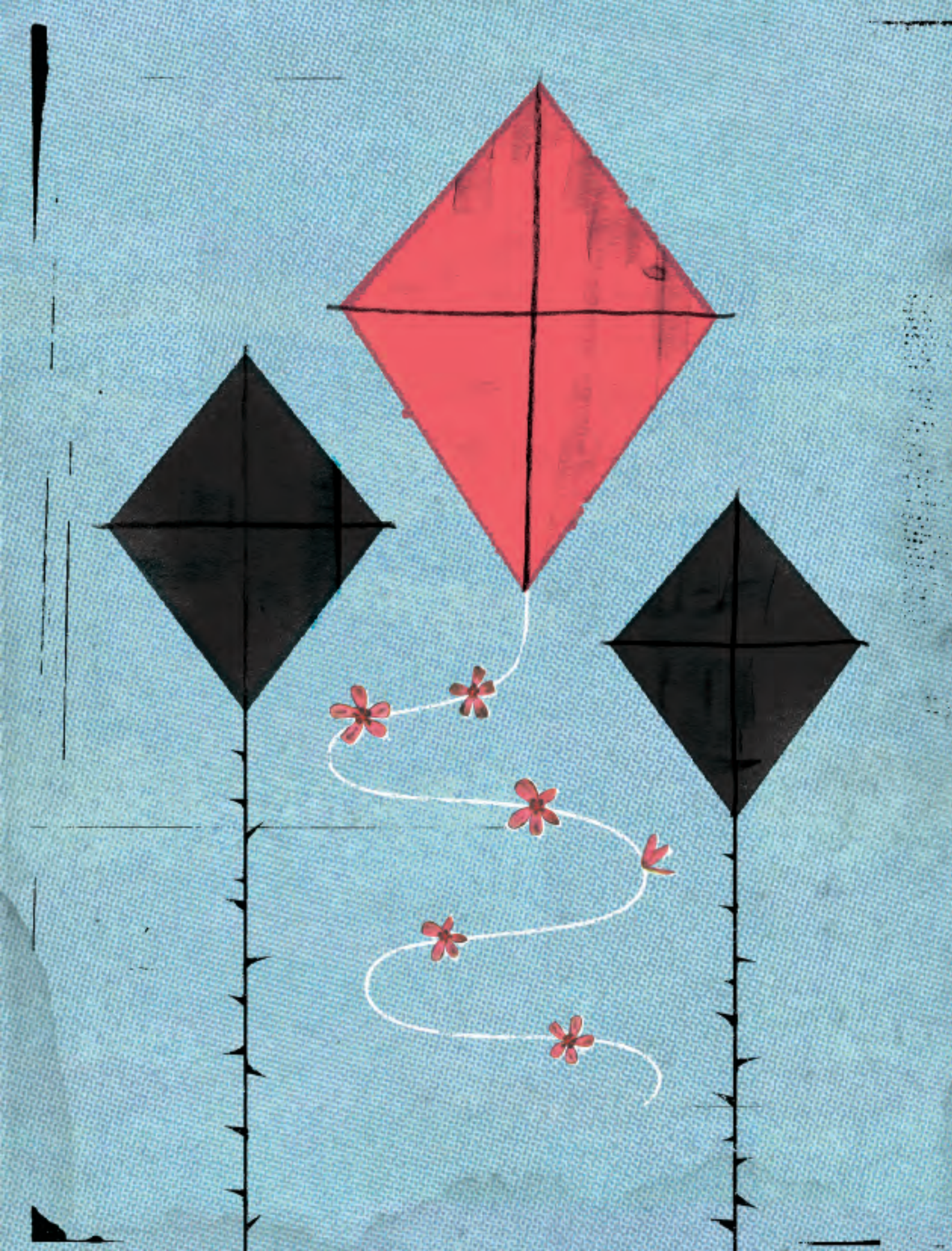
Greene is not the only one who sees the power of his new technique. HHMI investigator Michael O'Donnell at the Rockefeller University studies DNA replication—the process by which proteins copy DNA strands. He wanted to know

(continued on page 48)

by Brian Vastag
illustration by The Heads of State

HOPE FLOATS

With a new arsenal of robust models of ALS, drug development may move to the fast track.



HHMI investigator Nancy Bonini's sprawling laboratory at the University of Pennsylvania is filled with fruit flies.

But they're not swarming. They're sequestered in glass tubes stoppered with cotton. Endless boxes of the white-tufted tubes crowd the benches and stack high on the shelves, overflowing onto the floor.

Postdoctoral fellow Hyung-Jun Kim taps the bottoms of two tubes on a lab counter, agitating the roughly two dozen flies in each. In one tube, flies zip around and scale the glass, some nearly reaching the top. In the other, the flies are sluggish. Most crawl on the bottom; a few make feeble attempts to climb but don't get far. "They don't look as happy," remarks Bonini.

The source of the stark contrast in energy and climbing ability is surprising: a human gene that is involved in amyotrophic lateral sclerosis (ALS), known as Lou Gehrig's disease. The sluggish flies carry the gene and an abundance of the protein it produces, while the sprightly flies do not.

Fruit flies have been a favorite of developmental biologists since the 1970s, with their rapid reproduction and easily manipulated genome. In 1998, however, Bonini authored an idea that radically extended the scientific reach of the humble insect. She mused that inserting genes related to human brain diseases might yield critical insights into poorly understood neurodegenerative conditions, including Huntington's disease, Parkinson's disease, and ALS. "I saw it as, 'Hey, there are all these terrible diseases and nobody is really studying them in model organisms,'" Bonini says. "I knew it was a high-risk thing."

That risk is paying off. In August, Bonini and colleagues announced in *Nature* a genetic factor that contributes to ALS in some patients. Bonini collaborated with Aaron Gitler, a Penn colleague who studies ALS genetics in yeast. Both are keen to define additional genes involved in ALS. Says Gitler: "My hope is that in the next three to five years we find all of the genetic contributors to ALS."

Bonini and Gitler's powerful fly and yeast tools are proving themselves just as other advanced animal models to study the disease are coming online. At Yale Medical School, HHMI investigator Arthur Horwich has developed *Caenorhabditis elegans* roundworms as a model organism for studying nerve degeneration, while both Horwich and HHMI investigator Hugo Bellen at Baylor College of Medicine have bred colonies of mutant mice that reliably develop ALS. Meanwhile, at the Harvard Stem Cell Institute, HHMI early career scientist Kevin Eggan is incubating dishes of human motor neurons grown from the skin cells of patients with the disease. Never before have researchers had access to human motor neurons in the laboratory, let alone nerve cells harboring the disease.

During the past 5 to 10 years, there has "almost been a renaissance in model organism genetics, applying them to human brain diseases," says Bonini. "It's been really exciting."

Sorely Needed Methods

Collectively, these new weapons in the fight against ALS provide a huge leap in scientists' ability to divine the genetic and molecular origins of the disease, says Amelie Gubitz, program director for neurodegeneration at the National Institute of Neurological Disorders and Stroke (NINDS). The menagerie of ALS animal models should also speed the development of drugs, she says.

In the past 15 years or so, patients and their families have experienced disappointment after disappointment as two dozen initially promising ALS drugs ultimately failed to help patients. Most recently, in 2009, a NINDS safety board halted a large trial of the brain drug lithium after determining it provided no benefit to patients. Though there could be many reasons why the trials failed, says Gubitz, one possibility is that the drugs were tested in animal models that did not faithfully reproduce the disease. That's why there is now "a big push to integrate the new animal models into the drug discovery pipeline."

Gubitz is also pushing ALS researchers to collaborate more closely and to identify the strengths and weaknesses of each animal model. To that end, at the Society for Neuroscience meeting in November, Gubitz and Lucie Bruijn, of the ALS Association, gathered 125 scientists working with ALS animal models for an idea exchange. "We're trying to figure out which models should be made more broadly available," she says.

Clockwise from top left: Nancy Bonini, Arthur Horwich, Hugo Bellen, and Kevin Eggan. These HHMI scientists are contributing new models for studying ALS and testing new treatments.

Bonini: Nick Antony Horowich: Chris Jones Bellen: Misty Keasler Eggan: New York Stem Cell Foundation

New methods to study ALS are sorely needed, as the disease remains a frustrating, deadly mystery. It was first described in the mid-19th century, when French physicians linked deaths from muscle spasticity and wastage to shriveled nerve fibers in the spinal column. In the time since, progress on understanding the causes of ALS and related motor neuron diseases—such as spinal muscular atrophy, which, in its severest form, is a fatal disease in children—has been maddeningly slow.

On a gross level, motor neurons, the long nerve cells that control movement, degenerate and die. Patients are typically diagnosed between the ages of 40 and 70, and they rarely survive more than five years. (About 10 percent of patients, including cosmologist Stephen Hawking, have a slowly progressing form of the disease and can survive for decades.) Often, control of the legs goes first, and then paralysis marches upward, eventually shutting down the lungs. In other cases, the face is affected first. The disease leaves the intellect and emotions of patients intact as their bodies wither. "It's a devastating disease," says Bonini, and it affects about 2 in 100,000 people worldwide.

In 1991, an international team funded by the ALS Association and others, including HHMI, identified a form of ALS that runs in families. By 1993, the team had pinpointed mutations in a gene called *SOD1* as responsible for some of these inherited cases. But only a small proportion of ALS cases—perhaps 2 percent—appear to be caused by the inherited *SOD1* mutations, leaving researchers scratching their heads as to the cause of the vast majority of cases.

The search for treatments has been nearly fruitless, as well. Just one drug, riluzole (marketed under the brand name Rilutek), is approved to treat ALS, and it extends life by only a few months. A handful of other drugs are in clinical trials, but many more lie abandoned after failing in large studies. In one new treatment approach launched this year in two phase 1 trials, researchers

implant nerve or bone marrow stem cells into the spinal columns of ALS patients. The hope is that the stem cells will pump out protective growth factors that rescue or rebuild dying motor neurons, says the leader of one of the trials, Clive Svendsen, director of the Regenerative Medicine Institute at Cedars-Sinai Medical Center in Los Angeles. But it will be years before researchers know whether stem cell therapy helps ALS patients.

