Winter '14 Vol. 27 No. 1

HHMI BULLETIN

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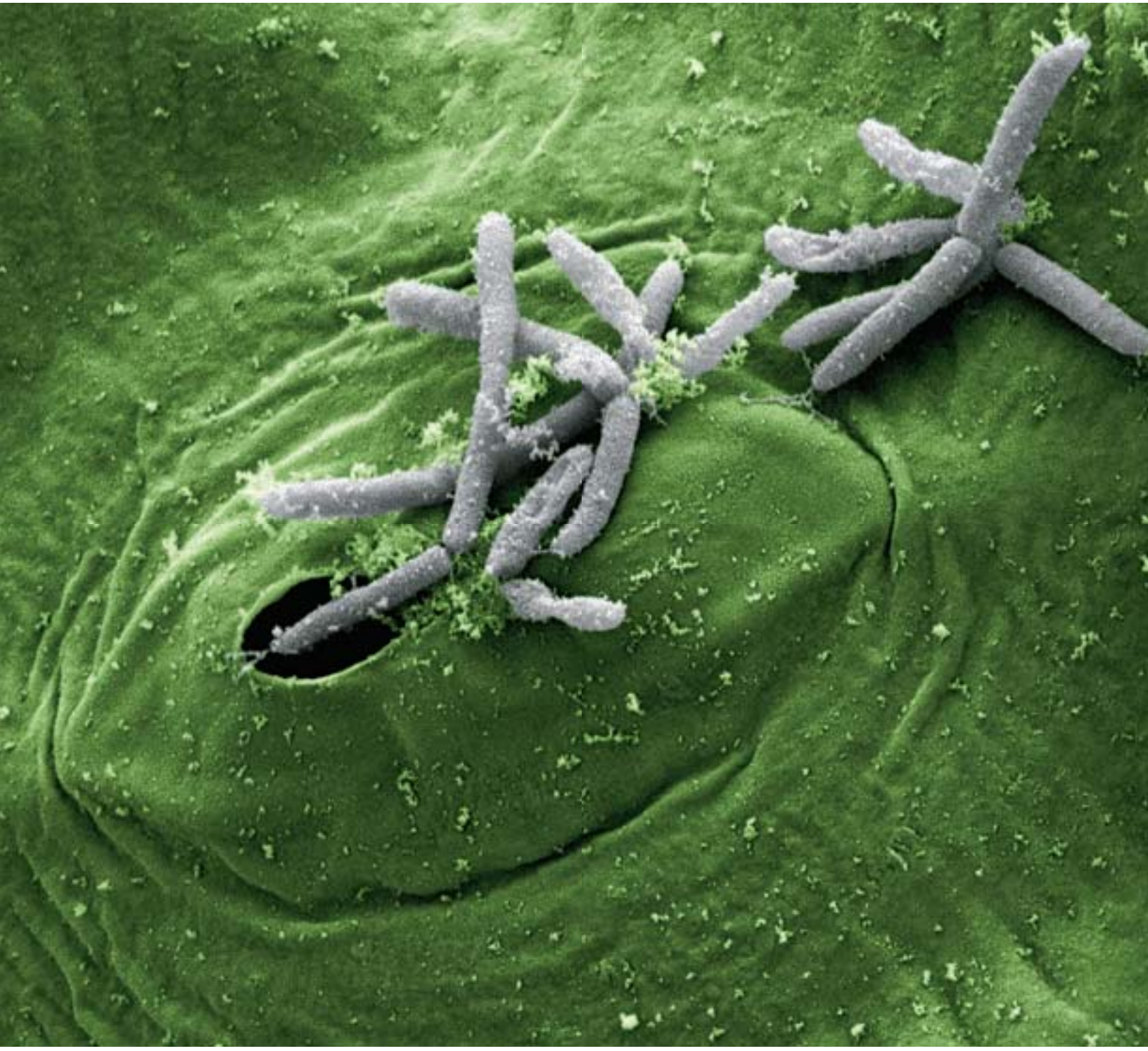
IN THIS ISSUE

Shipping News

Nobel-winning work on the basics of cellular transport



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This gang of insurgent *Pseudomonas syringae* bacteria (purple) is infiltrating a leaf through an opening called a stoma. The tiny pores—used for gas exchange during photosynthesis—are open during the day, providing a perfect route for bacteria to slip through a plant's outer protective barrier and take up residence inside. HHMI-GBMF Investigator Sheng Yang He discovered that when plants sense an assault, they shut their stomata to block the invaders.

James Kremer, Sheng Yang He

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Cover image:
Brendan Monroe

Contributors

Veteran science journalist **John Carey** ("Technical Wizardry," page 28) wrote for *Newsweek*, *National Wildlife*, and *Business Week* for three decades before going freelance in 2010. Along the way, he's chased after sea otters and loons, and explained everything from the genome project to why cholesterol numbers aren't destiny. Between assignments, he dabbles in triathlons.

Illustrator **Michael Kirkham** ("Defenses Up," page 22) lives in Edinburgh, Scotland, and thoroughly enjoys drawing pictures for clients all over the world. When not drawing, he likes to swim and row slightly leaky boats in the Lake District with his girlfriend and their two small children.

Brendan Monroe (cover and "Express Delivery," page 4) is an artist and illustrator living and working in Oakland, CA. He makes paintings about microscopic motion, comics about dreams, and blob creature wood sculptures.

New York City freelancer **Robin Marantz Henig** ("Straight Shooter," page 16) writes long-form features about science and medicine for *The New York Times Magazine*, where she is a contributing writer. Her most recent book is *Twentysomething: Why Do Young Adults Seem Stuck*, which she wrote with her twentysomething daughter, Samantha Henig.

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John Carey, Brendan Monroe , Michael Kirkham, Robin Henig

President's Letter

Alliance for Change

James Kegley

LAST OCTOBER'S Nobel Prize announcements were a thrilling celebration of the bold creativity that propels science. News reports brought us glimpses of pre-dawn phone calls to stunned researchers. For a few moments, the world was wowed by the sheer power of human discovery.

The celebration was all too short, however. We must applaud our scientific risk takers throughout the year. For it is the day-in, day-out curiosity, wonder, and unflagging persistence that fuels maverick researchers, in fields such as biology, chemistry, computer science, mathematics, and physics.

Big acknowledgements like the Nobel begin with questions whose answers may be found only in basic scientific research, done primarily for the discovery of knowledge. In the case of the 2013 Nobel Laureates in Physiology or Medicine, HHMI Investigators Randy Schekman and Thomas Südhof, along with James Rothman, worked independently to answer a fundamental question: How are proteins and other molecules transported into and out of cells? Their research, which you can read about in this issue of the *HHMI Bulletin*, spans different organisms and different systems, each revealing key molecular details of that process.

The work demonstrates the rewards of investing in basic discovery research. Schekman, for instance, decided to study cellular transport in yeast when he was starting his first lab; it was a departure from his graduate and postdoctoral work into a new and little-studied area. No one could have predicted that understanding the secretory pathway and how vesicles move in and out of cells would have implications in medicine. Yet today, thanks in part to Schekman's work, a third of insulin used by diabetics worldwide and the entire global supply of hepatitis B vaccine are produced in yeast.

But our idea pipeline is at serious risk. Federal and state funding for foundational research in universities have been repeatedly slashed, leaving many young scientists scrambling for support when they should be thinking big. Corporations once known for funding blue-sky research have largely cut back as well. Even private foundations have increasingly shifted toward applied disease research, at the expense of core knowledge.

To address the need for basic research support, HHMI has joined with five other private nonprofit organizations—the Gordon and Betty Moore Foundation, the Alfred P. Sloan Foundation, the Simons Foundation, the Kavli Foundation, and the Research Corporation for Science Advancement—to form the Science Philanthropy Alliance, with an aim to increase funding for discovery research. We hope that other philanthropists, as well as universities and government agencies, will join our efforts to channel new resources toward basic science.

This kind of high-risk, high-reward research could not be funded any other way. Like the best entrepreneurs, promising young scientists need the capital and time to pursue novel approaches and original thinking.

One approach that philanthropies can take to broaden investment in basic science is through partnerships, such as the initiative HHMI launched in 2011 with the Gordon and Betty Moore Foundation to help a talented group of plant scientists move their research in creative directions. You can read about some of their discoveries regarding plant immune defenses in this *Bulletin*. Their findings may help address food supply challenges facing the world today.

And in this issue, you can learn about Danny Reinberg, a biochemist who, with the help of an HHMI Collaborative Innovation Award, shifted his lab's focus in an unexpected direction: using the ant as a model organism to study epigenetics and its influence on animal behavior. In this case, giving Reinberg the freedom to step out of his comfort zone and collaborate with researchers with disparate expertise has paid off in a big way.

The public return on investment from the endeavors of these and other basic research scientists is real. Such research will continue to change the future and the quality of our lives if we can galvanize a new generation of scientists striving for unpredictable discoveries. It may be 20 or even 40 years before you read about the results in stories of Nobel Prize winners, but the impact will be profound—every day of the year.

Express Shipping

The winners of this year's Nobel Prize in Physiology or Medicine discovered how packages of molecules are ferried around and between cells.

BY SARAH C.P. WILLIAMS

THERE IS NOTHING STATIC or random about the inside of a cell. Miniscule molecular cities are crisscrossed with roads dotted with vehicles on the go; fluid components merge and separate. Every movement is deliberate—carefully packed spheres repetitively travel the same well-worn routes, and cargo is delivered to the right place time and again. The signals that guide the spheres, or vesicles, are hard to see, but they are there.

For the three winners of the 2013 Nobel Prize in Physiology or Medicine, no road signs guided their career paths, and no one had forged the trail ahead of them. In fact, each scientist, over the span of a few decades, began studying how cells direct membrane-bound vesicles specifically because he saw it as an untouched area of biology.

"I was looking for something that was novel and unexplored and important all at once," says HHMI Investigator Thomas Südhof of Stanford University. Südhof, HHMI Investigator Randy Schekman of the University of California, Berkeley, and James Rothman of Yale University developed new methods to study cells at the same time they uncovered the molecules that control the formation, trafficking, and final disassembly of vesicles.

On Their Way

In the 1970s, many scientists were focusing their attention on DNA and RNA, unraveling how the genetic molecules encoded the components of cells and the traits that make people unique. Schekman, as a graduate student, was caught up in the excitement of how cells copy DNA. He helped isolate the molecules responsible for DNA replication. Then, in 1974, he heard a lecture on the maze of membranes that wind throughout the interior of a cell, transporting materials from one organelle to another—for example, from the DNA-containing nucleus to the series of interconnected, flattened sacs called the endoplasmic reticulum, where proteins are made.

Through microscopes, scientists had observed rough details of the physical process by which vesicles form. First, a small section of one organelle's membrane begins to curve outward into the watery cytoplasm, capturing cargo proteins and lipids into a budding vesicle. Once the membranes have rearranged to form an entire sphere, the vesicle pinches off and moves through the cell's liquid interior. The vesicle then merges with the membrane of a different organelle, delivering its cargo in a "reverse budding" process. Sometimes the vesicle's target is the outer membrane of the cell, where it sends signaling molecules out to other cells. But what initiates vesicle budding? How do vesicles recognize the correct destination? And how do they package the right contents? Schekman wanted to know the answers to these questions, and he soon realized that few other scientists were trying to answer them.

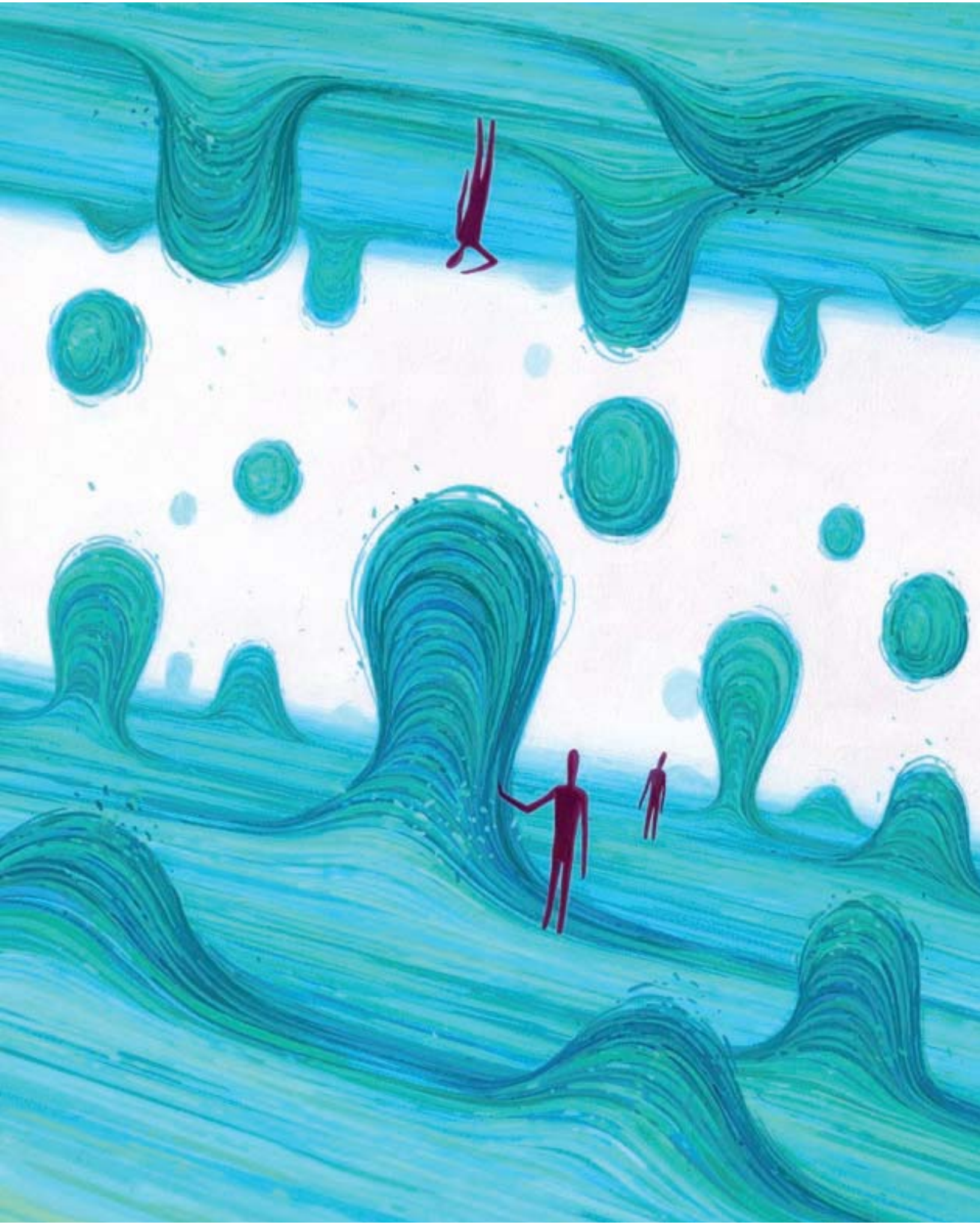
"People were really not looking at membranes from a mechanistic and molecular perspective at all," says Schekman. "So I thought that, given my training and interest, I could make a contribution."

Three years into that exploration, in 1978, Schekman says he had his one true "Eureka!" moment, which paved the way for all his subsequent work. With a graduate student, Peter Novick, he was growing cultures of yeast cells with random mutations, hoping to find mutants that failed to target vesicles, pointing toward a genetic key to vesicle biology. One afternoon, Novick was examining a new mutant that stood out.