Fingering Protein Clumps

"Oh, that's disturbing," Bonini proclaims. She's watching a big-screen monitor that displays microscope images of motor neurons autopsied from an ALS patient. Gitler, operating the microscope, points to the ugly brown smear that caused Bonini's reaction. It's a large clump of protein and it's not where it belongs.

In healthy motor neurons, this protein, TDP-43, accumulates only in the nucleus, where it's required for normal processing of RNA. But in the motor neurons of ALS patients, TDP-43 somehow escapes the nucleus and clumps in the cell body. "It's like a skein of yarn coming out of the nucleus," Gitler says. "It's just really striking. It's a dense aggregate."

In 2006, Penn Medicine neuroscientist Virginia Lee and colleagues reported finding clumps of TDP-43 in the motor neurons of ALS patients and in other nerve cells in patients with frontotemporal dementia, a condition that can cause sudden and baffling deviant behavior. The telltale clumps have also been found in athletes—including former professional football players—who later in life developed ALS-like disease and died. Subsequently, various research groups reported mutations in the *TDP-43* gene (also called *TARDBP*) in families in which multiple members had ALS.

The discovery attracted Bonini's attention. "TDP-43 is probably a big player in different types of neurodegeneration," she says. "Everyone is trying to figure out how it hurts neurons."

Around the same time as the TDP-43 discovery, Gitler arrived at Penn and set up a system to rapidly screen the effects of thousands of genes on yeast growth. When he spliced human *TDP-43* into yeast, sure enough, clumps formed and the cells died early. Likewise, Bonini added human *TDP-43* to her fruit flies, and the insects weakened, unable to move and climb normally. Those findings proved that yeast and fruit flies could serve as models of the deleterious effects of TDP-43 aggregation, an important confirmation. Bonini and Gitler hoped they could use the combined input of yeast and flies to address genes important for TDP-43 toxicity.

Gitler amped up a high-throughput genetic screening system, testing in *TDP-43* yeast thousands of genes to see which, if any, accelerated or slowed cell death. They soon got an intriguing hit—the yeast homologue of a gene previously implicated in several human neurodegenerative conditions, *ataxin-2*.

When Bonini added *ataxin-2* to her *TDP-43* flies, they became even sicker; the gene clearly enhanced the toxic effects of *TDP-43*. "That was a 'Whoa' moment," says Bonini. "So we just went after *ataxin-2*."

The pair enlisted the help of clinicians who treat ALS, testing samples from 900 patients and 900 controls for variations in *ataxin-2*. What they found was remarkable: Some 5 percent of patients carried an altered version, whereas just 1.4 percent of controls did, as they reported in *Nature*. "It's a risk factor for ALS," says Bonini.

The discovery also hints at a common mechanism of neuronal damage caused by interactions between ataxin-2 and TDP-43 proteins. Previously, a long form of *ataxin-2* had been implicated in a nerve disease called spinocerebellar ataxia type 2 (SCA-2). That disease, like ALS, also features clumps of TDP-43 protein in certain neurons. The 5 percent of ALS patients with abnormal *ataxin-2*, in contrast, carry a medium-long version of the gene. "It may be that interactions between these proteins are underlying different presentations of neurodegenerative disease, but the same pathway is involved," says Bonini. "There might be a continuum of damage caused by different versions of *ataxin-2*."

In a relatively short time, the pair has inched closer to understanding the causes of ALS. And even if they never fully understand what triggers the disease—a fear that Bonini expressed—their yeast and fly systems can help identify potential treatments. Already, they have identified several genes that appear to slow the toxic effects of *TDP-43* and *ataxin-2*. If confirmed, these protective genes could inspire drug development, Bonini says.

In the laboratory of Arthur Horwich, mice engineered to carry the human *SOD1* gene linked to hereditary ALS serve as subjects for drug testing. To generate ALS-like symptoms quickly in the animals, Horwich revved up expression of the SOD1 protein. "In mice you might need 50 to 100 times the concentration of [SOD1]," he says. "Then you get roughly the same level of toxicity you see accreted in humans over many decades." He adds, "Of all the neurological diseases, ALS is really the most accurately recapitulated in mice. We're beginning to see what the toxic effects are at the level of the neuron. We're trying to connect the dots."

One connection has become clear: in all types of ALS, whether linked to *SOD1* or *TDP-43*, clumps of protein aggregate where they shouldn't in motor neurons. In 2009, Horwich published two papers showing that worms or mice carrying mutant human *SOD1* produce aggregates in neurons similar to what ALS patients

"The field is making progress," Hugo Bellen says. "Every year we make strides toward better understanding. But it's been a very tough nut to crack."

produce. Intriguingly, *SOD1* mice show that motor neurons can, for a time, successfully clear the aggregates. But eventually, the cells' ability to disperse the clusters wears down and the nerves become dysfunctional. Horwich hopes some of the wide range of drugs he's testing on mice and worms rev up the cells' ability to clear the clumps. "If we can do that, the downstream toxicity is prevented and the animals walk away free," he says.

Bellen at Baylor College of Medicine is also trying to connect the molecular dots in mice bred with ALS-causing *SOD1* mutations. Over the past decade, he's focused on the junction between motor neurons and muscle cells, studying a protein that may one day serve as an early warning sign of ALS. The protein, called VAPB, directs proper development of the neuromuscular junction, and it appears to be depleted in ALS patients and *SOD1* mice, several other research groups have found. A critical segment of this protein also circulates in the blood and functions as a hormone, which makes it an ideal candidate for an early detection blood test. Bellen is now supplying his ALS mice with extra VAPB to see if the protein slows or reverses symptoms—a small, early step toward developing the protein as a drug.

Though progress identifying the causes of ALS has been slow and piecemeal, researchers are inching closer to a unified theory of the molecular mechanisms of the disease. "The field is making progress," Bellen says. "Every year we make strides toward better understanding. But it's been a very tough nut to crack."

Making Motor Neurons

Perhaps the newest means to study ALS will finally provide the scientific nutcracker researchers have long been searching for: dishes of human motor neurons grown from ALS patients. Until now, "never have we ever, ever been able to isolate a single motor neuron from a [nonfetal] source that could survive," says Kevin Eggan. He has perfected the molecular recipe for making the cells, a recipe that promises a nearly unlimited supply of motor neurons that can be used for basic physiological studies of the cells and for screens of potential new drugs.

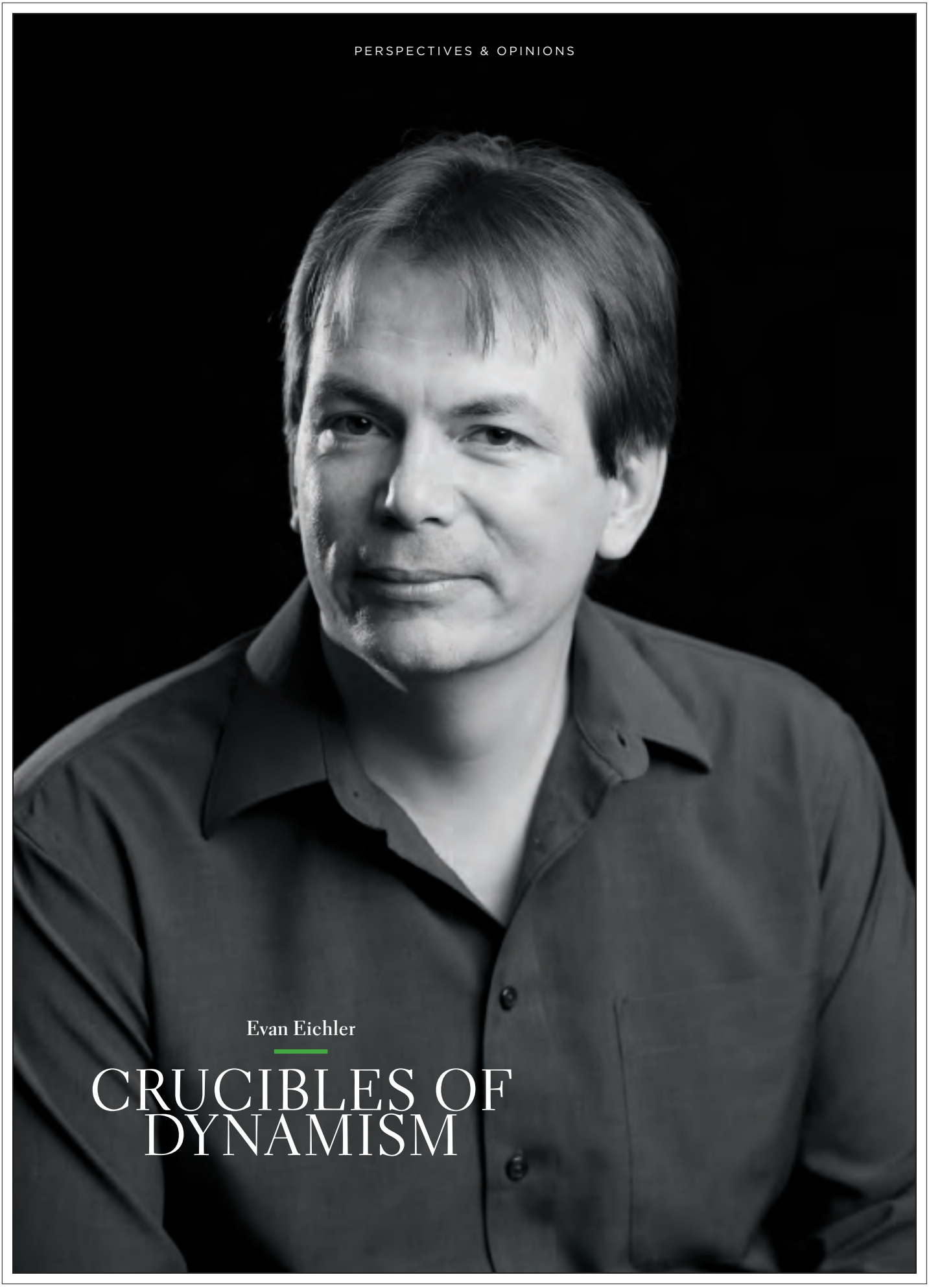
The recipe builds on two decades of work centered in the laboratory of developmental biologist and HHMI investigator Tom Jessell of Columbia University. Jessell became fascinated with motor neurons early in his career, as they're fundamentally different from the 10,000 other types of neurons. Motor neurons send long axons—up to three feet long—outside the spinal sheathing to every muscle in the body. "They're the sole means of communication between the central nervous system and the body," he says. "So in this feature they are distinct from all other CNS neurons."