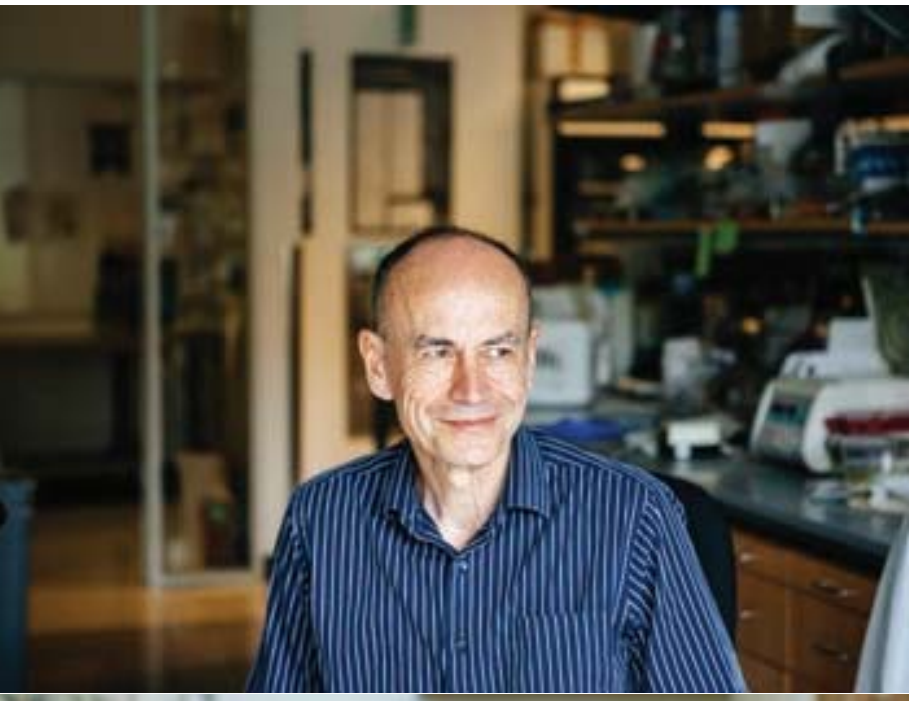
"I remember vividly to this day when Peter called me over to the microscope," Schekman says. "The cells were pockmarked with accumulated vesicles."

The cells, it turned out, had a mutation in a gene they called *SEC1*. While the cells could form vesicles, the vesicles were unable to fuse with their destination membrane to release their contents. Schekman had a starting point for

Brendan Monroe



Thomas Südhof (top) and Randy Schekman (bottom) have focused their careers on increasing fundamental understanding of how cellular transport works.



his molecular studies on membrane trafficking.

In the following years, the lab uncovered mutations representing 23 different *SEC* genes. Each blocked membrane traffic at a different stop light: Some were deficient in forming vesicles at the Golgi complex, an organelle that packages proteins to be secreted out of the cell. Some blocked vesicles at the endoplasmic reticulum. And some mutants, like *SEC1*, failed to allow vesicles to fuse with the cell's outer membrane. As a result, fully packed vesicles accumulated in the cell.

When asked what's been key to his success at unearthing so many components of the system—more than 50 genes now—Schekman doesn't hesitate. "Being adventurous and willing to gamble at something new and different," he says. "Scientists don't do that enough."

By this time—the 1980s—Rothman, then at Stanford, had also turned his attention to the choreographed movements of vesicles. His goal: to reconstitute in a beaker all of the components necessary for vesicle fusion. Whereas Schekman's approach was genetic, Rothman's was protein based.

"Very distinguished cell biologists cautioned me to not bother because it would never work," Rothman wrote in a 2002 *Nature Medicine* paper on his career.

But Rothman came up with a cell-free system that worked quite well, allowing him to follow the movement of radioactively labeled molecules from one membrane-bound compartment to the next within a Golgi complex. By studying his new system with electron microscopy, he elucidated even more details. He saw that the transport vesicles were enveloped in a coat of molecules, now known as a COP coat. And by disabling GTP, one of the cell's energy currencies, his lab group could keep this COP coat from disassembling at the vesicle's destination. Like Schekman, he'd discovered a molecular cause for a vesicle traffic jam.

By the 1990s, Rothman had discovered another key component of vesicle trafficking. Three SNARE proteins formed a complex that tethered transport vesicles to their destination and spurred the unzipping of a vesicle from the target membrane. One, synaptobrevin, was attached to the outside of vesicles themselves, while the other two, SNAP-25 and syntaxin, were found on the target cell's outer membranes.

As it turned out, many of the proteins that Rothman had discovered were encoded by genes that Schekman had pinpointed. The two researchers, each drawn to the little-studied world of membrane trafficking, had helped map the molecular signals that control the traffic inside the cell.

Brain Traffic

Vesicles aren't just for schlepping molecules around the interiors of cells, however. They also carry messages from cell to cell. In the brain, for example, vesicles full of neurotransmitters control the passage of signals from neuron to neuron. The proper functioning of these vesicles is responsible for a person's every thought, action, and memory. Südhof has spent 25 years studying how these vesicles fuse with such precision and speed as to keep up with the brain.

When a neuron receives an electrical impulse, the charged pulse travels down the length of the long cell to

Südhof: Leah Fasten Schekman: Hadar Goren Photography

> **"The longer I've worked on membrane trafficking, the more apparent it has become that it's incredibly important."**
> —THOMAS SÜDHOF

the presynaptic terminal, the end that reaches toward a neighboring neuron. At this terminal, calcium floods into the cell through channels that open in response to the impulse. Then, vesicles that are positioned, ready and waiting, at the presynaptic terminal fuse with the outer membrane and release neurotransmitters into the synapse, the space between the neuron and its neighbor. The neurotransmitters fill the synapse and bind to receptors on the membrane of the next neuron. In response to this binding, the cycle of neuron excitation begins in the second cell, where vesicles containing neurotransmitters will eventually fuse with the outer membrane, passing a signal to a third cell. The process continues as a message moves through the brain.

When Südhof launched his career, originally at the University of Texas Southwestern, he, like Rothman, had an ambitious goal: to purify and clone every protein in the presynaptic terminal, the area of the neuron where the vesicles gather. He spent more than a decade on this project, isolating one protein at a time. The growing list of components soon started to overlap the work of Rothman and Schekman—the same trio of SNARE proteins that Rothman had helped identify were present in the presynaptic terminal. They attached vesicles full of neurotransmitters to the terminal's membranes and helped initiate the fusion of the vesicle with the membrane.

"I think the biggest surprise has been the overall simplicity of the machinery that controls this," Südhof says. "I find it amazing that there's only a handful of proteins that do the job."

Südhof had another question, though: How does the flood of calcium into a neuron trigger the fusion of vesicles with the neuron's membrane? Since the 1990s, Südhof's lab group has studied this interaction between calcium and the SNARE proteins that control vesicle fusion.

"The work of mine that's most often mentioned are those initial studies," says Südhof. "But some of what I consider my lab's most important work has occurred in the past 15 years. It's required technologies that have only recently been developed." A combination of genetics, electrophysiology, and mouse behavioral assays has been key to pushing his work forward, he says. His team discovered that, before a calcium influx, vesicles are docked at the membranes of neurons, attached by a partially assembled complex of SNARE proteins. When calcium enters the cell, proteins called synaptotagmin and complexin respond to the calcium molecules and cause conformational changes to the SNARE proteins. Within microseconds, these changes trigger vesicle fusion with the membrane.

Right Turn

More recently, Schekman and Südhof have steered their careers in a new direction—using their findings on membrane trafficking to understand human diseases. Schekman's work has already been enormously helpful in the biotech world; today, one-third of human insulin is produced by yeast. Schekman was further inspired at an HHMI scientific meeting where he heard a presentation on Alzheimer's disease. "It became clear to me that there were issues of membrane traffic that may be malfunctioning in genetic forms of Alzheimer's," Schekman says.

He's shifted nearly his entire lab from studying membrane trafficking in yeast cells to studying the process in human cells—a huge undertaking, he says, that he wouldn't have been able to spearhead if not for his HHMI support.

And Südhof realized that a number of the genes involved in the formation of the presynaptic terminal and the presynaptic vesicles have been implicated in autism and schizophrenia. So his lab is taking a closer look at these disease-causing mutations. "I think that neuropsychiatric disorders are an enormous challenge right now, and this is a new angle we can look at," Südhof says. "The longer I've worked [on membrane trafficking], the more apparent it has become that it's incredibly important."

Südhof and Schekman, both HHMI investigators for more than 20 years, say their career paths are testaments to the incredible power of basic research. If there's a broader message that they want to convey as Nobel Laureates, that is it. "I have a concern that we are losing appreciation for studies that simply give us facts—the need to describe something in detail and dissect its components in order to understand how it works," says Südhof.

Schekman, who was editor-in-chief of the *Proceedings of the National Academy of Sciences* from 2006 through 2011 and is now the editor-in-chief of the open-access journal *eLife*, agrees: "Basic science is the foundation on which all technologies and medical applications are based," he says. "And I worry that young scientists are moving away from pursuing this kind of essential basic science."

He hopes that, if anything, his success offers a road sign that early career scientists will follow: Studying a basic biological system and charting your own path can lead to great things. ■

Romy Blümel

Champions in Education

THE RECESSION HIT Eugene, Oregon—including its schools—hard. The school district had cut $22 million from its 2011-2012 budget, laid off 100 teachers, and axed most advanced placement (AP) courses in biology, chemistry, and physics.

Parents were worried, says Tom Lininger, a University of Oregon law professor who led a group of faculty, all with children in Eugene and nearby budget-strapped school districts, to come up with a solution. HHMI Investigator Chris Doe, with two sons in the system, was among them.

"The school year had been reduced to 165 days; the national average is 180. Some parents were talking about moving their kids to private schools," Lininger adds. "We knew it was important to do something. We understood that, with limited resources, the school district had focused its interest on students who were at risk of dropping out."

Lininger and other campus representatives met with the superintendents of three Eugene-area school districts to outline a proposal that would offer 9th- through 12th-grade students specially designed courses at the university (legal considerations limited the university group's ability to teach in the schools). "We had no disrespect for local teachers," he says. "We just wanted to fill in some of the gaps."

The university made campus classrooms and labs available. Through a grant, the Wayne Morse Center for Law and Politics agreed to cover the $60-per-course enrollment fee for students from families that needed financial help. The first session offered courses in microeconomics and environmental science. Chemistry, political science, history, psychology, and statistics came next. Though not official AP courses, the content tracks AP requirements and students can take the AP exams.

Students, about 30 per course, meet on Sundays and on the school district's furlough and teacher improvement days. "The kids were all very motivated," says Doe, who taught environmental science. "They loved being pushed and challenged. They got both high school AP credit and college credit."

"The chance to take courses at the university was a huge gift," says Paige Kouba, who took Doe's course. "We were able to explore subjects that were otherwise unavailable to us," adds Kouba, now majoring in ecology at Harvard University. "It was downright frightening to see the education system constantly fighting budget cuts."

Doe, who studies how stem cells make neurons, chose to teach the two-semester course in environmental science, a field he knew little about. He scoured the Web and textbooks, "cramming" for three months to prepare 30 hours of lectures, 20-plus hours of lab experiments, and field trips to a local landfill and sewage treatment plant. "Preparing for the course and teaching it helped me get a better appreciation for the [rigor] involved in environmental science," he says.

It also changed his actions. "I became more personally aware of my own impact on the environment," he says. He traded the family's minivan for an electric vehicle, installed solar panels on his house, and took up composting.

Doe's efforts earned him a "Champion in Education" award from the Eugene and Springfield chambers of commerce. "People thought Chris walked on water," Lininger says.

"Teaching this course has made me realize that teaching high school can be really, really enjoyable," Doe says. "I have fun teaching these excited high school kids. It's changed my life." *–Jim Barlow*

Horses and Prairie

WHEN MICHAEL WELSH'S daughter was taking horseback riding lessons, he asked the instructor if he had any horses good for reining, a style of Western riding that sounded like great fun to the experienced horseman. A few months later, the instructor said he had a horse that Welsh should see.

"I walked into the barn, it was February, and the horse was woolly, gray, and short. I thought, ewww, I'm not gonna like this! Then I got on him and was hooked," says Welsh, an HHMI investigator who studies cystic fibrosis at the University of Iowa.

Welsh began to compete in reining, where the rider directs the horse through patterns of circles, spins, and stops. The horse should be willingly guided, and controlled speed raises the difficulty level. "A pattern might call for two large fast circles, then a small slow circle, where the horse drops from a full run to a rhythmic da-dum, da-dum, da-dum," says Welsh. "That's hard to do because the horse's adrenaline gets going."

One of Welsh's favorites is a sliding stop. Rider and horse circle one end of the arena, accelerate, and then stop, the horse's back feet sliding through the sand. "It feels like the horse just dropped out from under you, because its back end has gone down toward the ground. Sand sprays up on either side. It's completely thrilling," Welsh says.

He stopped competing seven years ago but still keeps a horse on his 80 acres south of Iowa City, for trail rides and to entertain his four grandchildren, ages 3 to 8. They either ride with him or he leads while they ride. "I'm hoping some of them might pick up horseback riding in 4-H," he says, just as his daughter did.

In recent years, his focus has shifted to 50 acres of rehabilitated tallgrass prairie at the center of his property. The prairie was once farmland that had been pushed too hard, washing away topsoil and exposing clay. "The land reminded me of a scarred old face," he says. "It became an interesting endeavor, to move it back toward what it had been."

So Welsh borrowed a tractor, harrow-plowed the land, and used a prairie drill to plant seeds. Once planted, maintaining the prairie has been fairly easy, he says. But it took some time. "The first year all I could see were weeds. I thought, oh, geez, what did I do?" The next year grasses emerged, and a few flowers. Now, "it just keeps going." Every few years he organizes a burn, for rejuvenation's sake and to give native plants a competitive advantage. Lab members come to observe or help with shovels and rakes to keep the roaring flames in control.

"It's relaxing to walk the prairie," he says. "It's a good place to walk with someone, unhurried, and talk. The prairie has a quiet beauty that can be appreciated from afar for its color, movement, and expanse and up close for its ecosystems of grasses, flowers, and insects."

– Lauren Arcuri Ware

Photo illustration by Braulio Amado Photo courtesy of Michael Welsh

Fungal Squatters

This fungus makes itself at home in the lungs, evading immune defenses and causing disease.

THE SOIL OF the Ohio and Mississippi River Valleys is home to a fungus called *Histoplasma capsulatum*, whose long filaments extend into the environment to collect nutrients. Once airborne, however, the fungus can be inhaled by humans or other animals. Inside the lungs, it must quickly shift gears—and shape—to survive.

Histoplasma responds rapidly to the elevated temperature of its new home. Its slender body morphs into a spherical cell, better suited for evading mammalian immune defenses. At the same time, the fungus turns on myriad disease-causing genes. Not everyone who is infected with *Histoplasma* gets sick, but some experience lung disease or vision loss. Up to 25,000 people in the midwestern United States, where the fungus is more abundant, develop life-threatening systemic complications each year.

Anita Sil, a microbiologist and HHMI early career scientist, is fascinated by the dramatic changes that *Histoplasma*—and related organisms known as thermally dimorphic fungi—undergo when they find themselves in the warm lungs of a host. Her team can trigger the same transformation by moving the fungi from room temperature to a warm incubator in the lab. Many shape-shifting fungi can cause disease, Sil says, but little is known about how they recognize that they've been taken up by a host and then adapt to manipulate the host's immune system.