Working with chicken and mouse embryos, Jessell figured out the sequence of genetic switches that turn on and off in embryonic nerve tissue to produce motor neurons. Building on that work, a postdoctoral fellow in Jessell's lab, Hynek Wichterle, discovered in 2002 that adding two small molecules to embryonic stem cells coaxes them to generate motor neurons with high efficiency. Wichterle and Jessell told Eggan about the advance over coffee, and the young Eggan—who was then deliberating a career path—decided to work on motor neurons and ALS. "Hynek's work distilled 20 years of developmental biology into a simple and reproducible molecular recipe for making a motor neuron," Eggan says. "These cells were not *like* motor neurons, they really *were* motor neurons. They were electrophysiologically active; they made synapses with other cells."

After Eggan landed a faculty position at Harvard, he and his colleagues had some early successes in making human motor neurons, but the difficulty in obtaining a sufficient supply of human embryos slowed the work. Then in 2006, while attending a stem cell conference in Whistler, British Columbia, Eggan heard about a second advance that would prove crucial to pushing the work forward. Shinya Yamanaka of Kyoto University told attendees that he had reprogrammed ordinary skin cells to act like embryonic stem cells. Yamanaka called the new cells induced pluripotent stem (iPS) cells. "I was sitting in the back row and this wave of realization washed over me," Eggan says. He visualized a path to an unlimited supply of motor neurons carrying all the genetic mutations found in ALS patients. Step 1: Obtain skin cells from patients. Step 2: Reprogram those cells into iPS cells. Step 3: Apply Wichterle's recipe to grow those embryonic-like cells into motor neurons.

Now, after collecting skin cells from several dozen ALS patients and transforming them, Eggan is confident that the

(continued on page 48)



Evan Eichler

CRUCIBLES OF DYNAMISM

Scott Aveman

Puzzling pockets of redundancy account for about 5 percent of the human genome. HHMI investigator Evan Eichler found a way to interpret what is happening in these areas of genetic repetition. The University of Washington researcher's discovery holds promise for our understanding of evolution and neurocognitive disease.

We're accustomed to thinking of evolution moving forward one base pair at a time: A single nucleotide is replaced in the ribbon of our DNA and a gene is gradually transformed from one function to another, or a disease emerges as the gene's function is perturbed by these small stepwise changes. But there is another, parallel story unfolding within our genes, which, until recently, no one was able to easily read. To all appearances, it's a more dramatic tale, with big events and rapid cataclysmic change.

If the single-nucleotide substitution view of genetic advances relates a drawing room drama of our evolution, the story my lab is uncovering is more like an action-adventure movie with multiple car crashes. We think it is in this part of the story where profound changes took place in our evolution. The regions we examine also appear to be a source of vulnerability to cognitive disease, where big genetic errors can lead to intellectual disability, autism, and schizophrenia, among other disorders.

Although it was long apparent that duplication reigned in parts of our genome, there was no way to accurately analyze these repeating elements. Our assays were based on hybridization, which relied on nucleotide probes to suss out complementary sequences, and such probes could not distinguish among identical regions. We could not count the multiple copies of genes accurately, and we could not relate genetic changes to function.

Using next-generation genetic sequencing and a new computational algorithm, my lab can now read about 70 percent of the chaotic chromosomal regions. The emerging picture is of regions that are crucibles of dynamism, where lots of change happens quickly, both adaptive and deleterious.

It appears that in the early common ancestor of humans and the great apes, certain genetic segments began hopping around the genome, inserting a duplicated version of themselves at new locations. This pattern is rarely seen in other mammals. While mice, cows, and dog genomes carry repeated genes, the repeats tend to follow one after the other. But our analysis of duplication in the genomes of macaque, orangutan, chimp, and human, published in *Nature* in 2005 and 2009, showed that in the early common ancestor, repeats may be more than a million bases apart.

When these hopscotching genes relocated—and we don't actually know how or why—they picked up whatever genetic code sat beside them, and then duplicated it and themselves somewhere else along the genome. We call these hopscotching genes "core duplicons" because they seem to be at the heart of most regions of dynamic genetic change. From their new roost, the duplicons gathered additional flanking regions, and again continued their march along the chromosome, creating complex regions of duplications within duplications at many new locations.

This architecture can create problems. Any time you have identical sequences, it's easier to trick DNA's recombination machinery. These repeating segments and the regions that flank them are susceptible to unequal crossing-over events. That is, chromosome pairs, no longer perfectly aligned, swap genetic material unequally during meiosis, leading to big changes when sperm and egg form. Some of the most common genetic causes of epilepsy and autism are due to rearrangements in these duplicated genes.

When we looked more closely into these regions, we saw an incredible amount of variation between individuals and some intriguing genes that have expanded in primate evolution. Although we have been studying these regions for 15 years, the extent of genetic variation still surprises me.

Of course, the big challenge now is to sort out just what this means, to link this more complex variability to phenotype—that is, any observable traits. Early data suggest that the genes within the core duplicons are expressed preferentially in neurons, particularly at sites of new nerve growth. We know that the large-scale copy number variation can explain about 17 percent of the cognitive disability among children we study from University of Washington Autism Center and other clinics around the world.

One has to wonder why this kind of structural vulnerability hasn't been selected against over the generations. The persistence of this genomic architecture suggests that these regions also confer important evolutionary benefit. I believe there's a balancing act at play, especially considering the point in our evolutionary history when the burst of duplications occurred.

The circumstantial data argue for something fundamental that we're missing about evolution and disease—that this structural variability contributes to what distinguishes us from other primates. Is that the whole story of how we evolved? Absolutely not. But it is an important part that I think has been overlooked. Over the next couple of years, we'll either prove or disprove that hypothesis.

INTERVIEW BY JENNI LAIDMAN. *Evan Eichler is a professor of genome sciences at the University of Washington.*

Q&A

Tell us something a postdoc said or did during an interview that made you sure he/she wasn't right for your lab.

One of this issue's feature articles explores the secrets of hiring good postdoctoral fellows, calling them "the backbone of a lab." But not every postdoc who applies for a spot is a good fit, and the interview process is key to finding the best match for a given lab. Here, four scientists share moments when they knew a potential postdoc wouldn't work out.

—EDITED BY SARAH C.P. WILLIAMS

Leslie B. Vosshall

HHMI INVESTIGATOR
THE ROCKEFELLER UNIVERSITY

"I prescreen my postdoc applicants with a written questionnaire before they set foot here for an interview. I ask potential postdocs what kind of project they would like to work on, among other questions. If the answer shows a fairly standard approach to problems and lacks creativity or a sense of excitement, I pass on the candidate. If the person comes up with projects that are in progress in the lab (but not generally publicized, meaning they have guessed what we are doing), or comes up with new interesting ideas, I will definitely consider inviting them for an interview."

Leonard I. Zon

HHMI INVESTIGATOR
CHILDREN'S HOSPITAL BOSTON

"I interviewed a postdoc candidate from France. He seemed pretty well qualified and was nice. At the end of the interview, the postdoc said he had one other request: "In France, we go on vacation in the summer. I must request 5 weeks of vacation per year, and that all of those weeks are to be taken in August–September." I must say that I was taken aback and couldn't hire him."

Bradley Cairns

HHMI INVESTIGATOR
UNIVERSITY OF UTAH SCHOOL OF MEDICINE

"My lab utilizes genomics to study transcription and chromatin, and statisticians are becoming very valuable for the analysis of genomics data. One postdoc we interviewed last year had been working on statistics involved in air pollution and traffic patterns and had done very good statistical work. When he arrived for his interview, however, he assumed we had read all of his and his mentor's papers, yet he had not read a single paper of ours. When challenged on this, he said he was confident he could master all the literature on vertebrate development, epigenetics, and transcription in a month, two at the most. To justify, he said that statisticians (himself in particular) were much smarter than molecular biologists. We are still laughing about that interview."

Lily Jan

HHMI INVESTIGATOR
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

"One potential postdoc appeared to pay more attention to whether studies—both his and ours—were published in high-profile journals than to the content of the studies themselves. This left us unsure that this very accomplished young scientist was right for our lab—though it's always difficult to gauge based on brief interactions. On the other hand, our experience has been very good with postdocs who came without flashy publications and interviewed well."

Vosshall: Allan Zepeda / AP, ©HHMI Zon: Courtesy of Leonard Zon Cairns: Paul Fetters Jan: Fred Mertz

chronicle

38 SCIENCE EDUCATION
Road Warrior

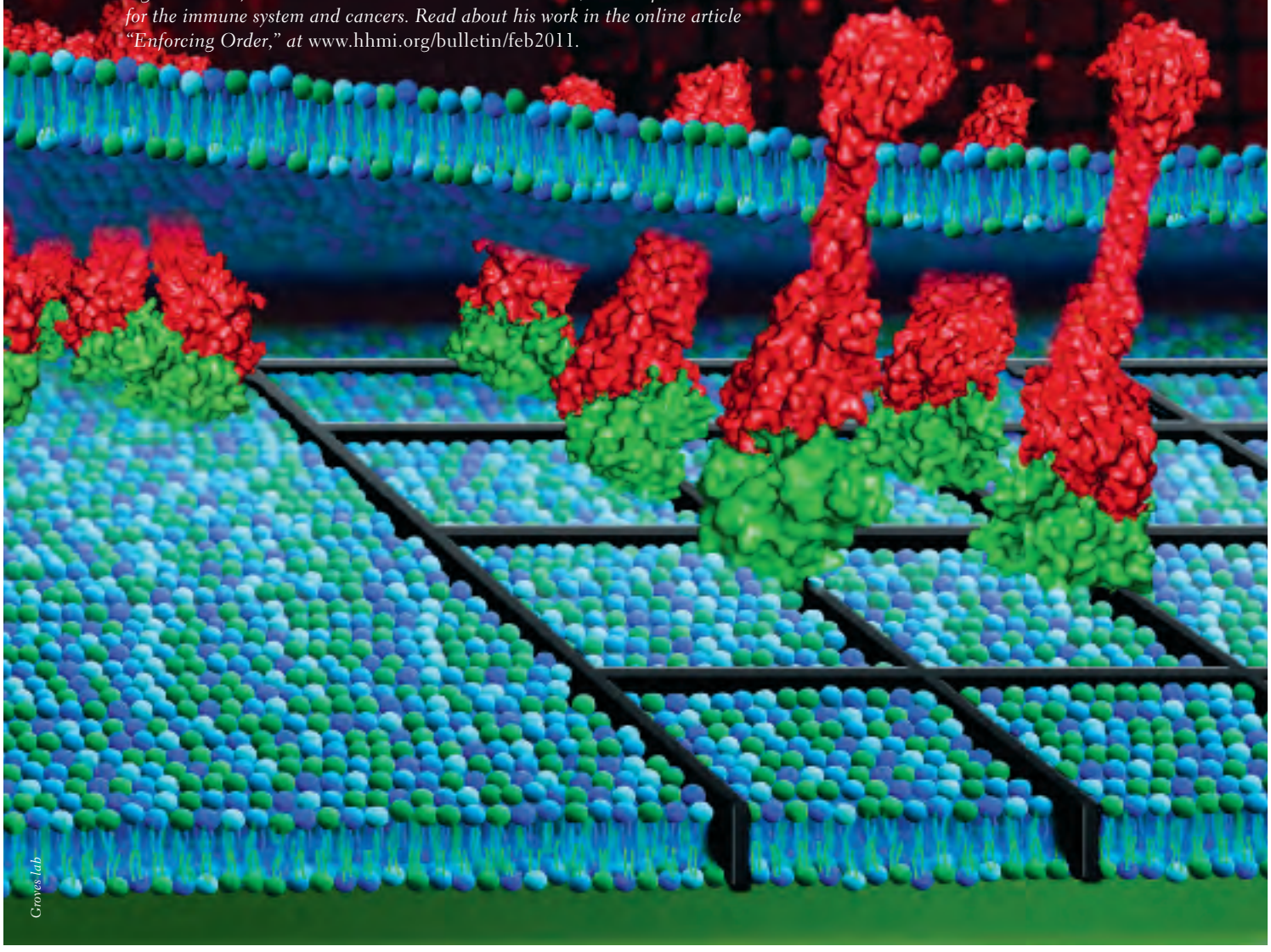
40 INSTITUTE NEWS
2010 Holiday Lectures on Science—Viral Outbreak: The Science of Emerging Disease / SEA Reaches New Shores / HHMI Launches International Competition for Early Career Scientists

42 LAB BOOK
Forgetting Fear / Releasing the Brakes on Cell Fate / Warming Malaria

45 ASK A SCIENTIST
How do internal stresses cause the external appearance of humans to change with things such as wrinkles? Can appearance predict general health?

46 NOTA BENE
National Medal of Science Goes to Lindquist and Benkovic / Six HHMI Researchers Elected to Institute of Medicine

Jay Groves turned his physical chemistry expertise on a biological question: does organization in a cell affect function? To find the answer, he creates gridded membranes, introduces proteins, and watches them behave as he adds blocks and hurdles. The results he's getting from his experiments—an example is depicted in these images—suggest just how important spatial organization of molecules inside cells is to human health, with implications for the immune system and cancers. Read about his work in the online article "Enforcing Order," at www.hhmi.org/bulletin/feb2011.



Groves lab

Road Warrior

SPREADING THE WORD THAT RESEARCH IS DOABLE IS IN MIKE YERKY'S DNA

TALL, LONG-HAIRED, AND DRESSED IN BLACK, MIKE DARWIN YERKY looks like a rock star. His appearance also fits with his informal moniker: road warrior.

Yerky travels in a minivan—packed with pipettes and gel rigs—to schools in upstate New York and beyond as the mobile division of the Cornell Institute for Biology Teachers (CIBT). Supported by an HHMI grant, CIBT brings college-level labs to high schools, conducts workshops for science teachers in Cornell's hometown of Ithaca, and runs a lending library where cash-strapped teachers can borrow expensive equipment.

A primatologist from Switzerland, Yerky took the middle name Darwin when he became a U.S. citizen days before his 40th birthday. Each year, he shares his love of science with about 2,000 students from special education groups, advanced placement classes, and everything in between. In November, he visited the Cuyahoga Valley Career Center, a vocational school in Brecksville, Ohio. His mission: teach a room full of half-interested teens how to do DNA electrophoresis, a sophisticated technique that uses an electric current to separate DNA strands in a gel slab for analysis.

"I don't think I can do this," says a girl named Danielle, as she uses a pipette to extract DNA from a microfuge tube. She and her classmates from nearby Garfield High School are visiting the career center as part of a health careers field trip.

"Yes, yes, you can," says Yerky, talking her through step by step. "Nice. See?" This is progress. Earlier, Danielle had been whispering and giggling with her friends, but Yerky managed to keep her and the rest of the class in check with some droll remarks delivered in his Teutonic accent.

"If you're between six and seven milliliters after adding the alcohol, you're in good shape," he announced at one point. "If you're way up, to 14, you may need to lay off the booze a little bit next time."

Sheri Zakarowsky, a science teacher at the career center who teaches anatomy for health careers, biochemistry for the culinary arts, and physics for engineering technology, had invited the suburban high school students for the day to gauge interest in a biotech program she hopes to start next year. She says she was pleased by the students' interest in Yerky's training.

VSA Partners

Teachers in Ithaca

Zakarowsky met Yerky when she attended CIBT's two-week workshop last summer. Every year, 40 to 50 new teachers go through the program. Shorter workshops during the school year attract another 50 or so. "It was grueling," Zakarowsky says. In one lab, she and other teachers performed DNA analysis on insects to determine the prevalence of parasitic bacteria. "But I learned a lot."

Yerky enjoys seeing the teachers come into their own during their time away from the classroom.

"We usually get people who are not that secure in how they teach science, and they soak up stuff like sponges. They exchange information and experiences. So you see them progress in their confidence level. 'Oh, yeah, I can do that now!'"

After a workshop, teachers can borrow from the CIBT lending library—a real boon, considering equipment for the DNA lab, for example, can run about $10,000. Another popular high school lab is a protein gel kit for analyzing and comparing protein bands according to their molecular weights, then establishing evolutionary relatedness among different groups. There are kits designed specifically for elementary teachers, too, like a "Bat Trunk" for learning nocturnal animals' habits and habitats, with interactive learning games, videos, and plenty of cool stuff to pass around: desiccated bat wings and skin, and bat masks and puppets. Teachers can also borrow microscopes and anatomic models.

Tips and Tricks

School visits give Yerky follow-up time with teachers after they return to the classroom. "They can see me present the lab again and take notes on what I do, jot down little tips and tricks that I point out that might not be in the written lab."

Carolyn Wilczynski, a science teacher at Binghamton High School's Regents Academy for at-risk kids in New York, attended her first CIBT summer workshop in 2000, as a rookie teacher. "It gave me a community of teachers that I could interact with all over the state," she says. "And a support system at Cornell. I could borrow equipment that wasn't available at the high school and ask questions." She also learned more about cellular molecular biology. "I had never used a pipettor before," she says. "I had never run DNA gel electrophoresis in any of my college courses."

Each year, Wilcyznski attends follow-up workshops in Ithaca to stay sharp. In the classroom, "I like talking with the kids, but the interaction with my peers is on a different level."

Wilczynski teaches about 60 at-risk students. "They might not otherwise meet a scientist in their lives," she says." Having someone like Yerky come in is really exciting for them. When they figure out the results of their lab—oh, my gosh, the joy! They're running to tell the secretary what they've done. I've taught the accelerated kids, but they're more reserved. They've done a lot of involved experiments already."

Back in Zakarowsky's class, the students finish running their gels. Yerky instructs them to come to the front of the room, one group at a time, to review their findings. "But first you have to disconnect and unplug everything," he instructs, with a wink. "We don't want you to leave with a free perm. Right?"

An all-female group of budding forensic scientists goes first. They've made a few technical mistakes and the output is a "little weak," says Yerky, but strong enough to see some results.

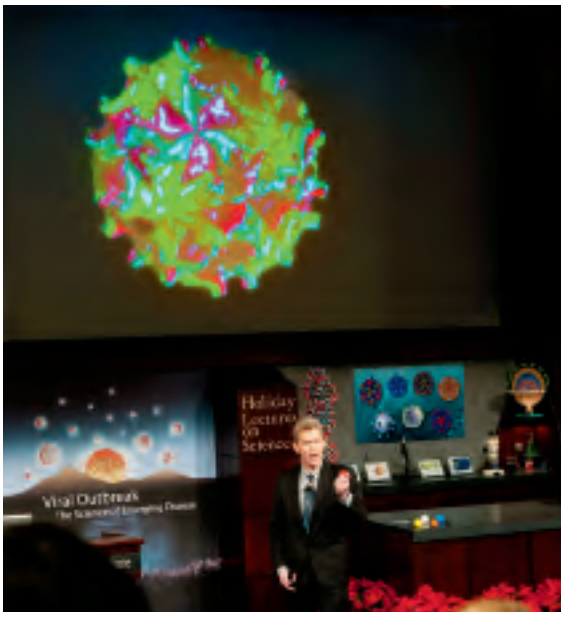
One girl, Barbara, looks crestfallen. "I was disappointed about my DNA—I got some, just not enough," she says. "But I really liked the experiment. I understood everything. And I thought it was all interesting." She wonders aloud whether she and her fellow group members can divide up their gel and take a piece home as a memento. But her chemistry teacher, Brad Lambert, proudly says he first wants to show it to the principal, "so he can see what we're doing."