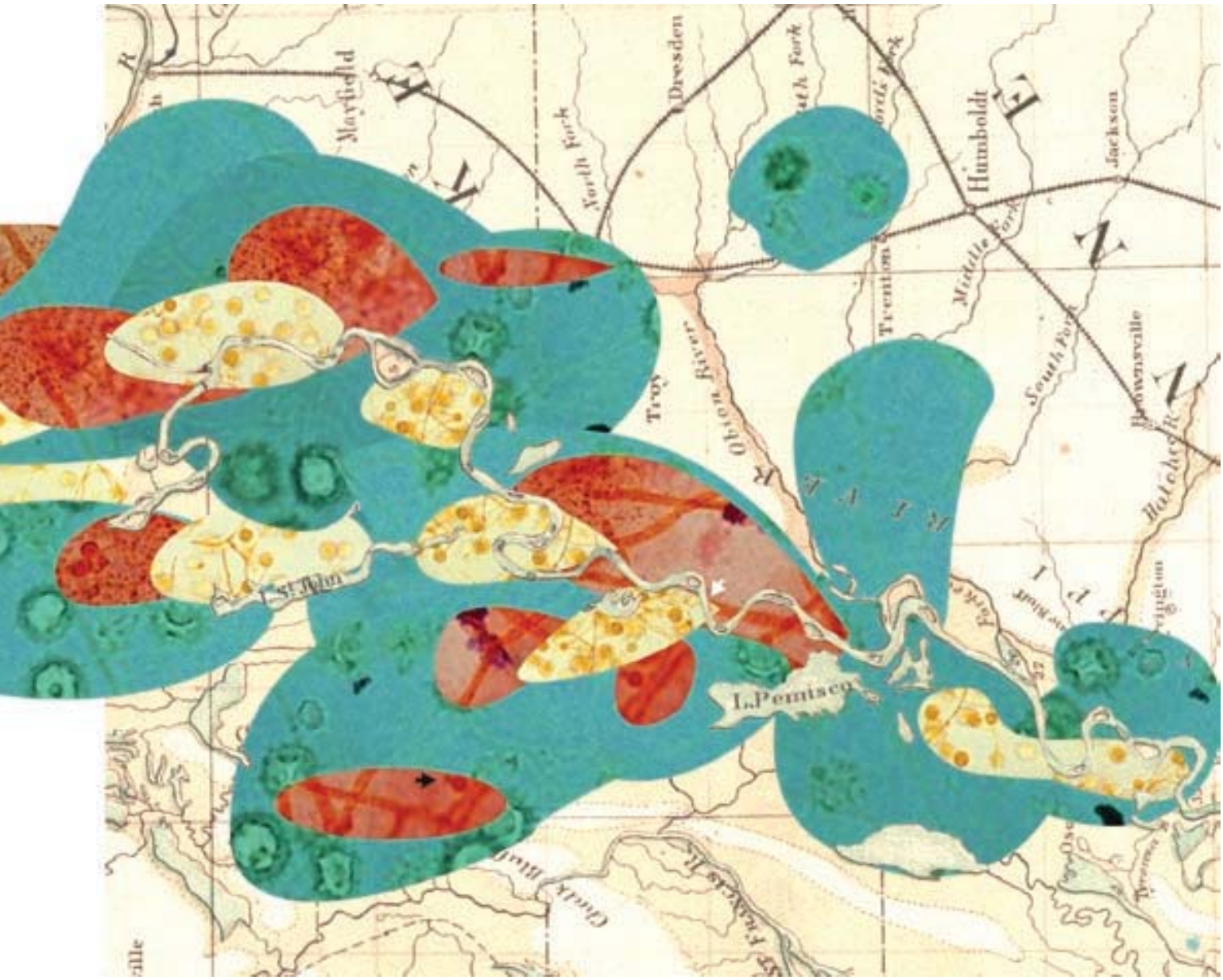
Sil has been working on that puzzle for more than a decade, at the University of California, San Francisco. Her lab is specially equipped to ensure safe handling and containment of the hazardous pathogen. Along with being dangerous for healthy people, *Histoplasma* is slow growing and difficult to study. "But there is so much we don't know," Sil says. Finding answers about *Histoplasma* and related fungi is crucial not only because of the organisms' unique biology, but also for human health. "It's very hard to prevent exposure to pathogens that live in the soil," Sil says. "Part of the solution is going to lie in better understanding their virulence mechanisms."

In 2008, Sil and her colleagues unearthed their first clues to the pathogen's strategy for switching to its virulent form (known as its yeast phase). They discovered three regulatory proteins—Ryp1, Ryp2, and Ryp3—that are necessary for *Histoplasma*'s change from its filamentous, soil-dwelling form to its spherical, yeast form.

By comparing those three Ryp proteins to similar proteins in other organisms, Sil and her colleagues predicted that the Ryp proteins control the fungi's transformation by altering gene activity. They analyzed the activity of each of the fungus's 9,000 genes and revealed more than 750 genes with activity specific to either the yeast or the filamentous form of the fungus. Genes on that list appear to influence many cellular features, including shape, structure, metabolism, and virulence.

Sil and her colleagues showed that the proteins physically interact with *Histoplasma* DNA, regulating gene activity directly. In addition, their experiments revealed a fourth protein, which they dubbed Ryp4, that is also necessary for *Histoplasma* to grow in its yeast phase.

In the July 2013 issue of the journal *PLOS Biology*, Sil and her colleagues reported how, at a warm temperature, the four Ryp proteins work together to enhance the activity of yeast-phase genes and to shut down genes specific to the filament phase. Now, her team is studying how the fungus senses the temperature increase that triggers its transformation. They've turned up a few signaling molecules



Clara Mata

"It's very hard to prevent exposure to pathogens that live in the soil. Part of the solution is going to lie in better understanding their virulence mechanisms."
—ANITA SIL

that may carry out this function.

A next step will be determining whether the molecules and pathways they have identified in *Histoplasma* are responsible for shape and virulence changes in other thermally dimorphic fungi.

"All of these organisms are evolutionarily related, but we don't really know whether they are using overlapping or distinct pathways," she says. "Now that we've developed the tools and ways of thinking about how to find these pathways in related organisms, we're especially interested in studying *Coccidioides*." This fungus is endemic in the southwestern United States and causes valley fever, a disease that often resolves on its own, but can become severe. The disease is on the rise in Sil's home state of California, as well as Arizona, Nevada, New Mexico, and Utah.

There's another big question that provokes Sil's curiosity: Why have these fungi evolved these remarkable capabilities? "These organisms do fine in the soil," she says. "They don't need a mammalian host to propagate. So how do these pathways benefit the organism in the environment?" There may be an advantage to hitching a ride in a mammalian host to a new habitat, she says, but *Histoplasma* and its relatives must wait for their host's death before they can return to the soil. She wonders whether temperature sensing might more directly enhance survival or reproduction in the soil. Identifying *Histoplasma*'s temperature-sensing pathways are a first step toward finding out.

–Jennifer Michalowski

Notes in the Margins

During brain development, the chemical marks that stud the length of DNA may be just as important as the genes themselves.

WHEN RESEARCHERS IN Terrence Sejnowski's laboratory added a new drug to the cocktail of compounds they were giving young mice, they didn't expect to see much effect on the rodents' brain cells. The mice were already receiving ketamine, a drug that altered brain cell development and induced neurological and behavioral signs of schizophrenia, helping the team study the roots of the disease.

When the mice received the additional drug, 5-azacytidine, in conjunction with ketamine, the effect was profound. The brain cell alterations and behavior changes produced by ketamine seemed to stop entirely.

"All of a sudden, this experiment became really exciting," says Sejnowski, an HHMI investigator at the Salk Institute for Biological Studies.

5-azacytidine is a methylation inhibitor; it halts the addition of methyl chemical groups to DNA, and changes how mice learn. "We hadn't anticipated any role of methylation in the underlying pathophysiology of schizophrenia or ketamine's effects," he says.

Luckily for him, a methylation expert was just down the hall. Joe Ecker, an HHMI-GBMF investigator at Salk, was an expert on methyl groups in plants and had begun to shift his attention toward mammalian biology. The timing for a collaboration was perfect.

Methylation—adding a methyl chemical group to the DNA of a plant or animal—doesn't change the information held in a gene. Instead, it influences which genes are expressed in a cell, and at what level. When a methyl group is tacked onto a gene, it keeps other regulatory molecules from binding to the gene and reading its code, so the gene's protein product is less likely to be made. Ecker, whose early research focused on methylation in the plant *Arabidopsis*, had developed a method to map the location of all methyl groups in a cell's DNA and had recent success in applying this method to human stem cells.

So Ecker, Sejnowski, and members of their labs, including staff scientist Margarita Behrens, teamed up to study whether methylation might be involved in learning and memory, or in brain development in mammals. Using Ecker's mapping methods on brain cells, they got another surprise. In mammals, most methylation is known to occur at DNA sites where there is a particular combination of nucleotides—a cytosine followed by a guanine. This is called CG methylation. But Ecker's mapping methods, applied to the brain cells of mice, told a different story: the scientists found unusual methylation levels at other nucleotide combinations—that is, non-CG methylation.

In humans, non-CG methylation had previously been detected only in stem cells—by Ecker's group. Moreover, researchers' prior efforts to map brain methylation made the erroneous assumption that all human DNA methylation occurred at CG sites. Because Ecker's method highlighted all methylation marks, it showed what others had missed.

Non-CG methylation marks, the team discovered, appear as the brain develops. "It was particularly striking that there are none of these marks in the fetal brain," says Ecker. "But as brain development progresses, there is a rapid increase in non-CG methylation."

The scientists mapped the patterns of non-CG methyl marks in several types of brain cells, throughout development, in a study reported August 9, 2013, in *Science*. In both humans and mice, non-CG methylation in brain cells increased between birth and adolescence. Moreover, the exact location of methylation in the genome was remarkably similar among humans, and even similar to the pattern seen in mice, Ecker says, suggesting that the marks affect fundamental processes in brain development or function.

"The increase in non-CG methylation occurs during a time in development when many new cellular connections are being made in the brain."

—JOE ECKER

Clara Terne

Watch an animation showing how methylation stops gene expression at www.hhmi.org/bulletin/winter2014.

"The increase in non-CG methylation occurs during a time in development when many new cellular connections are being made in the brain, and this is the exact period when things are known to go wrong in various diseases," he adds.

"This is so exciting because for a disease like schizophrenia, the genetic component is only around 50 percent heritable [based on twin studies], and nobody knows what explains the other 50 percent," says Sejnowski. "These findings open the door for a whole new area to delve into."

Methylation marks, which can be influenced by genetics and are passed between generations in mice, may also be influenced by environment. Methylation patterns can be altered by diet and chemical exposure, and may be influenced by other factors such as drugs. Further research could reveal a role in disease development, including schizophrenia.

To speed research in this area, Ecker, Sejnowski, and Behrens are sharing their maps, creating a public database that contains the full methylation patterns—or methylomes—of the individuals they've studied so far.

"We've just laid the foundation," says Sejnowski. "We're only beginning to understand all these mechanisms that are constantly at work in our brains while we're running around planning things, learning, remembering, and making decisions."

–Sarah C. P. Williams

Bench Report

Go online to see a 3-D map of the medulla: www.hhmi.org/bulletin/winter2014.

Motion Circuits

Mapping a fly brain reveals clues on how it sees movement.

PHYSICIST DMITRI CHKLOVSKII came to neuroscience ready to search for the brain's equivalent of Newton's laws—the basic equations that describe the mechanics of the physical universe. "We don't have the fundamental principles of how the brain works yet," he says. "We are starting to get the first glimpse of what they are."

For the past several years, Chklovskii has led a team of scientists at HHMI's Janelia Farm Research Campus on the Fly EM project—one of several team projects at Janelia. The project has two aims: to map all nervous system circuits in the fruit fly, using novel approaches to electron microscopy (EM), and to develop theoretical models of the brain to help understand how information is trafficked. "If we know the structure of something, its function is much easier to infer," he says.

The Fly EM project grew out of work from Chklovskii's group and now includes more than 30 team members, including electrical engineer and Janelia Lab Head Louis Scheffer.

The team has zeroed in on a region of the fly brain that processes vision, known as the optic medulla. Scientists already know the kind of brain cells there, how they fire, and what genes govern their function. The optic medulla was ripe for mapping to learn how the underlying circuits make sense of visual information.

To plot the circuits in the optic medulla, Chklovskii and his colleagues froze a fly brain, sliced it into very thin sections, and stained the sections with chemicals to visualize cell membranes and neural connections under an electron microscope. They took pictures of the part of each section corresponding to the optic medulla.

Using these images to build a three-dimensional, or 3-D, map was a monumental task: The team needed to identify cells and structures in each image, and then trace those cells from one image to the next. To accelerate the process, Chklovskii's and Scheffer's groups developed computer algorithms that automatically identify cells and trace their paths.

The automated approach sped the mapping effort, but it wasn't perfect. Sometimes the computer joined two neurons that should have been separate, or split a single neuron into two. About a dozen human "proofreaders" checked for errors and performed other manual steps, devoting more than 14,000 hours—the equivalent of one person working more than seven years, full time.

The team used an existing catalog of distinctly shaped cell types in the optic medulla to classify the neurons in their 3-D map by type, such as Mi1, Tm3, and T4. After identifying each neuron, they pinpointed its many connections, or synapses, by searching for the protein-dense structures that mark those junctions. In total, they mapped 379 cells, which made 8,637 connections.

With the 3-D map in hand, the team set out to learn more about the neural circuitry that detects motion. They relied on findings from Alexander Borst, a regular visitor to Janelia, and his colleagues at the Max Planck Institute of Neurobiology. The German group had demonstrated that T4 and T5 cells were essential in transferring visual information from the medulla to the lobula plate, the area downstream of the medulla in the fly's optic lobe.

Since Chklovskii and his colleagues had identified T4 cells in their 3-D map, they decided to start with T4 cells and work backwards in the circuit to the medulla. There, the scientists found two other types of neurons, Mi1 and Tm3, which connect to T4 cells.

The team discovered that each T4 cell connects to a cloud of Mi1 cells and a cloud of Tm3 cells. Furthermore, the groupings of Mi1 and Tm3 cells are offset from one another, suggesting that those sets of cells act en masse to spot an object moving across the eye. Based on these findings, Chklovskii predicted that Mi1 and Tm3 cells, in connection with T4 cells, constitute a neural circuit that detects motion.

The group published its results August 8, 2013, in the journal *Nature*. In the same issue, Borst and his colleagues reported findings that support Chklovskii's prediction. They measured activity of T4 cells by using

Kelsey Dake

Fly EM can now make maps five times faster than before, and continues to speed up the process about twofold each year.

fluorescent imaging reagents that respond to calcium levels, which fluctuate during neuronal activity. They found that the T4 and T5 cells exist in four forms—each sensitive to motion in one of the four cardinal directions.

Since completing those studies, the Fly EM group has improved the process substantially. Rather than slicing up the tissue and putting sections in the microscope, they now start with a block of tissue, image the top surface, and then use an ion beam to erode a thin layer. Then they image again and repeat the erosion. This approach, refined by Harald Hess and colleagues at Janelia, captures higher quality images that make mapping from image to image more accurate.

Fly EM can now make maps five times faster than before, and continues to speed up the process about twofold each year, says Scheffer. The human brain has about one million times more neurons than the fly, so it will take 25 years before they can map the human brain efficiently—if they can keep up the pace. But first, Scheffer says, there are plenty of fundamentals to be learned from dissecting fly circuits. *–R. John Davenport*

Danny Reinberg chose an unconventional model organism to study gene expression. In the process, he's revealed fascinating things about ants, behavior, and aging.

BY ROBIN MARANTZ HENIG

Straight Shooter

PHOTOGRAPHY BY MACKENZIE STROH

THE GOOFY, BESPECTACLED plant is the first thing you see in Danny Reinberg's office at the New York University (NYU) School of Medicine. It's how you know that this serious, accomplished scientist has an irreverent side. "You can see I love plants," says Reinberg, an HHMI investigator, gesturing toward the profuse greenery at his windows overlooking the East River. "But this one didn't make it." Instead of tossing the dead plant, though, his assistant, Michele Giunta, noticed that its bedraggled brown foliage bore an uncanny resemblance to a colleague from another lab. When she put a pair of Groucho glasses on it, the transformation was complete.

"He has a crazy haircut like this, and he wears weird glasses, and has a weird nose," Reinberg explains in his tuneful South American accent (he was born and raised in Chile). "She thought it was a perfect way to remind me constantly of him." It's also a good way to make anyone who walks in to see Reinberg break into a smile.

In his sunny office, Reinberg talks about his journey from a boyhood in Santiago—his father was a German Jew who moved to Chile just before World War II—to his position as a much-lauded biochemist whose curiosity led him to uncover key details of gene transcription, the process by which DNA is copied to RNA as the first step in protein synthesis.

He uses social ants as the model system for his most recent work on the epigenetic aspects of gene transcription. Although ant workers and queens have virtually identical genetic makeups, they express their genes differently. And that expression can change dramatically in response to a change in the environment—a worker can become a queen if the colony loses its queen, or one caste of workers can become a higher caste if the need arises.

Epigenetics is a trendy and complicated concept, so Reinberg has been giving lots of interviews to reporters who want to use his ant model as a way to explain it to their readers. But when we met in his office, he wanted to be sure our conversation went beyond ants to cover the previous 30 years of work that led him to epigenetics research—beginning with his student days in Chile amidst the political upheaval of Salvador Allende's presidency and the subsequent coup.

Supportive and Intense

From a young age, Reinberg set about defying expectations. As the oldest in a family of two boys and two girls, he was expected to take over the family business. But he hated working in his father's stores, where he sold jewelry and furniture during school breaks. Like most young people in his city, he was expected to live at home until he married, but he couldn't wait to get away from a household filled with discord. So at age 19, he moved into his grandfather's small apartment in Viña del Mar, one and a half hours away, and eventually studied science at the Pontificia Universidad Católica de Valparaíso. "My father hoped that if I didn't take over the family business, I'd at least become a doctor or a lawyer," he says. "But that's not what I wanted."

At the university, Reinberg loved all of science: physics, chemistry, and especially biology—the more the better. "In Chile no one worked on weekends," he says. "I was the only crazy person at the microscope on Saturdays and Sundays." He finished with a degree in cell biology in 1976 and started a doctoral program in Santiago in a histology/cell biology lab. When he found the science to be too descriptive, he switched to biochemistry. Through a string of lucky coincidences, he landed at the Albert Einstein College of Medicine, in the Bronx, and met the man who would become his mentor and lifelong friend, biochemist Jerry Hurwitz.

"He was a very ambitious, very smart kid," says Hurwitz, who is now at Memorial Sloan-Kettering Cancer Center. "Very aggressive. Always eager to do more things. Also very upbeat, very positive. And above all, a prodigious worker." Reinberg could be a critical and somewhat demanding co-worker, Hurwitz says, but he was most critical and demanding of himself. He pushed himself and was always looking around for new research topics that would allow him to make a real contribution to the scientific understanding of how genes are replicated, transcribed, and expressed.

With the good fortune of having landed in the lab of the father of protein enzymology, Reinberg set about learning everything he could about how to purify proteins and run assays. As Hurwitz puts it, he was steeped in the brute-force process of purification on a large scale. Reinberg worked hard at it, knowing it would serve him well no matter where he went in protein enzymology. "I knew if I wanted to continue in that field, I had acquired a skill very beneficial to my life as a scientist," he says.

Hurwitz did more than supply Reinberg with crucial lab skills. He also was a role model for how to run a lab, creating the collegial, cooperative, just-competitive-enough atmosphere that Reinberg has tried to copy in his own lab, first at Stony Brook University for a year, then at the University of Medicine and Dentistry of New Jersey (UMDNJ) from 1986 to 2006 (where in 1994 he was named an HHMI investigator), and then at NYU since 2006. "Danny really bends over backward to help out young scientists," says his wife, Lynne Vales, who first met Reinberg when she was doing graduate work in a different biochemistry lab at Albert Einstein. "He gets a kick out of boosting people's careers—the ones who are worthy of it." When asked to describe the atmosphere in his NYU lab, where Vales now works writing and editing grant applications and scientific papers, she says that two words come to mind: "supportive" and "intense."

Hurwitz had a tendency to bark at students and postdocs with some rather salty language, Reinberg recalls, but there was nothing he wouldn't do for them—something Reinberg discovered for himself soon after he earned his PhD in 1982. He went straight to the University of California, Berkeley, to do a postdoctoral fellowship—and lasted four months. California was a bad fit, he says. "It was too laid-

Danny Reinberg, in the ant room, uses two species of social ants to study epigenetics and behavior.

Scientists in Reinberg's lab are learning to stimulate epigenetic changes that convert one kind of worker ant to another.

back for me." Even nearby San Francisco wasn't urban enough; the bars closed at 11, and the city was always foggy.

Then one probably foggy Saturday morning in late 1982, Hurwitz telephoned out of the blue. "Come back, son," he said. "We'll take care of you."

The following year, Reinberg took a postdoc position in the lab of Robert Roeder at Manhattan's Rockefeller University, where he set about trying to characterize the complex process of gene transcription. (Vales also eventually went to Rockefeller to do a postdoc at a different lab. She and Reinberg got reacquainted there and married in 1986.) "Many labs were working on gene transcription, trying to identify factors that allow the enzyme that makes RNA to be recruited to specific genes," Reinberg says. His goal was to be the first to make the fullest identification of the proteins involved and how they function.

Transcription in a Test Tube

At the time, not much was known about the biochemistry of gene transcription in eukaryotes, though scientists knew it was set in motion by RNA polymerase II, an enzyme that travels along DNA to produce RNA along with other protein fractions. In Roeder's lab, Reinberg used the human HeLa cell line to help identify factors that are required for transcription, which he and Roeder published in 1987 in an extraordinary series of three papers in a single issue of *The Journal of Biological Chemistry*. In the first paper, they described two transcription factors, TFIIE and TFIIB, that initiate the process at all promoter sites, the stretches of DNA adjoining genes that spark their transcription. In the second paper they described the actions of two more transcription factors, TFIIA and TFIID. In the third paper they described another factor, TFIIS, involved in the

Reinberg's group can make carpenter ant foragers and fighters switch roles.

elongation of the RNA chain being transcribed.

By the time these papers appeared, Reinberg had established his own laboratory at Stony Brook. Years later, Reinberg's group discovered other factors required for transcription, TFIIF and TFIIH, and finally reconstituted transcription in a test tube using DNA strands—so-called naked DNA. They went on to reconstitute transcription working with DNA in the form of chromatin, as occurs naturally in the nucleus of a cell.

His lab was a spirited place to work. "Danny speaks his mind," says Gary LeRoy, who was a grad student in the UMDNJ lab from 1995 to 2000 and recently re-joined Reinberg at NYU as a research scientist. His directness earned him the nickname "Chili Pepper," but LeRoy says he liked Reinberg's approach—especially because he so obviously cared about his students.

Stories of the lighthearted moments in the lab tend to be a bit nerdy. Like the time in the late 1990s when LeRoy was doing "bucket biochemistry" (formally known as biochemical fractionation) to try to isolate an enzyme. LeRoy was in the cold room and Reinberg was standing outside, looking in through the window, waving. LeRoy would look up and see Reinberg waving, go back to his work, look up again and see Reinberg still waving. Finally he came out of the cold room to ask what was going on. "I'm waving goodbye to your protein if you think you're going to put it on that gigantic column," Reinberg said. The story makes LeRoy laugh in the retelling, but it's clearly a joke only a biochemist could love.

Other stories reveal something more serious about the kind of scientist and mentor Reinberg is. LeRoy talks about the time he asked Reinberg why he had accepted him as a grad student. "Because you had ideas," Reinberg told him. "They were very immature ideas which you will never do in my laboratory, but at least you had ideas."

During his early UMDNJ years, Reinberg continued to live in Manhattan, enjoying the city's lively social life. He and Vales moved to New Jersey in 1990, but it wasn't until 1999, when they walked into a 120-year-old farmhouse in the New Jersey town of Warren and instantly fell in love with it, that Reinberg fully embraced suburbia and became an avid gardener. When he moved his lab to NYU in 2006, he stayed in New Jersey—the house and its garden were too beautiful to leave behind—and once again became a commuter.

During this time, Reinberg kept working on chromatin, the complex of nucleic acids and proteins that condenses into chromosomes during cell division. He helped to refine the understanding of how gene transcription is enabled and disabled through modifications to histones, the proteins that are the main component of chromatin. These studies led him to epigenetics. He was wondering how he could design an epigenetic study of social behavior at the organism level when the answer arrived in an unusual way—on a hot bus stuck in traffic in Mexico.

Social Creatures

It was 2004, and Reinberg had flown into Mexico City for a scientific conference that was an hour from the airport. Because of anti-government protests on the streets, the bus ride to the meeting site stretched to two hours, then three, then four. Luckily he was sitting with his friend Shelley Berger, director of the epigenetics program at the University of Pennsylvania. As the bus crawled through the snarled traffic, they chatted about the question he'd been mulling: what model systems would work best to study epigenetics? He rejected yeast, the organism Berger worked with. He rejected worms. "I told her that I wanted to work on something that is social," he says, but that bees were too much of a hassle; you need

to suit up to handle them, and you get stung anyway. That led Berger to describe some fascinating behavior she'd observed among leaf-cutter ants during a family trip to Costa Rica. "That's the system we want to work on," Reinberg remembers thinking. "Ants!"

Reinberg went home and did what he usually does when he gets excited about something: he bought books on the subject and read voraciously. What makes ants ideal for epigenetics research, he found, is that in any one colony the ants are genetically identical, yet they have different behaviors, lifespans, and brain size. How does the social environment during development affect how those genes are expressed? And does a change in the environment change gene expression even in adulthood, after an ant's fate seems to have been sealed?

Berger agreed that ant societies would be an excellent model for studying how epigenetic changes are linked to changes in behavior, reproduction, aging, and neurobiology. Fortunately, just as Berger and Reinberg were talking about working together, HHMI launched the HHMI Collaborative Innovation Awards (HCIA).

In 2008, Reinberg and Berger received a large HCIA award and could begin sequencing the genomes of two species of social ants: *Camponotus floridanus* (a carpenter ant from Florida) and *Harpegnathos saltator* (a.k.a. Jerdon's jumping ant). They also were able to bring in another collaborator, Jürgen Liebig, of Arizona State University, an evolutionary biologist interested in how insect societies maintain the division of labor.

The timing of the HCIA grant program was propitious, allowing Reinberg's lab to launch the new project and benefit from the different mindsets of his collaborators. As Berger describes it, Liebig has a big-picture view of ant society typical of evolutionary biologists, and she and Reinberg are more interested in the details, as biochemists usually are. "We really challenge each other," she says, especially Reinberg, who loves to ask provocative questions.

After they sequenced the ant genomes, the real work could begin: studying epigenetic changes in response to changes in the environment. Reinberg set up an ant room off the lab's main corridor—essentially, a series of shelves holding big Tupperware containers, each home to a colony. Any time a lab worker puts on gloves and reaches in with tweezers to retrieve an ant, the colony gets worked up, ants scurrying up the plastic walls like convicts trying to make a jail break.

When that gloved hand reaches into a *Harpegnathos* colony to remove the queen, it provokes an especially dramatic change. Queens have a social role and lifespan quite different from other females in a colony; they spend their lives laying eggs and can live for as long as 12 years. The female worker ants, on the other hand, are rendered sterile in the presence of the queen and live on average just one year. (The males in the colony—haploids, with only half the full genome complement—are not much more than sperm with legs; they live only as long as it takes them to fertilize the queen's eggs, and then they die.) The brains of queens and workers are different, too: once the queen establishes the colony, she doesn't need to see or engage in any activity other than egg-laying, so her brain shrinks accordingly, while worker brains are more fully developed.

In the *Harpegnathos* colonies in Reinberg's ant room, removing the queen creates a change in the other ants: the once-cooperative workers start fighting to see who will become the new queen. (In the *Camponotus* colonies, no such power struggle occurs; remove the queen and the whole colony dies.) Eventually a few workers become dominant, and they develop the physical traits of the queen—that is, they become capable of reproducing. They're called gamergates, or, as Reinberg calls them, pseudoqueens.

Reinberg's team has identified several intriguing biochemical changes in pseudoqueens, including increased gene expression in at least two genes associated with longevity in mammals, one for the enzyme telomerase, the other for the enzyme sirtuin-1. Preliminary findings suggest that the pseudoqueens live longer than a typical worker, too (though not quite as long as a true queen, probably just three to four years). Could activation of telomerase or sirtuin-1 help explain the several-fold increase in lifespan in queens?

"Danny is very rigorous, dispassionate, and deeply questioning."
—SHELLEY BERGER

As for the *Camponotus*, even though removing the queen is too drastic, the HCIA team has found a way to induce other interesting changes. *Camponotus* has two worker castes, major and minor, that differ in size (the majors are bigger) and behaviors (the majors are fighters, the minors are foragers). Scientists in Reinberg's lab are learning to stimulate epigenetic changes that convert one kind of worker to another. They can induce minors to become majors in the *Camponotus*, just as they are learning to create pseudoqueens in the *Harpegnathos*. "We can propagate *Harpegnathos* in the lab," Reinberg says, "which means we can start injecting the embryos or larvae with different things that may affect gene expression." He wants to keep the details vague for now, saying only that the genes are "important for the transition from workers to gamergates, aging, etc."

Berger has enjoyed working with Reinberg. "Danny is very rigorous, dispassionate, and deeply questioning," she says. "He loves the science. He loves big questions. The attraction of this project is what fantastic questions there are to ask."

As rigorous and demanding as he is as a co-worker, Berger is quick to add, Reinberg is also "a great guy, lots of fun at meetings, someone who knows everybody and wants to interact with everybody." Which is why she plans to join a big group of Reinberg's colleagues and friends for a blow-out 60th birthday party in January. "I wouldn't miss it for the world," she says. "I'm trying to think of a couple of good stories to tell."

The birthday celebration will begin with an all-day symposium presented in Reinberg's honor, after which a shuttle will take attendees to a floating restaurant in the East River for the festivities: a jazz band and cocktails, followed by dinner and roasts until midnight. Lab manager Heike Pelka is coordinating the event, with 120 guests, some coming from as far away as China. She doesn't know exactly what to expect, but she says one speaker will be delivering a talk called "Keeping Danny Activated." From the looks of it, what keeps him activated is science—the chance to do it, share it, talk about it, and then do it some more. ■

They might not look very tough, but plants are armed and ready to fend off attackers.