"We usually get people who are not that secure in how they teach science, and they soak up stuff like sponges. They exchange information and experiences. So you see them progress in their confidence level. 'Oh, yeah, I can do that now!'"

MIKE YERKY

As Barbara leaves, she says goodbye. "Thank you for everything. Wonderful lab."

"You're welcome," Yerky replies. "Maybe I'll see you at Cornell a couple years from now." ■ –LAURA PUTRE



2010 HOLIDAY LECTURES ON SCIENCE

Viral Outbreak: The Science of Emerging Disease

VIRUSES THAT KILL BY CAUSING SARS AND DENGUE FEVER. VIRUSES that fell parrots and destroy colonies of bees. Almost 200 high school students from across the Washington, D.C., area learned firsthand how scientists study the emergence and spread of these and other deadly viruses on December 2–3 at the 2010 Holiday Lectures on Science. At least 3,000 others watched the live webcast of the lecture series, Viral Outbreak: The Science of Emerging Disease.

Eva Harris, a professor at the University of California, Berkeley, told students about her experience studying dengue fever with scientists and clinicians in Nicaragua and their efforts to improve detection and treatment. Harris showed a music video produced by local musicians that helped educate the public on how to control mosquitoes, which carry the virus.

The students also learned how scientists identify previously unknown viruses from HHMI investigator Joseph L. DeRisi, of the University of California, San Francisco. DeRisi showed them how he distinguishes new threats from older viruses by using a microchip technology he developed called Virochip. He also described the different types of viruses and gave each student a brightly colored, racquetball-sized virus model created on a three-dimensional printer in his lab.

After the lectures, students got to try their hands at virus identification, working in small teams to analyze virus data from DeRisi's lab, or talk with public health experts who work to control the spread of mosquito-borne diseases in the United States, such as dengue fever and West Nile virus.

In addition to the students, 14 teachers from the United States and Canada attended the lectures and spent the next few days brainstorming to create related lessons as a resource for other teachers. ■

Top left: *Students learn about virus threats.* Top right: *Joe DeRisi shows a Nodamura virus, a common virus that infects mammals and insects.* Bottom left: *Eva Harris talks about her work in Nicaragua.* Bottom right: *Students help each other identify mystery viruses using microarray data from Joe DeRisi's lab.*

Paul Vetters

SEA Reaches New Shores

HHMI'S SCIENCE EDUCATION ALLIANCE ANNOUNCES NEW MEMBERS.

HHMI HAS SELECTED A DIVERSE GROUP OF 12 colleges and universities to become part of its Science Education Alliance (SEA). The institutions will join 36 other schools in offering a year-long course that gives first-year students the opportunity to do sophisticated, hands-on research.

The 12 new alliance members include large research universities and small colleges from urban centers and rural towns. Another 14 schools—including a consortium of four Maine colleges—will join SEA as associate members. Faculty from associate member schools, like those at full-member institutions, will attend training sessions to implement the phage course but have the option of offering students an abbreviated version. Director Tuajuanda Jordan is excited that the initiative will now reach students in 29 states and Puerto Rico. "The SEA tide continues to roll."

The course, called the National Genomics Research Initiative (NGRI), is taught in two parts. In the first part, students isolate bacterial viruses, called phage, from local soil samples, purify them, and extract their DNA. In the second part, the students use bioinformatics techniques to analyze the phage's genome. So far, almost 1,700 students have participated in the NGRI course at 39 schools. During the first two years, the students sequenced and annotated 37 phage and then deposited the data in the national GenBank database. Another 50 are expected to be completed this year, Jordan says.

"This experience makes excellent students that much more excited, and it makes students who weren't sure about their level of interest in the life sciences a lot more engaged," says Sean B. Carroll, HHMI's vice president for science education.

The new group will go through training in the coming months and will offer the NGRI course in classrooms beginning in the fall of 2011. ■

2011 SCIENCE EDUCATION ALLIANCE MEMBERS

BROWN UNIVERSITY Providence, RI
CARTHAGE COLLEGE Kenosha, WI
COLLEGE OF ST. SCHOLASTICA Duluth, MN
GEORGIA GWINNETT COLLEGE Lawrenceville, GA
JOHNS HOPKINS UNIVERSITY Baltimore, MD
MONTCLAIR STATE UNIVERSITY Montclair, NJ
OHIO STATE UNIVERSITY Columbus, OH
OUACHITA BAPTIST UNIVERSITY Arkadelphia, AR
SOUTHERN CONNECTICUT STATE UNIVERSITY New Haven, CT
UNIVERSITY OF FLORIDA Gainesville, FL
WASHINGTON STATE UNIVERSITY Pullman, WA
XAVIER UNIVERSITY OF LOUISIANA New Orleans, LA

ASSOCIATE MEMBERS

DEL MAR COLLEGE Corpus Christi, TX
GETTYSBURG COLLEGE Gettysburg, PA
HAMPDEN-SYDNEY COLLEGE Hampden-Sydney, WV
ILLINOIS WESLEYAN UNIVERSITY Bloomington, IL
MIAMI UNIVERSITY Oxford, OH
MOREHOUSE COLLEGE Atlanta, GA
OKLAHOMA STATE UNIVERSITY Stillwater, OK
PROVIDENCE COLLEGE Providence, RI
SMITH COLLEGE Northampton, MA
SOUTHERN MAINE COMMUNITY COLLEGE South Portland, ME
TRINITY COLLEGE Hartford, CT
UNIVERSITY OF MAINE HONORS COLLEGE Orono, ME
UNIVERSITY OF MAINE at Fort Kent, ME
UNIVERSITY OF MAINE at Machias, ME

HHMI Launches International Competition for Early Career Scientists

HHMI OPENED AN INTERNATIONAL COMPETITION IN DECEMBER aimed at helping up to 35 early career scientists establish independent research programs. Scientists trained in the United States who are now running a lab in any eligible country may apply.

"Science is an international endeavor," says HHMI President Robert Tjian, "and HHMI wants to help develop the next generation of scientific talent worldwide."

HHMI has committed $24 million for the International Early Career Scientist Program. Each selected scientist will receive $650,000 over five years, with eligibility for a five-year extension of the grant. The competition is open to scientists who have trained in the United States at the graduate, medical, or postdoctoral level; have run their own labs for fewer than seven years; and work on biomedical research in a university or nonprofit research institution in one of 18 eligible countries: Argentina, Brazil, Chile, China, Czech Republic, Egypt, Hungary, India, Italy, Mexico, Poland, Portugal, Russia, South Africa, South Korea, Spain, Taiwan, and Turkey.

The program will complement HHMI's 2008 initiative supporting 50 early career scientists in the United States. Early career support can be even more important internationally, because many scientists working outside the United States do not receive the large startup packages available at many U.S. universities. If the program is successful, it could form the basis for a larger international endeavor.

"HHMI's investigators are deeply involved in the international research community, and we want to make sure that community is as robust as possible," says HHMI vice president and chief scientific officer Jack E. Dixon. Since 1991, HHMI has spent more than $145 million to fund international scientists working in specific geographic areas, including Canada, Latin America, and Eastern Europe, or in a specific field of research, such as parasitology or infectious disease.

In a June survey of HHMI investigators and early career scientists, 73 percent of those who responded reported international collaborations and 62 percent said they have international postdoctoral students in their laboratories.

The competition is open until February 23, 2011. Interested scientists can submit their applications on HHMI's website, www.hhmi.org/research/application/iecs2011. Panels of distinguished biomedical researchers will evaluate the applications; finalists will be invited to give a presentation on their work at HHMI's Janelia Farm Research Campus in November 2011. ■

FOR MORE INFORMATION: To learn more about the International Early Career Scientist Program, visit www.hhmi.org/research/competitions.

Forgetting Fear

A COMPOUND GIVEN AT JUST THE RIGHT TIME MAY MAKE MICE FORGET TO BE AFRAID.

The ultimate memory eraser—a way to avoid posttraumatic stress disorder—is now a reality for mice. HHMI investigator Richard Huganir has discovered a protein critical for erasing memory of a traumatic event.

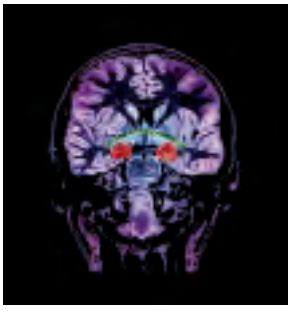
When the brain learns something—a new skill or a fear response, for example—neurons gain surface receptors at synaptic connections that strengthen signals from neighboring neurons. When fear is learned, this strengthening happens in an area of the brain known as the lateral amygdala.

To find a way to block this memory formation, Huganir and collaborators at the Johns Hopkins University School of Medicine imparted fear in mice by giving them a mild shock while playing a specific tone. When the mice heard the tone again—for weeks afterward—they froze.

The researchers examined the animals' brains at various times postshock. After a few hours, the number of glutamate receptors—common in neuron signaling—had increased at synapses in the lateral amygdala. Twenty-four hours later though, most of these receptors had lost a subunit, turning them into more rare calcium-penetrable AMPA receptors (CP-AMPARs). This finding was a surprise, especially since, a few days later, the receptors regained the subunit, returning to the more common form.

The team discovered that as CP-AMPARs the receptors are especially unstable and can be selectively removed from synapses by activation of another brain receptor, mGluR1. They hypothesized that removing CP-AMPARs at just the right time could prevent the memory linking the tone and the shock. The receptors' rarity meant that other parts of the brain wouldn't be affected.

Their hypothesis was right; a behavioral therapy known to activate the mGluR1 pathway removed these rare receptors and erased the fear memory. Now, the researchers are working to develop drugs that could activate mGluR1 to erase the memory in place of behavioral therapy.



The amygdala, shown here in red, is the area of the brain that learns fear.

The only caveat: the compound would have to be administered in a brief window of time after the event, when CP-AMPARs are present.

Huganir thinks the results, published in *Science* on November 18, 2010, will likely translate to humans. If so, there might be a period of time after a traumatic event when the brain can be coaxed to forget. ■

–SARAH C.P. WILLIAMS

IN BRIEF

NEW TIMELINE FOR PANCREATIC CANCER

Pancreatic cancer, once thought to be among the fastest-growing cancers, has been revealed to have a long, slow progression of more than 20 years. Scientists pieced together the new timeline by tracking the accumulation of cancer-causing mutations. The finding opens the possibility of detecting the cancer early.

HHMI investigator Bert Vogelstein, of the Johns Hopkins University School of Medicine, was a lead researcher on the study. He says there were two theories on why pancreatic cancers—with fewer than 5 percent of patients surviving 5 years after diagnosis—are so lethal. First, that they are a fast-growing, aggressive cancer. Second, that symptoms don't appear until the disease is far along.

"We were surprised and pleased to discover that this second theory is correct, at least for a major fraction of tumors," says Vogelstein.

He and his colleagues collected samples from seven autopsied pancreatic tumors as well as metastatic lesions from other organs. The team then sequenced each sample's DNA. Because mutations accumulate at a steady rate, the scientists could track how long it had taken for a primary tumor to form after the first cancer-related mutation, metastasize to another location with new mutations, and kill the patient.

The researchers concluded, in the October 28, 2010, issue of *Nature*, that it averaged close to 12 years from the first mutation to the formation of a mature pancreatic tumor. Then, on average, 7 years elapsed before the lesion acquired the ability to colonize other organs, and another 2.7 years went by before the patient died.

"It gives us hope that we will eventually be able to reduce morbidity and mortality from pancreatic cancer through earlier detection," says Vogelstein.

RETT SYNDROME LINKED TO SIGNALING MOLECULE

A set of neurons in the brain has been newly implicated in Rett syndrome—a disease of the nervous system that can cause autism-like symptoms, breathing problems, and a shortened lifespan. It was already known that a mutation in a gene called *MeCP2*, located on the X chromosome, causes the syndrome, which appears primarily in girls. But scientists couldn't explain how the mutant MeCP2 protein leads to the symptoms.

A team of scientists led by HHMI investigator Huda Zoghbi, of the Baylor College of Medicine, has discovered that losing *MeCP2* causes a shortage of a signaling molecule called GABA. Deleting MeCP2 protein from GABA-expressing neurons, which make up 15 to 20 percent of all neurons, was enough to cause the symptoms of Rett syndrome in mice.

Looking closer at the neurons, the researchers found that without MeCP2, levels of an enzyme that helps produce GABA dropped by 30 percent. GABA is responsible for preventing neural overactivity. Without it, the neural network becomes too active, the team found, leading to the symptoms of Rett syndrome. The results appear in the November 11, 2010, issue of *Nature*.

Zoghbi calls the results encouraging. "It gives you a path forward," she says. "If you can improve GABA signaling pharmacologically, you might improve features of Rett syndrome."

LESSONS FROM A MODEL FLATWORM

The flatworm *Schistosoma*, which infects 200 million people a year around the world with a chronic parasitic disease, is notoriously hard to study in the lab. But planarians, considered a "cousin" of schistosomes, are well established as a model organism. So when HHMI investigator Phillip Newmark wanted to know how to block reproduction

Living Art Enterprises / Photo Researchers, Inc.

Releasing the Brakes on Cell Fate

REMOVING ONE TRANSCRIPTION FACTOR ALLOWS OTHERS TO DRIVE CELLULAR DESTINY.

Converting one cell type directly into another is a kind of modern-day alchemy, an ultimate goal in biological research. But unlike turning base metals into gold, changing a cell's identity is feasible, new research shows.

While many scientists attempt to convert cell types by beginning with the clean slate of a stem cell, HHMI investigator Oliver Hobert wanted to skip a step and change one cell to another without using stem cells. But in his study organism, the nematode *Caenorhabditis elegans*, the factors required to produce neurons from stem cells had no effect when expressed in other cell types.

Hobert and his colleagues at the Columbia University College of Physicians and Surgeons hypothesized that the problem wasn't the factors—it was that cells have a protective mechanism in place to prevent reprogramming.

To find the key that makes cells impervious to reprogramming, Hobert's lab group individually blocked expression of different genes in thousands of *C. elegans* worms and then induced expression of a neuron factor. In one line, which could not make a protein called Lin-53, the germ cells—precursors to sperm and egg cells—changed to neuron cells.

Lin-53 plays a role in controlling gene expression in a cell by altering its chromatin, or DNA packaging. But the protein has never before been linked to the ability to reprogram a cell. Hobert's team was able to convert the germ cells to three different types of neurons by using different neuron factors. In each case, within 6 hours of receiving the Lin-53 block and a neuron-specific factor, the cell changed its gene expression and underwent morphological changes. The results appeared online in *Science* on December 9, 2010.

In normal nematodes, there's only one ASE neuron (green dot), but researchers have now coaxed other cells to turn into ASE neurons.

"This is a potential first step toward being able to generate any cell type you want," says Hobert. "By removing Lin-53, we have put a foot in the door to allow other transcription factors to drive cell fate." His team has also found ways to convert skin cells into neurons by removing a separate factor, also involved in chromatin biology. Hobert thinks that each tissue type has its own reprogramming deterrent in place. Releasing these brakes allows cell switching to speed ahead. ■

–SARAH C.P. WILLIAMS

Hobert Lab

IN BRIEF

of schistosomes, he turned to planarians. His findings could now lead back to ways to thwart schistosome infections.

Some schistosomes and planarians have complicated sex lives—they can switch between sexual and asexual reproduction depending on the environment or life-cycle stage. Newmark and his colleagues at the University of Illinois at Urbana–Champaign suspected that this switch was triggered by a signal from the brain, since planarians without heads (famous for their regeneration skills) reabsorb their reproductive organs.

The researchers knew that enzymes called convertases are required to produce neuropeptides, the molecules they suspected played a role in signaling. So they deleted a convertase called pc2 from planarians. Sure enough, the flatworms' reproductive tissues reverted to a more primitive, asexual state, just like they'd seen in planarians without heads.

Then the scientists searched the planarian genome for genes encoding neuropeptides. They found around 50 genes, which helped them narrow their focus to one that's expressed only in sexually reproducing flatworms. Disrupting this gene had the same effects as deleting pc2, the team reports in the October 12, 2010, issue of *PLoS Biology*. They have also identified similar hormones in *Schistosoma*, which could lead to a new way to block the reproduction cycle of the infectious flatworm.

TURNING ON STEM CELL MULTIPLICATION

An overlooked layer of cells that line blood vessels and are found in bone marrow and other tissue is key to coaxing adult stem cells to multiply, a new study shows. The endothelium, thought to be largely a source of oxygen and nutrients to tissues, also releases growth factors.

In March 2010, HHMI investigator Shahin Rafii of the Weill Cornell Medical College published evidence showing that endothelial cells in bone marrow express growth factors. Now, he and his collaborators have found the pathway that controls expression of these growth factors.

When the researchers triggered activation of the biochemical pathway Akt in the endothelium, the number of stem cells in the bone marrow increased 10-fold. When they switched off activation, the number of stem cells decreased. The results appear in the November 2010 issue of *Nature Cell Biology.*

In a second study, the team discovered that endothelial cells in the liver also prompt stem cells to expand. That finding appeared in *Nature* on November 11, 2010. In addition, Rafii and his collaborators found evidence that activation of the endothelium is vital to tissue regeneration in the lungs and pancreas.

A next question, Rafii says, will be to determine the mechanisms by which activated endothelial cells trigger stem cell growth in each organ. "The challenge that lies ahead is to discover the organ-specific growth factors produced by the endothelial cells," he says.

CLOCKING TEMPERATURES

Fluctuations in body temperature are intricately tied to the 24-hour circadian rhythm of gene expressions in mammals, new research shows. While scientists knew that the internal clocks of plants, bacteria, and cold-blooded animals could be controlled by temperature, the new study by HHMI investigator Joseph Takahashi is the first to conclude that temperature can be used as a universal internal timing signal in mammals.

The suprachiasmatic nucleus (SCN), a tiny structure deep in the mammalian brain, is known as the body's master clock. It receives light input from our eyes and sends out signals to the rest of the body to synchronize functions to a daily schedule. Over the past couple of years, Takahashi's lab group at University of Texas

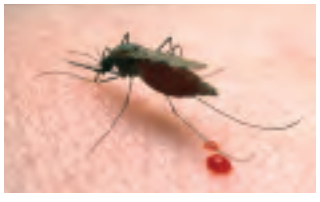
Warming Malaria

CLIMATE CHANGE IS EXPANDING THE DISEASE-CAUSING PATHOGEN'S COMFORT ZONE.

As temperatures rise in the East African highlands, so does the number of malaria cases. This conclusion of a new study by HHMI scientists suggests that areas not historically affected by malaria epidemics are at increasing risk of outbreaks due to rising global temperatures.

The retrospective study, led by HHMI investigator Mercedes Pascual of the University of Michigan, focused on a highland region of western Kenya. Here, a tea plantation made up of several estates, with a combined population of 50,000, kept detailed hospital admissions data, including confirmed monthly malaria cases, spanning 1966–2002.

"There aren't many data sets on malaria that span such a long time period and are of such good quality," says Pascual. The wealth of data combined with local temperature records made the new analysis possible.



The mosquito Anopheles gambiae *spreads the malaria parasite in Africa.*

At a physiological level, the effect of temperature on the malaria parasite has been well-studied: the parasite reproduces more quickly at higher temperatures, and the mosquitoes that carry the pathogen develop faster and bite more often. Because of this link, the Kenyan highlands are considered a "fringe region" for malaria transmission—their cooler climate has historically kept malaria levels much lower than in nearby warmer lowlands.

Pascual wondered whether long-term shifts in temperature had already changed the incidence of malaria in this fringe region. Her team isolated the effect of temperature—as opposed to drug resistance and land-use changes, among other factors—and revealed that warmer temperatures explain a significant fraction of the increase in malaria cases from the 1970s through the 1990s. The work appeared online in *Proceedings of the Royal Society B* on November 10, 2010.