BY NICOLE KRESGE

Defenses Up

ILLUSTRATION BY MICHAEL KIRKHAM

A FEW HUNDRED bacteria rest atop a lone grain of sand. Suddenly, a gust of wind scoops up the particle and its occupants, hurtling them toward an unsuspecting plant. The impact causes a tiny abrasion on a leaf and the bacterial passengers disembark to colonize their new home.

The plant is ready to defend itself—approximately 15 percent of its genome is dedicated to immune responses. Microscopic pores will clamp shut to prevent bacteria from entering the plant. Proteins will guard particularly valuable molecular targets. Leaves and stems will sacrifice diseased cells to prevent the microbes from spreading.

The bacteria won't back down without a fight, however. They pack syringe-like weaponry that injects dozens of toxins directly into plant cells. These molecules will shut down the plant's immune system and take control of its cellular machinery. What initially seemed like a quick and easy conquest for the bacteria could go either way.

Every day, plants are confronted by a daunting array of pathogens, from microscopic viruses to single-celled protozoa. Generally, the plant emerges victorious. But when it doesn't, the food supply can be hit hard. "Disease and insects account for up to a 30 percent [agricultural] yield loss globally," says HHMI-GBMF Investigator Sheng Yang He at Michigan State University. "Every year you can just count on it."

Preventing even a portion of those crop losses is a critical goal for feeding our expanding global population. Much of what occurs in the plant-pathogen arms race is a black box, but slowly, researchers like He are uncovering the details to learn how to enhance plant resistance to invaders. Some of the facts they're unearthing are already being used to help protect the world's crops.

Cellular Sentries

Unlike animals, plants do not have armies of circulating immune cells to fend off invaders. Instead, each cell of the plant must face down every intruder it encounters. As a result, much of the research that goes into boosting plant immunity centers on the events that occur inside the cells that are under attack.

Sheng Yang He is trying to disarm pathogens that deliver toxins into plant cells.

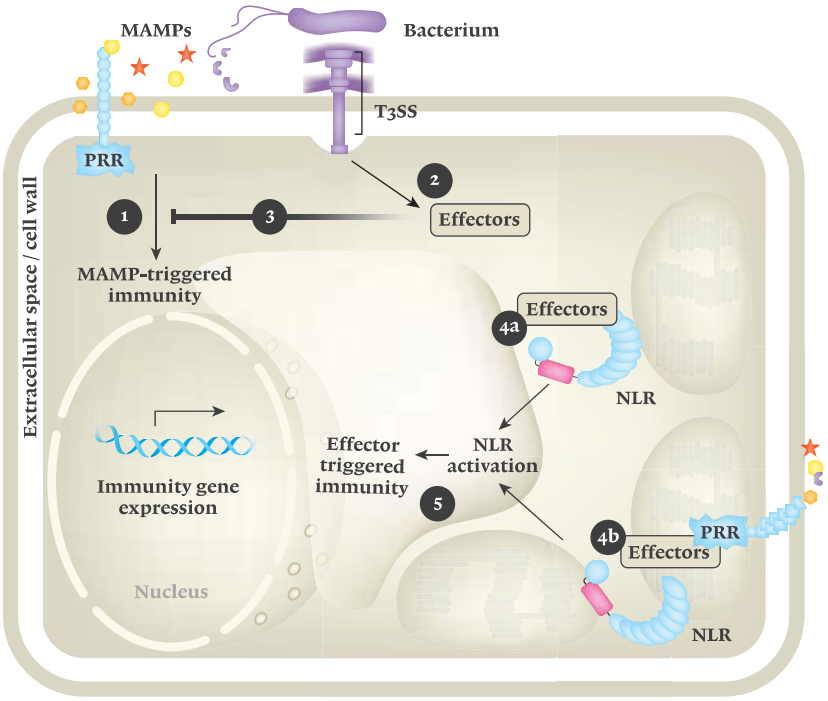
Pathogens typically invade by breaching a plant's outer protective "skin" through surface wounds or via pores called stomata—tiny, mouth-like pores that regulate the exchange of gases between the plant and the atmosphere as part of photosynthesis. These entry points lead to cavities between the cells where gas exchange occurs. It is here that the pathogen encounters the plant's first line of defense—a series of sentinel-like molecules called pattern-recognition receptors that stand watch on the surface of the plant cell. Jutting from the cell's membrane, these proteins detect general biological characteristics of pathogens. Known as "microbe-associated molecular patterns," or MAMPs, the telltale traits can be anything from the sugar molecules that make up a bacterium's cell wall to the flagellin proteins that form its whip-like tail.

"MAMPs are compounds that plants, for the most part, don't make," explains Fred Ausubel, a geneticist at Harvard Medical School. "So [plants] can use these molecules to differentiate their own cells from pathogen cells."

After spotting a pathogen's MAMP, the pattern-recognition receptors will sound the alarm by sending signals to the interior of the plant cell. What follows is a series of events—known as MAMP-triggered immunity—aimed at preventing the invading pathogen from colonizing the plant.

For example, stomata, which coat the underside of leaves, close to keep additional microbes from entering the plant. Generally, stomata are closed at night but open during the day, when they provide a perfect entry point for opportunistic microbes. Using the plant model organism *Arabidopsis thaliana*, Sheng Yang He and his Michigan State team showed that the cells that form stomata contain pattern-recognition receptors in their membranes. When they detect a pathogen, these "guard" cells swell, closing up the pore. He's group is doing genetic screens to pinpoint the proteins that control this opening and closing, with the aim of eventually fortifying the response in plants.

Cristian Danna, a postdoctoral fellow in Ausubel's lab, recently discovered another MAMP-triggered cellular defense tactic. The presence of bacterial flagellin causes plant cells to suck nutrients out of their apoplastic space—the area between cells. "This makes sense when you think about the mode of infection of most bacterial pathogens," says Ausubel. "They don't actually enter plant cells. They grow and multiply in the apoplastic space where they are dependent on the plant for nutrients to grow." By turning



The Plant Immune System
Pattern recognition receptors (PRRs) on the surface of the plant cell detect general pathogen characteristics known as "microbe-associated molecular patterns" (MAMPs). The PRRs then sound the alarm and initiate a series of events known as MAMP-triggered immunity (step 1). Bacteria use a syringe-like apparatus called the type III secretion system (T3SS) to inject toxic effector proteins into plant cells (step 2). The effectors block MAMP-triggered immunity (step 3). Via a second line of defense, plants use NLR proteins to disarm the effectors. Some NLR proteins recognize specific effectors (step 4a). Others guard cellular machinery, such as PRRs, that is targeted by effectors (4b). Once activated, the NLR proteins unleash a surge of antimicrobial molecules and cell death signals in a response known as effector-triggered immunity (step 5).

on amino acid and sugar transporters, the plant can starve the bacteria by removing nutrients from where the bacteria live.

A Clandestine Campaign

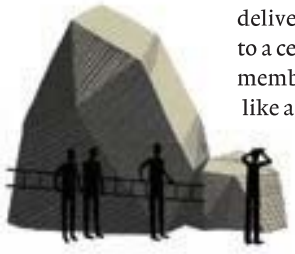
If starvation tactics and stomata on lockdown were enough to deter all pathogens, researchers would have a pretty easy time fortifying plant defenses to boost crop yield. However, that's not the case. In the late 1990s, He and other researchers discovered that some bacteria, such as *Pseudomonas syringae*, contain a syringe-like apparatus for injecting toxic proteins into plant cells. Called the type III secretion system (T3SS), this impressive machine is also found in animal pathogens. It consists of about 20 proteins assembled into a hollow complex that stretches from a bacterium's cytoplasm, through its membranes, across the plant's thick cell wall, and into the plant's plasma membrane. The resulting channel allows a wave of toxic foot soldiers, known as "effectors," to march directly from the bacterium into the plant cell's cytoplasm.

Each of the roughly 15 to 30 effectors that bacteria deliver into the plant cell carries a tag that directs it to a certain cellular location–for example, the plasma membrane, chloroplasts, nuclei, or mitochondria. "It's like a zip code that gets them to the various places," explains plant biologist Brian Staskawicz. His group at the University of California, Berkeley, was the first to identify a bacterial effector. Once deployed, the effectors set about terminating MAMP-triggered immune responses, diverting nutrients, and making it easier for the pathogen to colonize the rest of the plant, often by mimicking or inhibiting the cell's functions. Several effectors from *P. syringae* target the MAMP receptors at the plant cell membrane. Another effector, called HopM1, goes after an *Arabidopsis* protein that ups a plant's defenses, possibly by moving antimicrobial compounds to the cell wall.

T3SS may be the chink in a pathogen's armor that researchers have been looking for. Taking out the syringe-like structure could effectively halt the delivery of all toxic proteins into the plant cell. Many research groups are screening libraries of chemical compounds that could inhibit T3SS. He's team is taking a different approach, screening 4,000 medicinal plant extracts to see if any affect this particular weapon. "You would think that if the T3SS is so important, and if plants are smart, they would have evolved something to target it for defense," He says. "The hypothesis may be wrong, but I think it's worth trying." If he's right, his extracts could yield a very effective pesticide.

The Permanent Arms Race

Once their primary blockades have been breached, plants have a second line of defense: They unleash a squad of "resistance" proteins that disarm the invading effectors. Scientists now have a good handle on the identities of many of these molecules.



Adapted from Dangl, JL et al. *Science* 341 (6147) p 746-751, 2013

Jeff Dangl is studying disease-resistance proteins in plants.

"People have been selecting for disease-resistant plants since the dawn of agriculture," explains Jeff Dangl, an HHMI-GBMF investigator at the University of North Carolina at Chapel Hill. "What in fact they were selecting for, and what's been bred into all of our food, are disease-resistance genes." These plants have allowed our crop yields to remain high in the face of pathogen invasion, explains Dangl. With the genes that produce disease-resistance proteins in hand, scientists like Dangl hope to deploy them in a more targeted fashion to improve crop yield.

With the genes that produce disease-resistant proteins in hand, scientists like Dangl hope to deploy them in a more targeted fashion to improve crop yield.

Most disease-resistance genes code for a family of molecules called nucleotide-binding leucine-rich repeat receptor, or NLR, proteins. These proteins are found in everything from moss to tomatoes. NLR proteins detect effectors. In some cases, they do it by sensing the action of the pathogen effector on its host target, like a surveillance antenna. In other cases, they bind directly to the effector. Both cases activate the NLR protein, producing a suite of cellular responses that block pathogen replication. "There's a permanent arms race between the pathogen and the plant," explains HHMI-GBMF Investigator Jorge Dubcovsky. Pathogens are constantly evolving new effectors and effector combinations that are not detected by NLR proteins, and plants are continuously evolving new NLR proteins that recognize the novel effectors.

Dubcovsky, who uses genetics to build stronger varieties of wheat, is entering this arms race. A major project in his University of California, Davis, lab focuses on helping wheat build its defenses against rust fungus. In the 1950s, an outbreak of the fungus wiped out about 40 percent of the U.S. wheat harvest. Scientists have since bred disease-resistant wheat cultivars (strains), but a new race of rust able to defeat the deployed defense genes showed up in Uganda in 1999. The new rust variant, called Ug99, has now spread to South Africa and Iran, among other countries.

Jorge Dubcovsky is helping wheat build its defenses against rust fungus.

"This is kind of personal because when I released my first wheat variety as a breeder, the very next year it was destroyed by rust," explains Dubcovsky. Since then, he's developed several varieties of wheat containing combinations of resistance genes that protect against current rust races. He's also cloned two wheat genes—*Yr36* and *Sr35*—that confer resistance to rust. The *Yr36* gene, cloned in 2009, has already been added to several varieties of wheat released in California and other parts of the world. Dubcovsky published the identity of the *Sr35* gene in *Science* in August 2013. In the same issue of the journal, Australia's Commonwealth Scientific and Industrial Research Organisation reported on the identification of another Ug99 resistance gene. Both genes can now be bred into wheat to decrease the chances of a new strain of Ug99 from cropping up.

"The combination of rust resistance genes is similar to the AIDS strategy where you attack the pathogen with a cocktail of things that target different viral pathways," explains Dubcovsky. "It's very unlikely that the pathogen can mutate simultaneously at all these pathways."

Staskawicz, at Berkeley, is hoping to create a similar cocktail to protect tomatoes against bacterial spot disease, one of the most destructive pathogens affecting field and greenhouse crops. His team is searching for effectors that are conserved in several strains of the bacteria and using them to find the corresponding disease-resistance genes. They've also taken a bacterial spot resistance gene from pepper plants and placed it in tomatoes. Field tests in Florida showed promising results. Eventually, farmers growing these modified plants will not have to use harmful copper-based pesticides to protect tomatoes against bacterial spot disease.

Some plants and microbes get along just fine. Learn how Dangl and He are illuminating those relationships at www.hhmi.org/bulletin/winter2014.

Xinnian Dong discovered that parts of a plant's immune response run on a circadian clock.

Tactical Defense

Although plant breeders have been tweaking disease-resistance genes to boost plant immunity for over a hundred years by using the simple rules of genetics, exactly how their NLR proteins stop effectors remained a mystery until about 13 years ago. Plants have far fewer disease-resistance proteins than bacteria and other pathogens have effectors, so it's unlikely that each NLR protein recognizes just one specific effector. In 2001, Dangl and Jonathan Jones, a plant biologist at the Sainsbury Laboratory in the United Kingdom, proposed an alternative theory.

According to what they detailed as "the guard hypothesis," instead of zeroing in on specific effectors, many NLR proteins guard important cellular machinery that microbes want to exploit or shut down. This allows plants to recognize groups of pathogen effectors that go after the same targets. For example, when a bacterial or fungal effector tries to disable a particular plant protein, the NLR watching over it will spring into action. "Effectors usually do biochemistry," says Dangl. "And that biochemistry is both their virulence function and the thing that gets them into trouble."

Currently, Dangl's team is involved in what he refers to as a "big funnel approach" to defining a huge diversity of effector targets in *Arabidopsis*. "The idea is to find out what really happens when a pathogen infects a plant," explains Petra Epple, a research associate in Dangl's lab.

Epple and her colleagues in the Dangl lab mapped the *Arabidopsis* targets for two pathogens—the Gram-negative bacterium *P. syringae* and the fungus-like pathogen *Hyaloperonospora arabidopsidis*—and discovered that they seem to converge on a set of core cellular proteins involved in MAMP-triggered immunity. "That can't be random," says Dangl, "and statistical analyses back us up." His team plans to figure out how each effector interacts with these core proteins.

Damage Control

Once activated, a plant's NLR proteins unleash a surge of antimicrobial molecules and cell death signals that help the surrounding tissue resist the invading pathogen and minimize damage to the plant. This response, termed effector-triggered immunity, is a shorter, faster version of the immunity caused by MAMP molecules.

Xinnian Dong, an HHMI-GBMF investigator at Duke University, discovered that one way a plant controls the spread of effector-triggered immunity is through the production of salicylic acid (the active ingredient in aspirin) at the infection site. By interacting with different receptors, salicylic acid promotes death in infected cells and prevents it in healthy ones.

Dong also made the surprising finding that the circadian clock regulates pro-cell death genes. "We were really puzzled by this for a long time," she admits. That is, until she looked at the life cycle of *H. arabidopsidis*. This pathogen forms spores in the evening and sends them out to colonize plants when the sun rises. In response, the plant anticipates infection in the morning and enhances its resistance accordingly.

Dong has a hunch that plants also have a humidity-controlled circadian clock that triggers cell death when threat of invasion is high. "Humidity is very important for pathogen infection," she explains. "We know that when something gets wet, it gets moldy." Perhaps plants can anticipate humidity changes and will ramp up their cell death genes to prepare for high mold counts when the air is moist.

These circadian connections underscore the fact that plants don't grow in isolated, controlled environments in which they are attacked by a single pathogen. With this in mind, many plant immunologists are taking a more holistic approach to their research and incorporating a plant's surroundings in their studies. For example, He and Dangl are looking at how plants interact with the collection of microbes that grow in and around them [see Web Extra sidebar, "Community Life"].

Piecing together the information from inside and outside the plant, scientists are slowly beginning to understand how plants ward off pathogens, and they are applying that information to agriculture. "I think we are at a point where we have enough knowledge now to effectively deploy the plant immune system," says Dangl. By helping plants stay mean and green, farmers may no longer have to relinquish a portion of their crops to disease. ■

Jim Bounds / AP © HHMI

For more information: To learn more about the HHMI-GBMF program, see www.hhmi.org/news/GBMF.

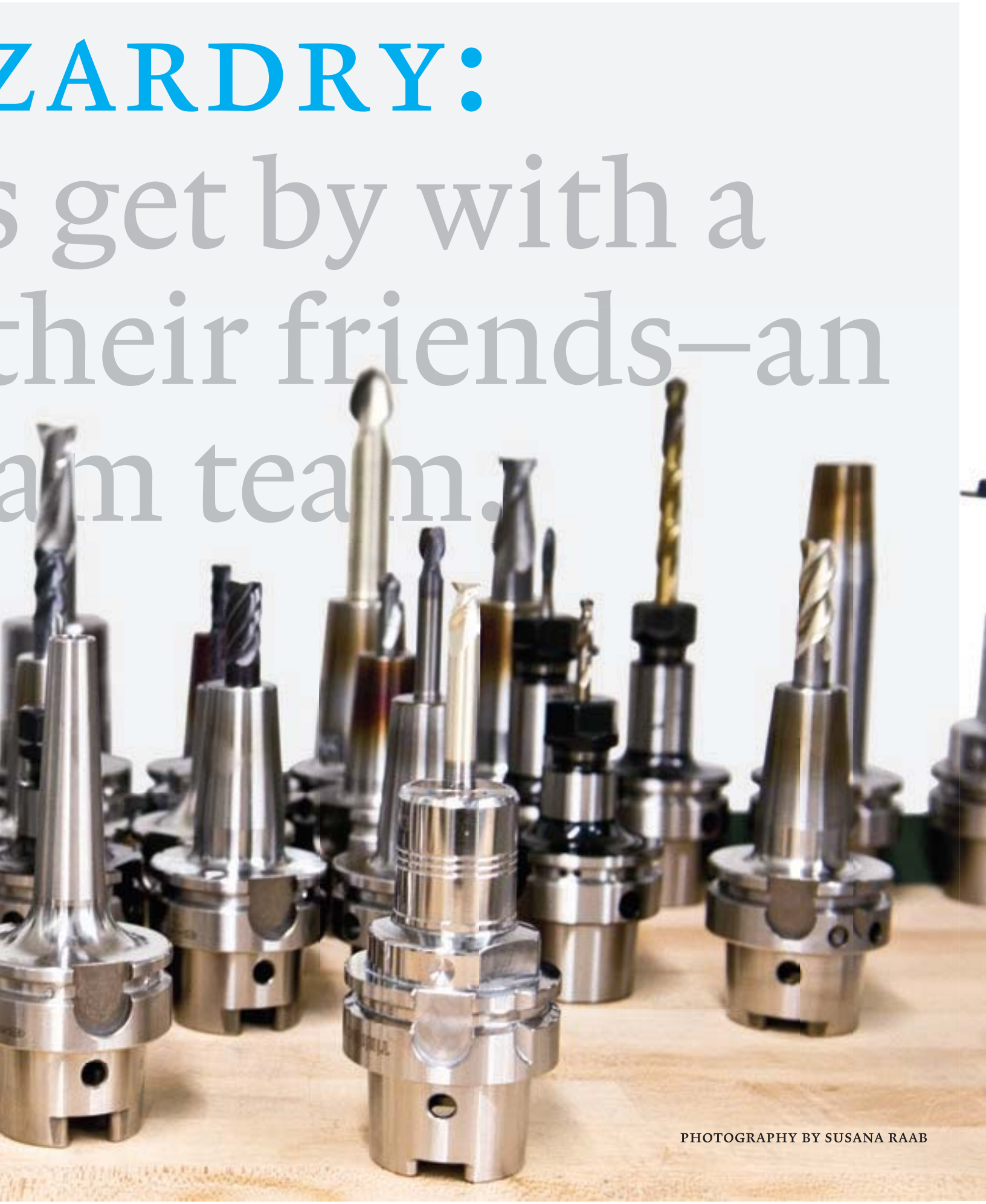
TECHNICAL WIZ

Janelia scientists
little help from t
engineering drea

BY JOHN CAREY

ZARDRY:

s get by with a
their friends—an
am team.



PHOTOGRAPHY BY SUSANA RAAB

FOR HER PHD research, Gwyneth Card needed to master an unusual skill—fly wrangling. Card, at the California Institute of Technology (Caltech), wanted to know how fruit flies flee from predators.

To find out, Card painstakingly herded flies down a tiny tunnel and manually opened a gate to release them one at a time onto a platform. She then played the role of "predator," pulling a string to send a threatening black disc zooming down a rod toward the fly. "I was the apparatus," she says. "It was like a one-woman fly show."

Card's fly legerdemain, combined with high-speed photography, revealed that fruit flies don't always flee with just a simple jump. Instead, they often perform a more complex sequence of movements, coordinating the position of their legs and preparing their wings. But that discovery raised new questions about what's going on in the tiny fly brains. To answer them, Card knew she had to move far beyond manual fly herding. "We needed to observe thousands of flies a day," she says. Only then could she test flies with a vast number of genetic variations and pin down the nerve circuits involved in the escape behavior.

So in 2010, Card moved to HHMI's Janelia Farm Research Campus and began to dream of an automated fly-scaring apparatus. "I had this whole elaborate plan of all the crazy things I wanted to build," she says.

Card came to the right place. Janelia was explicitly created to tackle areas of science that, if well-funded, had the potential to transform science in the next 10–30 years. After holding a series of workshops and consulting with advisors, HHMI leadership tightened its focus to two complementary areas: identifying how neural circuits process information, and developing imaging technologies and computational methods. "HHMI did an analysis and asked, What is holding the field of neuroscience back?" says Reed George, now senior director of Scientific Services. "The most obvious answer was instrumentation." As a result, Janelia was established with a focus not just on neurobiology, but also on the development of imaging systems, reagents, computer algorithms, and other tools.

Ryan Williamson, Gwyneth Card, Brian Coop, and Tanya Tabachnick (l–r) created a "virtual flyswatter" to study nerve circuits involved in escape behavior.

"Just knowing they are there means that when a crazy thought floats across my mind, I don't dismiss it."
—ROIAN EGNOR

HHMI hired microscope builder Eric Betzig and software experts, and brought in Executive Director Gerry Rubin's treasure trove—a library of more than 7,000 genetically distinct strains of fruit fly. It's been a successful strategy. The cutting-edge microscopes from Betzig's and others' groups, the software algorithms, and the reagents that have been developed are already advancing science both within and outside of Janelia.

But Reed George and others realized that the individual scientists also needed a broad range of specific, customized tools. So in the windowless rooms along Janelia's service corridor, deep in the heart of the building, HHMI built a high-tech machine shop and then hired a machinist and an engineer. In the years since Janelia's opening in 2006, that original shop has grown under George's guidance into a state-of-the-art facility. Called Instrument Design & Fabrication (ID&F), the facility has giant machines for cutting delicate parts from hunks of metal, 3-D printers, and 17 engineers and fabrication experts who have a passion for science. "ID&F is an awesome resource," says Janelia Lab Head Anthony Leonardo, who studies dragonflies. "I came here in part because of ID&F; these guys are more skilled than anyone I've worked with in the past."

A Test of Ingenuity

Those skills are now more important than ever. Many of today's experiments, such as recording from the brains of flies as they move freely in response to threats or other stimuli, just can't be done with commercially available equipment, says Michael Dickinson, a neuroscientist at the University of Washington, who recognized early on the value of bringing engineers into his lab.

The grants that many university scientists get, however, don't usually include funds for hiring engineers. A few other research labs have skilled equipment and tool builders, "but it's not on the same scale as at Janelia," says Leonardo. "It gives the scientists a huge advantage," says Dickinson, who was Card's graduate advisor at Caltech and

is now a regular visitor to Janelia.

And it's a rare scientist who can build his or her own sophisticated equipment. "I understand the biological questions, but I would have no clue how to make a door go up and down," says Lab Head Ulrike Heberlein.

When Card brought her ambitious fly wrangling plans to the engineers, they "were game for it," she recalls. "They love to challenge themselves." Lead mechanical engineer Tanya Tabachnik, who had built machinery capable of packaging virtually any product in cardboard or plastic wrap before coming to Janelia, devised tiny tunnels for flies to traverse from their vials to the test platform. Working with members of Card's lab, Brian Coop (who had previously designed, among other devices, prosthetic arms for a company that supplies veterans with artificial limbs) created a trap door that automatically releases one fly at a time onto the platform. He also constructed a virtual reality system that "attacks" the fly. "It's a lot of work, just to scare a fly," says Coop.

It also takes the right touch. The trap door often shut on a fly instead of allowing it through. Splat! "It squished a lot of flies," Coop recalls. The fly juice damaged sensors and made a mess. So Coop figured out how to adjust the timing so the door closes more slowly and the flies are, at worst, momentarily pinned down instead of annihilated.

Card's lab is now using the intricate apparatus, dubbed FlyPez, to startle 300 flies a day, and hopes to triple that number. So far, Card has learned that identical threats can trigger either the hardwired jump to safety or the more complex sequence of movements. Now her lab group is busy charting the neural circuits involved, which will add another piece to the puzzle of how the brain works.

The other 42 lab heads at Janelia are pursuing similar advances in knowledge—and many are also relying on tools, instruments, and devices fashioned by the ID&F team. The engineers have designed tiny tweezers to grab inch-long fish without harming a fin, and devices to gently hold flies by their necks. They've customized microscopes to image the entire head of a mouse and devised powerful strobe lights to capture precise images of dragonflies in flight. They've built microdrives for inserting electrodes into mouse and fly brains with amazing accuracy and saved researchers from anesthesia-caused headaches during animal surgeries by sucking the fumes away from the operating table.

The engineers' ingenuity is constantly being tested. Before coming to Janelia, instrument design specialist Jason Osborne had built parts for the space shuttle. But when Lab Head Vivek Jayaraman asked ID&F for a customized treadmill for fruit flies, Osborne faced a constraint that hadn't come up in the space program: delicate insect legs. "You don't want to break the fly's little legs if he's cruising along and then has to stop all of a sudden," he says. The solution ID&F and the lab came up with was modernizing an old idea: suspending a little foam ball on a column of air for the fly to run on. The ball responds quickly, with little inertia, to the fly's every move, and the ball's precise motion can be monitored. The treadmill has helped Vivek's lab group learn how flies perceive and respond to motion—and Osborne and colleague Gus Lott earned slots as co-authors on a 2010 *Nature Methods* paper describing the technique and its results.

Engineer Jason Osborne (r) is helping Roian Egnor develop video trackers and microphone arrays to monitor social behavior in mice.

Applied Wizardry

ID&F's work tends to fall into a few main categories. "There's the stuff we [scientists] couldn't possibly do technically, and the stuff I could do but would take me 100 times longer," says Lab Head Roian Egnor. Another important chunk of work involves making simple pieces, like brackets and cables, to boost the scientists' productivity.

The common thread in these projects is using technical wizardry to make researchers' lives easier and to advance the science. To study how odors affect behavior, for instance, Matt Smear, a research specialist in Egnor's lab group, designed a device to blow smells toward mice running on treadmills. But even after weeks of fine-tuning,

Engineer Jeff Jordan and postdoc Huai-Ti Lin are devising a new way to serve up "fly bait" for hungry dragonflies.

the odor plume wasn't wafting correctly to the mice's noses. "So I call Jason," says Egnor. "He comes up and in literally five minutes finds a solution."

Just down the hall, in Anthony Leonardo's lab, researchers are studying how dragonfly brains orchestrate the complex flight maneuvers needed to nab prey. His lab team has built tiny backpacks that capture and transmit signals from neurons. But once a dragonfly is fitted with nerve probes and a backpack, the window for testing its behavior is short—and there's no guarantee that the dragonfly will be interested in chasing a live bug. "With real prey, the odds are small that predator and prey will meet in that intricate ballet," says Leonardo. "So we want to have prey at the time the animals are interested in behaving."

The best answer seemed to be a fake bug that would fly around and reliably trigger dragonfly attacks. But how to create such a robot? Leonardo handed the problem to ID&F's Jeff Jordan. Realizing that a tiny, radio-controlled fly would be too expensive and challenging, Jordan says, "We had to think out of the box." He came up with the idea of a bead at the intersection of two strings. It works, to a point. Dragonflies attack until they learn that the whole reason for predation—a meal—is missing. So Jordan is now working on a more sophisticated multi-string system in which the "prey" will be a tasty morsel—perhaps even a real fruit fly—mounted on a tiny barbed rod.

And somebody's got to sort the fruit flies. Normally, that job would fall to graduate students. But Janelia's lab groups are small, and they don't have large numbers of graduate students. "A lot of things that might be done by throwing more labor at them, we can't do here," explains Saul Kravitz, who recently joined Janelia as senior director for Advanced Computation and Technology and now oversees ID&F. Besides, this sort of labor is tedious. Mistakes are made. So ID&F systems engineer Peter Polidoro, working with Janelia's Applied Physics and Instrumentation Group, has been developing a sophisticated fruit fly sorter. Starting with 10 vials of flies on a rack, the system chills the insects to put them to sleep, tips the vials' contents onto a ramp, and jiggles the flies down the ramp and past a camera, which uses image processing software to tell the flies apart by gender. Then, little puffs of air blow males into one container and females into another.

"I really like the idea of building machines to do things and collect data automatically to free up the scientists," says Polidoro. And, as with most of the tools devised at Janelia, ID&F makes all the hardware and software available open source so that researchers outside of Janelia can put them to use.

The Sweet Spot

The ID&F tasks that most excite both engineers and scientists, though, are the ones that open the door to new avenues of research. "There's a sweet spot for Janelia projects," says Egnor. "If it seems logical that experiments should be done, but no one is doing them because they're

For a glimpse into the world of ID&F at Janelia, see the slideshow at www.hhmi.org/bulletin/winter2014.

hard, those are Janelia projects." Egnor is tackling one such effort—exploring the brain circuitry that enables animals to navigate complex social environments. "We need two pieces of technology that are hard," she explains. First, they need to detect exactly what an animal is doing, "not just that George is sniffing another animal, but that he's sniffing the left haunch of Fred, and Fred is the subordinate male, and Fred and George just had a fight," she says. "We need the whole social context." With ID&F's help, her lab is developing video trackers and microphone arrays surrounding a special cage to accurately record and map every animal's behavior and vocalizations. "We're getting close," says Egnor.