The researchers are now looking at fringe regions of eastern Africa and India—including deserts, where low rainfall has typically limited malaria—to study the changing prevalence of the disease not just in the long term but also from year to year. Establishing a link between climate and malaria cases at these interannual time scales could help predict epidemics.

"Climate's effect on epidemic malaria does not mean that interventions cannot be effective," stresses Pascual. "It might just mean a bigger control problem." And understanding climate's effect, she says, might be key to this control. ■ –SARAH C.P. WILLIAMS

IN BRIEF

Southwestern Medical Center at Dallas and others have shown that virtually every cell in the body contains a circadian clock.

To study how the expression of cellular clocks waxed and waned over the course of a day in mice, Takahashi and his colleagues attached a luminescent protein to *Per2*, an important clock gene. Then they studied rhythms in *Per2* expression in cultured lung and pituitary tissue from mice. When they changed the temperature from 36°C to 38.5°C, within the normal range of body temperature for mice, the cycle of *Per2* was strongly reset to the temperature signal. In contrast, the SCN clock was resistant to the same temperature signals—explaining why mammals are largely insensitive to environmental temperature cycles at the organismal level.

Cell-cell communication in the SCN is responsible for temperature resistance, the researchers concluded on October 15, 2010, in *Science*. Thus, the SCN clock in the brain can transform 24-hour light-dark cycle information into an internal body temperature signal that syncs cellular clocks throughout the organism.

TRACING THE ROOTS OF HAITI'S CHOLERA OUTBREAK

The strain of cholera that began sweeping through Haiti in October 2010 originated in South Asia, according to a study co-authored by HHMI investigator Matthew Waldor. The finding supports the idea that the cholera arrived on the island through human activities, rather than washing up environmentally from Latin America.

To determine the origin of the cholera that has killed more than 2,500 people in Haiti as of mid-December 2010, Waldor and his colleagues at Harvard Medical School obtained two samples of *Vibrio cholerae*, the bacterium that causes the intestinal disease, from two Haitian patients. In collaboration with Pacific Biosciences, a company that manufactures DNA sequencing machines, the scientists quickly sequenced the genomes of the cholera bacteria.

The DNA sequences revealed that the two samples of *V. cholerae* were virtually identical. When compared with 26 other *V. cholerae* strains, the samples matched strains currently circulating in South Asia, which have different genetic markers than strains in South America.

"The most likely explanation for the sudden appearance of cholera in Haiti, the authors conclude, is transmission of the bacteria by an infected human, food, or other contaminated items from a region outside of Latin America to Haiti.

The analysis, which appeared online December 9, 2010, in the *New England Journal of Medicine* also found that the strain, while potentially more virulent than average, should respond to tetracycline antibiotics.

AGING MITOCHONDRIA RELATED TO DIABETES RISK

Weakened mitochondria are the reason a person's risk for type 2 diabetes increases with age, according to research by HHMI investigator Gerald Shulman.

Mitochondria generate energy and are particularly active in muscle cells. Schulman's lab group at the Yale School of Medicine had already shown that elderly individuals have lower mitochondrial activity in their muscles, which is associated with higher fat content and insulin resistance.

To test the hypothesis that oxidative damage in mitochondria was responsible, the researchers overexpressed the human catalase gene, which encodes an enzyme that protects against oxidative injury. The latest work shows that, similar to humans, older mice have reduced mitochondrial function, increased fat in their muscles, and insulin resistance. In contrast, mice with higher levels of catalase were protected from these age-associated changes.

The results, published in the December 1, 2010, issue of *Cell Metabolism*, offer further evidence that altered mitochondrial function plays a role in age-related insulin resistance and suggests that reducing oxidative damage in mitochondria may be a way to prevent age-related type 2 diabetes.

Dan Salaman / London School of Hygiene and Tropical Medicine

Q A

How do internal stresses cause the external appearance of humans to change with things such as wrinkles? Can appearance predict general health?

Asked by Joe, from Pennsylvania

In nature, external appearance absolutely predicts general health and is one of the driving forces of survival of the fittest. Within one species of multicolored parakeets, for example, females prefer to mate with the male that sports the brightest colored feathers. While it seems purely aesthetic and random to fixate on such features, the male with the brightest colored feathers is in fact the healthiest bird; his vibrant hues indicate he has the highest levels of important vitamins in his body. Humans, as well, are programmed to gravitate toward healthier individuals when choosing a mate.

The largest organ in the human body—the skin—is a remarkable conveyor of health. Physicians are trained to look to the skin for the external signs of diseases, which can sometimes be very subtle. For example, jaundice, a yellowing of the skin, results from a buildup of a chemical called bilirubin, which is generated from the breakdown of red blood cells. In a healthy body, it's excreted by the liver through the feces and urine. When the liver is diseased, however, bilirubin piles up in the blood and eventually deposits in the skin.

Another internal condition that changes appearance is Addison's disease, which is an inability of the adrenal glands to produce adequate amounts of certain hormones. In response, the brain ramps up production of adrenal-stimulating hormones to try to turn on the adrenal gland. This adrenal-stimulating hormone is structurally similar to a pigmenting hormone. As a result, people with Addison's disease often experience darkening of their skin.

To understand how external stressors from the environment—like smoke and UV radiation—can alter the skin, let's examine the skin's anatomy. The epidermis, the top layer, contains sheets of skin cells. Immediately below that is the dermis, which contains blood vessels, nerves, and the proteins that give the skin its structure and texture. One such protein, called collagen, is continuously created and broken down in the skin. Both UV radiation and tobacco smoke increase the rate of collagen destruction. The body tries to repair this damage, but the process is never 100 percent perfect. Over time, with repetitive exposures to UV radiation or cigarette smoke, the skin loses structural integrity—as it loses collagen—and wrinkles form.

UV radiation can cause even more severe changes in appearance if it leads to skin cancer. The radiation from the sun's rays can damage DNA inside skin cells, eventually damaging it enough so that the cells grow uncontrollably, forming a cancer.

Each time the body replaces collagen in its natural turnover cycles, it produces slightly less. So even someone who never smokes and who consistently wears sunscreen will eventually get some wrinkles with age. Eventually, the dermis has less collagen, as well as less elastin, a protein that helps keep the epidermis stretched tightly over the body.

ANSWER RESEARCHED BY NEFTHI SANDEEP, *a third-year resident in pediatrics at Columbia University Medical Center.*

FURTHER READING

Skin aging induced by ultraviolet exposure and tobacco smoking: evidence from epidemiological and molecular studies. Yin L, Morita A, Tsuji T. Photodermatol Photoimmunol Photomed. 2001 Aug;17(4):178–83.

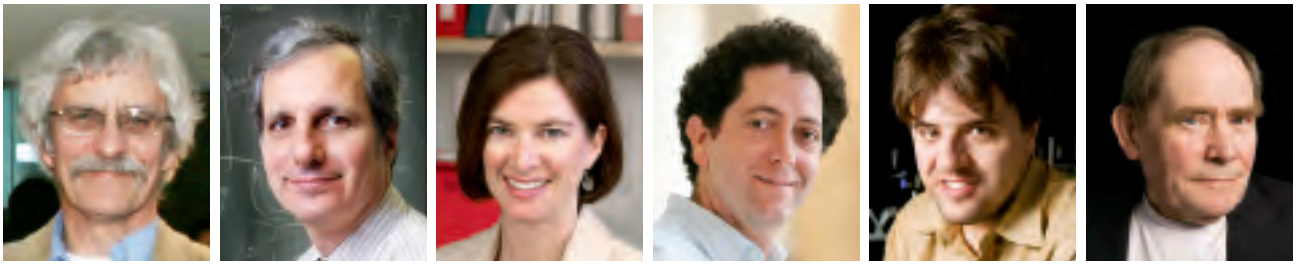
Pathophysiology of premature skin aging induced by ultraviolet light. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. N Engl J Med. 1997 Nov 13;337(20):1419–28.

Matrix metalloproteinase-1 and skin ageing in smokers. Lahmann C, Bergemann J, Harrison G, Young AR. Lancet. 2001 Mar 24;357(9260):935–6.

Science is all about asking questions, exploring the problems that confound or intrigue us. But answers can't always be found in a classroom or textbook. At HHMI's *Ask a Scientist* website, working scientists tackle your tough questions about human biology, diseases, evolution, animals, and genetics. Visit www.hhmi.org/askascientist to browse an archive of questions and answers, find helpful Web links, or toss your question into the mix. What's been puzzling you lately?

SPOTLIGHT

Six HHMI Researchers Elected to Institute of Medicine



LEFT TO RIGHT: CRESSWELL, DARNELL, DOUDNA, SHOKAT, DEISSEROTH, BRENNER

Four HHMI investigators and an HHMI early career scientist were elected to the National Academy of Sciences' Institute of Medicine. The investigators are **Peter Cresswell**, Yale University School of Medicine; **Robert B. Darnell**, The Rockefeller University; **Jennifer A. Doudna**, University of California, Berkeley; and **Kevan M. Shokat**, University of California, San Francisco. The early career scientist is **Karl Deisseroth**, Stanford University. In addition, **Sydney Brenner**, a senior fellow at HHMI's Janelia Farm Research Campus, was named a foreign associate of the institute.

A total of 9 HHMI investigators, 2 HHMI international research scholars, and a K-RITH advisory board member were named fellows of the American Association for the Advancement of Science. The investigators are FREDERICK W. ALT, Children's Hospital Boston; JASON G. CYSTER, University of California, San Francisco; ROBERT B. DARNELL, The Rockefeller University; GIDEON DREYFUSS, University of Pennsylvania School of Medicine; YISHI JIN, University of California, San Diego; JEANNIE T. LEE, Massachusetts General Hospital; STUART H. ORKIN, Children's Hospital Boston; LI-HUEI TSAI, Massachusetts Institute of Technology; and JOHN D. YORK, Duke University. The international research scholars are CHARLES M. BOONE, University of Toronto, and FREDA D. MILLER, Hospital for Sick Children, Toronto. The K-RITH board member is BARRY R. BLOOM, Harvard School of Public Health.

JAMES P. ALLISON, an HHMI investigator at Memorial Sloan-Kettering Cancer Center, won the 2010 Richard V. Smalley, MD Memorial Award, from the International Society for Biological Therapy of Cancer at the society's 2010 annual meeting. Allison's research focuses on the link between the immune system and cancers.

NICOLAS ALTEMOSE, a Duke University undergraduate who participated in HHMI's Exceptional Research Opportunities Program (EXROP) in 2009, won a Marshall Scholarship. With support from his EXROP award, Altemose worked with HHMI professor Huntington F. Willard, director of the Institute for Genome Sciences and Policy at Duke. With the Marshall Scholarship, Altemose will continue genetic research at the University of Oxford.

TERESA C. BALSER, who leads the HHMI-supported "Mad Bio Boot Camp" at the University of Wisconsin–Madison, was named U.S. Professor of the Year by the Carnegie Foundation for the Advancement of Teaching and the Council for Advancement and Support of Education. Balser, an associate professor of soil science, teaches the one-week boot camp in biology for incoming freshmen each summer.

Three HHMI investigators were among 14 American scientists named members of the European Molecular Biology Organization in October. They are ELAINE FUCHS, The Rockefeller University; MICHAEL R. GREEN, University of Massachusetts Medical School; and THOMAS M. JESSELL, Columbia University. New members are elected annually based on their scientific accomplishments.

DAVID GINSBURG, an HHMI investigator at the University of Michigan Medical School, received a Distinguished Research in the Biomedical Sciences Award from the Association of American Medical Colleges. The annual award recognizes clinical or laboratory research by a medical school faculty member. Ginsburg was chosen for his research on inherited blood clotting disorders.

HHMI professor JO HANDELSMAN, of Yale University, won the 2011 D.C. White Research and Mentoring Award from the American Society for Microbiology. Recipients are chosen annually for

Cresswell: Courtesy of Peter Cresswell Darnell: Courtesy of The Rockefeller University Doudna: Michael Marsland / Yale University Shokat: George Nikitin / AP, ©HHMI Deisseroth: L.A. Cicero / Stanford News Service Brenner: Paul Fetters

their scientific accomplishments and their dedication to mentoring. Handelsman has been a regular co-organizer of the Summer Institute on Undergraduate Education in Biology, sponsored by the National Academy of Sciences and HHMI. She focuses on bringing creativity and experimentation to the classroom.

HHMI investigator **ERIC R. KANDEL**, of the Columbia University College of Physicians and Surgeons, was awarded the 2010 Catcher in the Rye Humanitarian Award from the American Academy of Child & Adolescent Psychiatry. Kandel was chosen for the impact that his research on learning and memory has had in the field of child and adolescent psychiatry.

YE-JIN KANG, an undergraduate student at Rice University and participant in Rice's HHMI-funded "Beyond Traditional Borders" program, was named a 2010 Rhodes Scholar. The scholarship, awarded to 32 Americans, provides funding for two or three years of studies at the University of Oxford. Kang plans to study global health science and global governance and diplomacy.

HHMI investigator **STEPHEN R. QUAKE**, of Stanford University, won the 2010 Pioneers in Miniaturisation Prize from the journal *Lab on a Chip* and Corning Incorporated. Quake's lab integrates biology, physics, and biotechnology to develop tools for the fields of biology and medicine. Their systems, relying on microfluidics, allow biological tests and assays to be performed at a small scale. Quake was presented the annual award at the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences in October 2010.

HHMI international research scholar **ALEJANDRO F. SCHINDER**, of the Leloir Institute Foundation, won a Guggenheim Latin American and Caribbean Fellowship. Schinder studies how nerve cells that mature in an adult differ from those formed in early development.

HHMI President **ROBERT TJIAN** received the 2010 Glenn T. Seaborg Medal from the University of California, Los Angeles (UCLA). The annual medal is given for accomplishments in chemistry or biochemistry and is named for the 1951 recipient of the Nobel Prize in Chemistry, a UCLA graduate known for his characterization of the elements. Tjian also was honored with the 2010 Grand Prix Charles-Léopold Mayer from the French Academy of Sciences, an award given each year to a researcher for outstanding work in the biological sciences. Tjian was chosen for his research on how genes are turned on and off.

Two HHMI early career scientists, **AMY J. WAGERS** of Harvard University and **MICHAEL T. LAUB** of the Massachusetts Institute of Technology, were named recipients of the Presidential Early Career Award for Scientists and Engineers. Ten federal departments and agencies nominate worthy early career scientists each year. Wagers, who studies blood and muscle stem cells, was nominated by the National Institutes of Health while Laub, whose work focuses on regulatory circuits in bacteria, received his nomination from the National Science Foundation.

HHMI investigator **WAYNE M. YOKOYAMA**, of Washington University in St. Louis School of Medicine, won the Lee C. Howley Sr. Prize for Arthritis Research from the Arthritis Foundation. The annual award is considered the most prestigious award for arthritis research. Yokoyama studies natural killer cells, important players in the immune system. When natural killer cells fail to recognize the body's own cells, they cause inflammation, such as that seen in arthritis.

SPOTLIGHT

National Medals of Science Go to Lindquist and Benkovic

SUSAN LINDQUIST

STEPHEN BENKOVIC

In November, HHMI investigator **Susan Lindquist**, of the Massachusetts Institute of Technology, was presented a National Medal of Science from President Obama in a ceremony at the White House. The National Medal, the nation's highest scientific honor bestowed by the government, was established in 1959 by Congress. Lindquist was recognized for her studies of protein folding and how misfolded proteins and aggregates of proteins can influence human health. Her research has led to an increased understanding of prions and of neurodegenerative diseases. ¶ Ten other scientists also received medals, including **Stephen J. Benkovic** of Pennsylvania State University, who is a member of HHMI's scientific review board. Benkovic studies how enzymes drive chemical reactions inside cells.

Lindquist: Cheryl Senter / AP, ©HHMI Benkovic: Greg Grieco

CONTINUED FROM PAGE 17
(THE NEXT GENERATION)

Good mentors find ways to train their postdocs to excel at mentoring when they launch their own labs. Luger pairs postdocs with an undergraduate, rotation, or graduate student, and she checks in with the student to see how the relationship is working.

One Luger postdoc learned an invaluable lesson about adjusting mentoring approaches. Paired first with an undergraduate of similar personality, the postdoc delivered criticisms directly with no "gift-wrapping." His next student, however, shriveled under what she considered offensive feedback.

Luger warns her postdocs to allow for a "training period" for the students, who often cannot keep up intellectually with a postdoc at the top of his or her game. She has a favorite exercise for reminding them that trainees are not mini-me's: "Take a page from a paper, and replace every third noun and the occasional verb with the word 'Yakutat.' That's pretty much an undergraduate's experience when they first talk to their new mentors."

Patrick Kanold trained as a postdoc in the Harvard Medical School lab of Carla Shatz, now director of Bio-X at Stanford University in California and member of HHMI's medical advisory board. He says her skilled but understated mentoring prepared him to easily set up his own group at University of Maryland in College Park.

"She gave me room to develop my own thoughts and questions with subtle little pushes. I was being directed, but at the time I didn't notice," he says. "By building my own experimental set-ups and training grad students through guided mentoring, I was being given really important experiences that I carried with me."

Bardwell is often reminded of his experiences shooting unexplored rivers. His contacts tell him that Papua New Guinea's Jimi River awaits a first descent by humans. He'd love to give it a shot, with the right team beside him. The same goes for his lab—a well-chosen team with the right training makes for a gratifying journey. ■

WEB EXTRA: For more on how these lab leaders prepare their postdocs to run a lab, visit www.hhmi.org/bulletin/feb2011.

CONTINUED FROM PAGE 27
(BEHIND THE CURTAIN)

how fast proteins produce new copies. Biochemical experiments in his lab had hinted that replisomes, the proteins involved in replication, move at different rates on different DNA strands. The lab was stumped as to how to quantify this variation until O'Donnell heard Greene talk about DNA curtains.

"The one disadvantage of single molecule work is that you're looking at a single molecule," says O'Donnell. "Greene's method got around that. I didn't want to look at one molecule and find out later that I was observing something that is not general."

O'Donnell's DNA curtains showed that individual replisomes' movement rates vary as much as 5-fold, his team reported in *Proceedings of the National Academy of Sciences* on August 11, 2009. Now they have a working average and a way to test what factors can shift that average.

As for Greene, he's ready to add complexity to his curtains. "We'd like to make arrays where the DNA is crisscrossed or supercoiled," he says. True to his love of molecular crashes, he also plans to see how RecBCD and other motor proteins behave when they encounter not just one protein in their path but a whole stretch of roadblocks. "If you think about what DNA looks like in the nucleus, it's jam-packed with proteins, so we would really like to mimic that environment by putting lots and lots of proteins on the DNA." On thousands of DNA strands, this will mean many thousands of collisions. It will be a fascinating show to watch. ■

WEB EXTRA: To see videos of DNA curtains in action, visit www.hhmi.org/bulletin/feb2011.

CONTINUED FROM PAGE 33
(HOPE FLOATS)

resulting cells "are real, functional motor neurons. We had to go to great lengths to show that," he says. The Harvard Stem Cell Institute is preparing to provide the cells to qualified researchers.

Valerie Estess, director of research for Project ALS, which helps fund Eggan's work, calls the advance "ALS in a dish." She says that, already, researchers at Harvard and at Columbia University are using the cells to screen for potential new treatments. "We can now model ALS more accurately," she says. "We hope that the drugs that emerge from the screens are contenders."

Of course, that's the ultimate goal for ALS researchers—to provide treatments for patients who now have few options. Yeast, worms, flies, mice, and, now, human neurons in a dish all offer platforms to quickly test drug candidates against the range of defects that lead to ALS. "It's great to have an arsenal of models to study disease," says NINDS's Gubitz. "Every model mirrors a specific aspect of disease, and they're much more powerful when combined."

And so with this solid foundation for discovery, ALS researchers expect an avalanche of advances in the coming years. Says Bonini: "You can't predict where the big breakthroughs are going to come from, which is why it's important to take many different approaches. It's really important to cast a wide net." Because, in the end, "the whole reason we're doing this is to make an impact for patients." ■