The second requirement may be even tougher: measuring activity from neurons without affecting the animals' behavior. It's possible to implant electrodes in mouse brains to record activity. But if an animal looks implanted, any other self-respecting mouse will attack it. "It thinks, you've got that funny thing on your head and you're moving slow, and I'm going to beat you up," says Egnor. So ID&F lead electrical engineer Steven Sawtelle worked with the lab team to design a chip, smaller and thinner than a fingernail, that can be slipped under the skin on a mouse's head. It beams signals from electrodes in the brain to a receiving computer for analysis. Adam Taylor and Ben Arthur in Janelia's Scientific Computing group are helping Egnor's lab develop advanced software for synchronizing the flood of data from the video trackers, microphones, and neurons.

Egnor would never have attempted the complex effort without ID&F and the software team, she says. "Just knowing they are there means that when a crazy thought floats across my mind, I don't dismiss it."

Speeding Science

These success stories don't mean that interactions between scientists and ID&F are always wrinkle free. With multiple requests to juggle, engineers find that projects sometimes take longer than scientists expected. Scientists, in turn, sometimes move the goal posts, dragging out project timelines. And even with 17 engineers, there's far more demand for ID&F's services than can be met. "The biggest challenge is simply scheduling all the requests," says Coop.

Yet, to a person, the engineers say they thrive on the pressure and love being able to contribute to the science. "I'm so passionate and so excited about the research here," says Osborne. Jordan turned down a higher paying job designing satellite-tracking backpacks because, he says, the "mission and goal here are much more of a draw than money." The engineers are content to have their contributions only briefly mentioned in papers or presentations. "I work on something I think is extremely complicated and cool and amazing, and the scientists give a talk and you realize it was a very small piece," says Sawtelle. "But the scientists are the artists. We are the tool builders."

At the same time, they also say they have more to offer. "We want the scientists to use us more for things that are really challenging and less for just cables and brackets," says Tabachnik. Her suggestion to the researchers: "Bring us in at the beginning of a project so we can dream with you."

Another suggestion, which Janelia's management is exploring, involves making individual projects more broadly applicable. "We are building a portfolio of reusable components that can really accelerate the science, as opposed to everyone using his or her own very specialized solutions and starting from scratch every time," explains Kravitz.

Such an approach could also boost scientific progress outside of Janelia. Both scientists and engineers hope that sophisticated tools such as the automated fly sorter, Card's fly frightener, or the "fly bar"—a technological tour de force being used by Heberlein to study the neurobiology of alcohol addiction and motivation—will be replicated in other labs or offered by companies as commercial products. "The goal is always to create something the whole field can adopt," says Card.

And in fact, just like Betzig's microscopes and Rubin's genetic libraries, a few of ID&F's creations, such as a custom two-photon laser scanning microscope, have begun to find homes with researchers elsewhere. More widespread adoption, however, may require more time—and more scientific discoveries at Janelia. The onus is on the scientists, explains Parvez Ahammad, a junior fellow who studies neural circuits in fruit flies. "We have to show that something is worth studying," he says. "If we find interesting biological insights, people will want to replicate what we did and would need the same designs and technologies."

For now, Janelia's scientists mainly see ID&F as one of the crucial shared resources that speed up their research and boost the chances of new discoveries. "If I had one thing I needed to make and had a year to do it, I probably could get it done painfully," says Lab Head Albert Lee. "But when I have many things and want them quickly, and want the best, that's when I need ID&F." ■

Steven Sawtelle applies his expertise in electrical engineering to design microcircuits and specialized chips.

Perspectives & Opinions

It's BRAIN Time

Seeking a Google Earth view of the brain

When President Obama mentioned brain research in his 2013 State of the Union address, then launched the BRAIN (Brain Research through Advancing Innovative Neurotechnologies) Initiative soon after, people had questions. Is there a plan? Why this, why now? Where will the money come from? HHMI Investigator Cornelia Bargmann, who co-chairs the NIH-supported BRAIN working group with HHMI Investigator William Newsome, explains why now is the time.

Cornelia Bargmann

—

Head of the Laboratory of Neural Circuits and Behavior, The Rockefeller University

LIKE MOST NEUROSCIENTISTS, I was surprised and excited when President Obama hailed efforts to map the human brain in his State of the Union address. It's not often in basic science that we hear the President say that what we're doing is important and should be accelerated. The goal of the BRAIN Initiative isn't a passive map of brain structure, but an active map that shows the flow of traffic as well as the roads. The idea of constructing a full record of neural activity across the millions of circuits in the brain originated in a 2012 paper in *Neuron* by a group of scientists supported by the Kavli Foundation.

The new initiative caught everyone off balance and gave rise to two completely reasonable concerns: Where's the plan? Where's the money? Research in the United States is under stress already. With ongoing federal budget restrictions, biomedical research is in a world of pain. If I thought this project would be used as an excuse to drain money away from all the important basic science going on, I wouldn't be involved.

We hope to see the BRAIN Initiative bring more resources into science. With programs like the Apollo space missions and Human Genome Project, new resources came with the national will to see those projects succeed. For the BRAIN Initiative, the federal government will spend $100 million the first year, several philanthropic organizations have committed resources to overlapping projects, and private industry is interested as well. If energy builds for these ideas, there are many ways to promote them.

Technologically, the timing is right. In the past, we've learned a great deal about the brain at the level of single neurons and synapses, and by whole-brain imaging.

But somewhere between those two extremes is the level at which information is actually encoded to give rise to complex thoughts and behaviors—emotions, actions, perceptions, decisions. We know it's not one neuron, or one region, that's involved, and it's not the whole brain. Some of the activity is localized and some is distributed, and it's all changing in time and space.

A challenge of our time is to understand the shape of that intermediate level of circuits and systems of activity in the brain. Today, we can record hundreds of neurons at a time. But that's still not enough; the number of neurons involved in any particular mental process may number in the high thousands to millions. These neurons aren't just "on" or "off"; they have dynamic patterns of activity and dynamic relationships with each other. The tools developed by the BRAIN Initiative are aimed at capturing those network properties and dynamics, understanding which features are important, and finding out how they're generated and what they mean.

We think we can leap to the next level of analysis because of progress in many areas of science: in recording neural activity with genetically encoded indicators; in developing optics and related instrumentation; in engineering very fast, sensitive cameras that can capture a huge amount of information in a short time. Today, optogenetics enables us to perturb neural activity or manipulate it to see how it affects behavior. That's a big shift in the field from an observational to a causal science. At the same time, Silicon Valley companies now have the ability to analyze large, complex data sets.

All those things are happening already, but they're not as good as they need to be. We need to scale up these methods 100- or 1,000-fold. We need deeper theoretical approaches. We need more insight into the powerful but still incompletely understood pictures we get from human brain imaging.

The BRAIN Initiative is about accelerating technology development, and our interim report for NIH spending on this program in fiscal year 2014, prepared with extensive input from the scientific community, sets out nine high-priority research areas of focus, including a plan for disseminating knowledge and training.

It's been disappointing that drug development for brain disorders and psychiatric diseases has seen little progress over recent years, when the need is so great. Many pharmaceutical companies have pulled out of neuroscience drug development, and many promising avenues have failed, despite a lot of effort. Ask industry why they're backing away from these problems, and they say it's because we don't understand enough about the brain. Those are our marching orders.

—Interview by Cori Vanchieri

For more information on the BRAIN Initiative, go to www.nih.gov/science/brain.

Mike McGregor

To fully map the brain, Cornelia Bargmann says, we need to invest in technologies.

Perspectives & Opinions

Q&A

If you could revive one extinct species, what would you choose and why?

More than 99.9 percent of the species that have existed on Earth are extinct. As several groups attempt to resurrect birds and frogs we thought were gone for good, four scientists reveal the creatures they would bring back if they could.

–Edited by Nicole Kresge

Irving Epstein
HHMI Professor
Brandeis University

I'd go for the quagga (*Equus quagga quagga*), an African plains zebra that had stripes on only the front half of its body. It went extinct in the wild by 1878, and the last captive specimen died in an Amsterdam zoo in 1883. I offer three reasons:

1. I've always loved the name, and its plural, quaggas, makes a great seven-letter word for Scrabble.

2. The quagga was the first extinct animal to have its DNA analyzed. Scientists in South Africa are trying to recreate the quagga by selectively breeding present-day plains zebras.

3. I'm fascinated with what it takes to produce patterns of stripes or spots in living organisms. Scientists in Japan have done some clever experiments with zebrafish: They ablate portions of a pattern in an embryo and then watch how the pattern reconstitutes itself as the fish grows.

Sean Carroll
HHMI Vice President for Science Education

It would have to be a dinosaur. Instead of the infamous *Tyrannosaurus rex*, however, I would love to see one of the giant sauropods grazing on the landscape. Although not the largest, *Diplodocus* is one of the best known and would be an impressive sight at 10 tons and 100 feet or so, head to tail. Given time and room to roam, this long-necked creature would give rise to many more species. I think Michael Crichton might have already had this idea....

Gwyneth Card
Lab Head
Janelia Farm Research Campus

It would be tempting to revive a species purely for scientific purposes, such as *Archaeopteryx* (did these pre-birds fly?) or an early hominid species (did Neanderthals speak?). But my inner five-year-old can't resist choosing *Tyrannosaurus rex*. I spent hours as a child poring over dinosaur books, and now I study the neural basis of animal behavior—how could I pass up the chance to see this awe-inspiring prehistoric carnivore in action? Besides, I want to know if, as recent discoveries suggest, this fearsome predator was, in fact, covered in fluffy feathers.

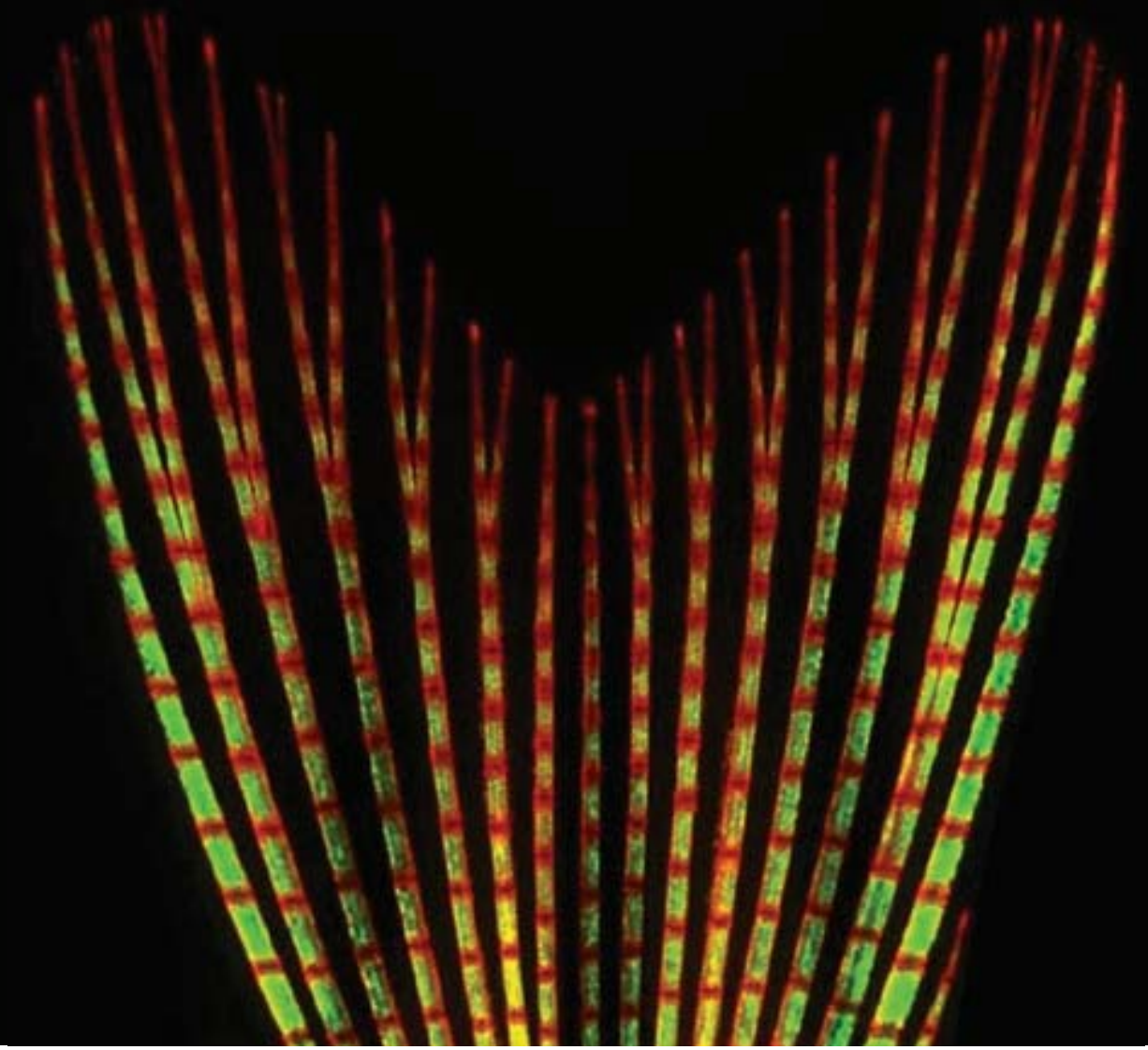
Fyodor Kondrashov
HHMI International Early Career Scientist
Center for Genomic Regulation, Barcelona, Spain

I would revive an ancestral species—something that's a direct ancestor of present-day lineages. In evolution, many traits are lost and gained, and we have only been able to study this process by comparing related species, never an existing species and its direct ancestor. It would be especially enlightening to revive the organism—whatever it was—that gave rise to all eukaryotes, as its nature is one of the most interesting and least understood. Reviving this ancestor would allow us to learn important aspects of its biology and, crucially, give insights into the biology of all living eukaryotes.

Epstein: Robert E. Klein Card: Matt Staley Carroll: James Kegley Kondrashov: Kevin Wolf

Chronicle

Zebrafish have the amazing ability to regrow their lost fins. But some zebrafish, females in particular, are better at regeneration than others. HHMI Early Career Scientist Ken Poss traced these gendered growth differences to a cluster of needle-like structures used by male zebrafish to grasp their partners during mating. The pointy protuberances produce a protein that short-circuits pectoral fin production in males. Poss's discovery suggests that male zebrafish have traded an ancient ability to regenerate tissue for a new way to enhance their reproductive success. You can learn more about Poss's findings in "Regenerate or Mate," on page 44.



Ken Poss

Chronicle / *Science Education*

An Ambitious Mission

With the revamped AP Biology curriculum in place, some teachers and students are struggling to adapt.

IN SEPTEMBER, A student in Stephen Traphagen's Advanced Placement (AP) Biology class asked him for an answer key to an assignment on human heredity. Instead of giving her one, Traphagen, who teaches at Rolling Meadows High School in Rolling Meadows, IL, told her about a NASA spacecraft.

The unmanned spacecraft was meant to search for signs of water—and former life—on Mars. But when it got there, it nearly crashed, then boomeranged into space forever. Engineers had crossed up English and metric measurements of thruster power, and no one had reviewed the data. "It's better for you to learn to check your own work than for me to give you an answer," Traphagen told the student.

The next day, the student's lab partner asked Traphagen for an answer key. "He doesn't do answer keys," Traphagen heard the young woman tell her friend with annoyance. "Something about crashing into Mars."

In 2012, the nation's AP Biology teachers embarked on a mission as ambitious as NASA's: to teach a new curriculum aimed at training students to solve problems the way scientists do. Many students and teachers, however, are struggling to adapt. To help them get up to speed, two groups—the organization that directs AP courses and an HHMI-supported collaboration—have been training teachers. In the early part of this transition, the jury on these programs is still out.

The reform effort emerged from years of education research showing that students learn science best if they read, write, think, and experiment conceptually, as scientists do, rather than memorize a menagerie of facts. The College Board, which designs the nation's AP curricula and exams, followed the advice of an influential 2002 National Research Council (NRC) panel and began overhauling several AP courses. The first to be completed was AP Biology; the revamped curriculum launched nationally in the fall of 2012.

The old AP Biology curriculum required students to memorize from a shallow swath of biology—the enzymes of glycolysis or details of photosynthesis, for example. The new one requires that students grasp pervasive concepts that unify biology, such as evolution. They must also understand the methods scientists use to answer questions. To discover how temperature spikes affect photosynthesis, for instance, students must be able to search the scientific literature, design an experiment to obtain data, and crunch the numbers to reveal answers. "The change in curriculum is very exciting, and it was due," says Jaclyn Reeves-Pepin, executive director of the National Association of Biology Teachers (NABT).

But those changes were extensive. "There was a lot of gnashing of teeth that we've gone too far, too fast," says David Knuffke, an AP Biology teacher from Deer Park High School in Deer Park, NY. He maintains the College Board's popular online listserv for AP Biology teachers. The teachers' angst was borne out by the first year's AP exam results. Just 5 percent of students achieved the top score of 5, as opposed to about 20 percent in past years.

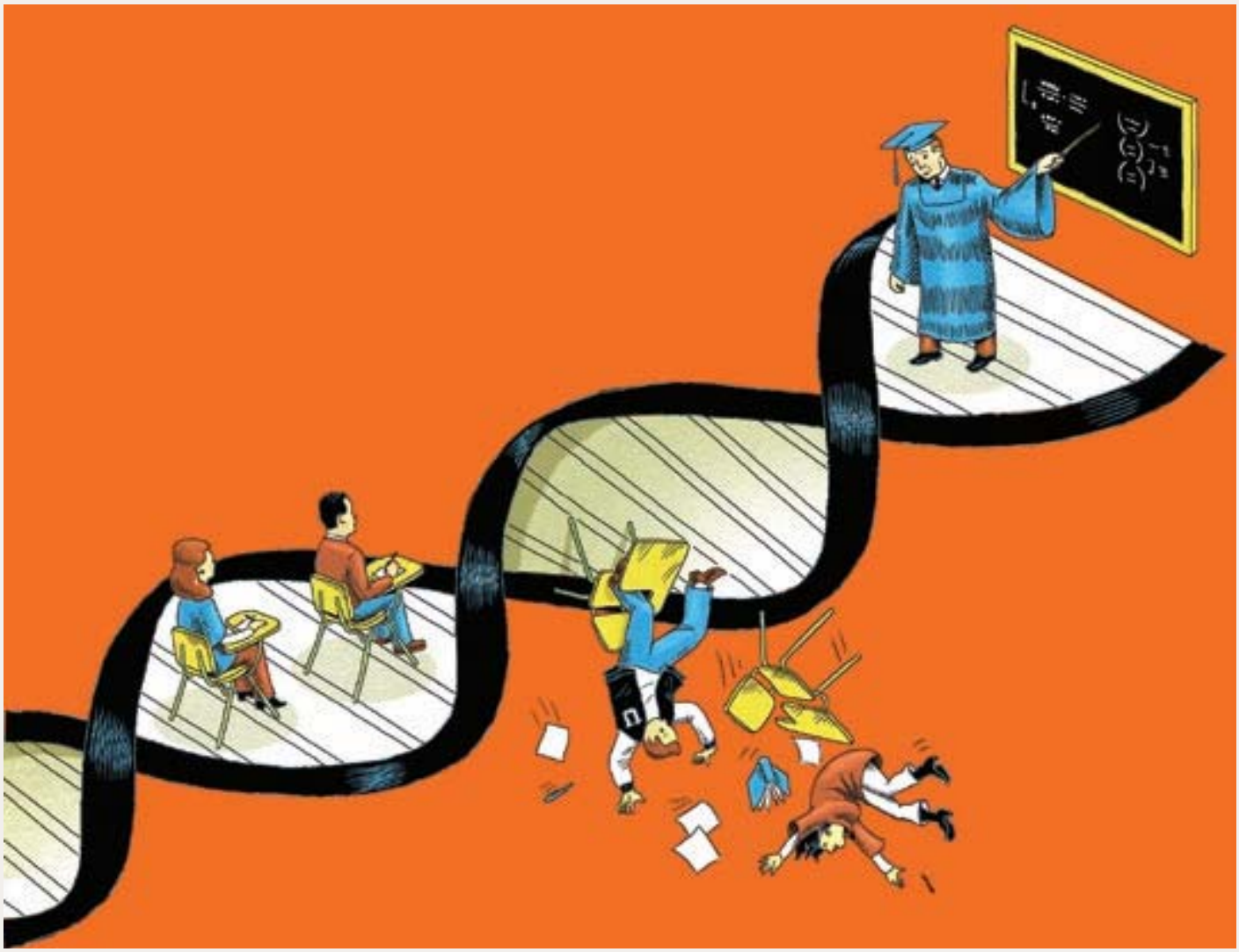
Scores dropped because students performed poorly on questions that required scientific reasoning and the use of mathematics to solve biological problems, says Bill Wood, a University of Colorado molecular biologist and educator who sat on the NRC panel and helped the College Board develop the new AP Biology exam. "It's a wake-up call," Wood says. "Teachers will need to learn [how to] teach to the new framework."

Dennis Liu, who heads education outreach at HHMI, says that lower scores might be fine because they realistically reflect students' grasp of the higher-level concepts and thinking. Plus, with the overhaul of a national test, disruption is inevitable.

But Liu and Wood agree that there's room for improvement via training. When teachers get training and stick with the new curriculum, the results can be transformative. "I haven't run across any students who can't succeed in the new framework," says Traphagen, who went through the HHMI-supported training.

To help the nation's 9,000 AP Biology teachers adopt the new curriculum, the College Board created print and online materials for use in developing syllabi and practice exams. It also offers weeklong professional development workshops, called AP Biology Summer Institutes, which cover instructional methods and specific lab lessons. More than 5,000 AP Biology teachers attended a Summer Institute in 2012, just as the new curriculum was launching, and nearly 6,000 have participated in one-day workshops. The College Board and the National Science Foundation are doing a large-scale, three-year study to see if professional development efforts are working, but no results are in yet, according to spokesperson Deborah Davis.

NABT, the Biological Sciences Curriculum Study (BSCS), and HHMI have developed a training program as well: a three-year program for AP Biology teachers who are willing to teach and mentor their colleagues locally and regionally. At an in-depth, weeklong summer workshop the first year, and a one-day workshop at the NABT conference that fall, a select group of teachers immersed themselves in the new AP Biology curriculum. They learned effective new teaching methods, such as challenging students to interpret charts and graphs, and hands-on labs where students take the lead in solving scientific problems. At a weeklong summer workshop the second year, they learned leadership skills and how



Wesley Bedrosian

For more information on science education reform, see "Calling All Teachers" and "A 21st Century Cook Book," *HHMI Bulletin*, November 2011.

When teachers get training and stick with the new curriculum, the results can be transformative.

to effectively teach their colleagues. NABT and BSCS also provide support through professional development workshops and regional networks of AP Biology teachers.

Participants say the program, called the AP Biology Leadership Academy, empowers them to improve the teaching skills of their colleagues back home. When it comes to professional development, "the limiting factor most often is people's sense of confidence, agency, and safety, and that's only going to happen in small groups and face-to-face meetings," says Traphagen, who completed the program and has trained 20 colleagues in the Chicago area and more than 200 in workshops at professional conferences.

It won't be easy to scale up in-depth training programs to a level sufficient to transform AP Biology classrooms nationwide. But it could happen if the newly trained teachers help spread the word about effective new teaching methods, says HHMI's Liu.

He hopes this will happen through teacher-led workshops held within regional networks. To date, the 46 teachers in the first training cohort have collectively given 21 workshops to more than 270 other teachers, and most reported that they were doing more informal mentoring, as well.

In Ohio, Colorado, and Michigan, AP Biology teachers from the program have revitalized statewide networks of biology teachers, in part to support each other as they adapt to teaching the new curriculum. "We've learned that it's hard, and these networks don't gel on their own," Liu says. "They need ongoing support."

HHMI is eager to provide it. "If we could get one regional network of teachers going, then I would call the overall model a success," Liu says.

Some, including Reeves-Pepin, are impatient for success and eager for large-scale change. "I appreciate the ripple effect," says Reeves-Pepin, "but I want change—and the sooner the better." *–Dan Ferber*

Chronicle / *Toolbox*

The History of the World in an App

The popular EarthViewer app breathes life into all 4.5 billion years of the place we call home.

IF YOU COULD hop into a time machine and travel back 100 years, how would our planet be different? What about 100 million years, or even a billion? Could you find New York City on a globe that, thanks to continental shifts, looks nothing like the one we know today? With HHMI's EarthViewer app, hundreds of thousands of people are asking and answering questions like these for themselves.

For decades, HHMI has supported biomedical research and science education related to life on Earth. Recently, the Institute has started to think more about the "Earth" part of that mission, developing educational resources for the classroom, including a short film about the Mesozoic extinction and a DVD package of materials on the history of life on our 4.5 billion-year-old planet. But HHMI wanted to create something interactive, to really engage students, says Satoshi Amagai, a senior program officer in the Educational Resources Group. "We [wanted to have] an app that could [bring users] through time to see how the Earth has changed," he says.

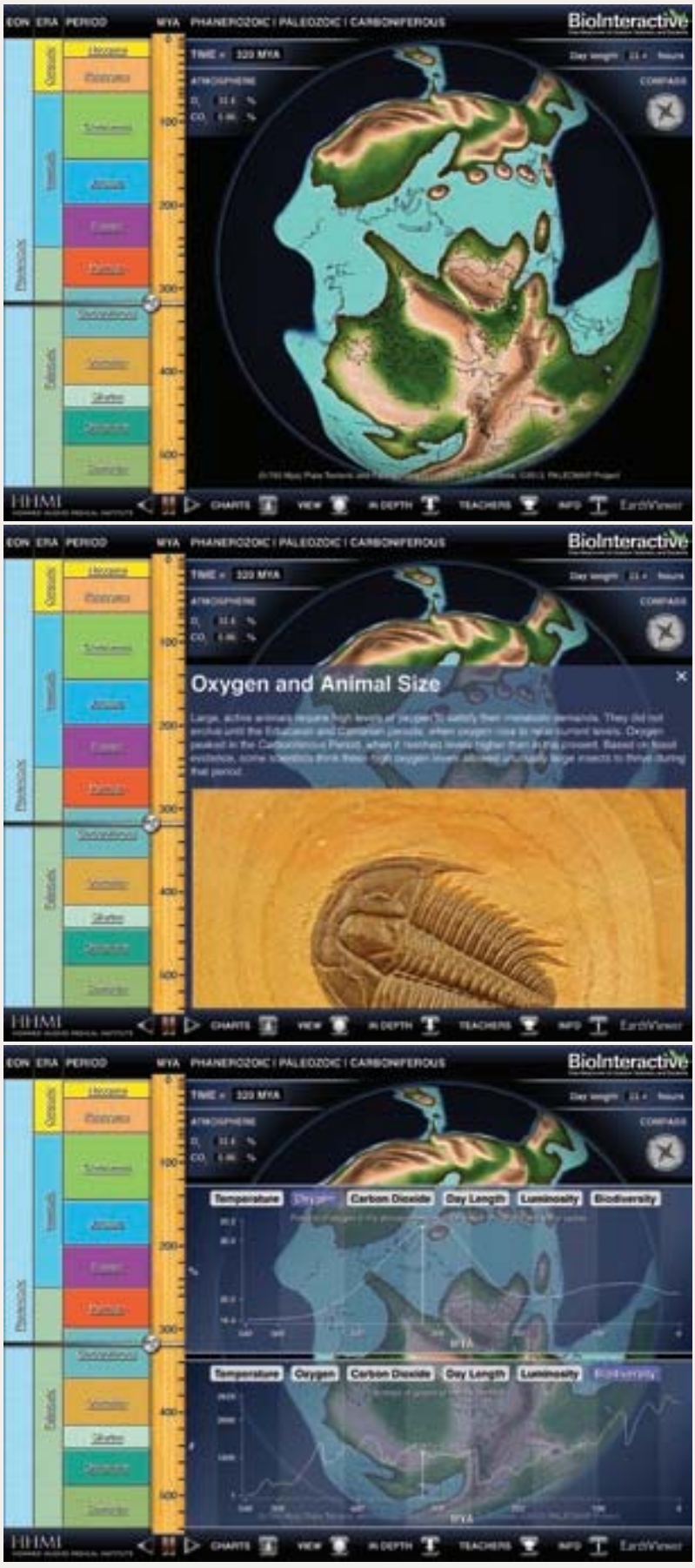
The EarthViewer app does just that. Designed for high school students to tap and pinch their way through billions of years of history, the app showcases everything from a century's worth of climate change data to the changing oxygen levels over four billion years. Students can spin a virtual globe to zoom in on specific areas of the planet to study fossil records and solar luminosity, for example, and dig deep into the raw data if they want more information. Like a Swiss Army knife, the app houses many different tools that can be used for a variety of purposes.

For high school science teacher Dave Kenyon of Paw Paw, MI, the app gives new depth to his lessons about how continents shift over time. For years, he has shown students Petoskey stones—fossilized coral that's native to Michigan—to help illustrate the idea that parts of the state were once located in warm, shallow salt water. Now, he enhances that lesson by showing students the EarthViewer app. They can see that Michigan was located south of the equator hundreds of millions of years ago, and then drag their way through the timeline to see how the land shifts thousands of miles to its current location. "Before the app," says Kenyon, "students would nod their heads [when I explained continental shift] and say, 'Okay, yeah.' But now they can really see that change. They get it."

With a scrollable, zoomable timeline on one side of the app and a spinnable three-dimensional globe on the other, students can select the things they're most interested in tracking—from world temperature to locations of modern cities to carbon dioxide levels—and watch them shift over time. They can check out information on specific fossil sites and study in-depth features of major geological events in Earth's history. Students can even layer on different types of data—continental shift and atmospheric conditions, for example—to get a more holistic understanding of how the world has changed over time.

So far, the app seems to be resonating with students and teachers: When HHMI released the iPad app in January 2013, it was featured in the iTunes App Store, and it rocketed up the charts. "If you get featured on the iTunes Store, fasten your seatbelts,"

"Before the app, students would nod their heads [when I explained continental shift] and say, 'Okay, yeah.' But now they can really see that change. They get it."
—DAVE KENYON



To learn more about the EarthViewer app, visit www.hhmi.org/biointeractive/earthviewer.

jokes Mark Nielsen, a science education fellow at HHMI who, among others, helped to develop the app. By the end of 2013, the app, which became available for Android tablet computers in September, had more than a quarter-million downloads.

For Laura Dinerman, who teaches environmental science at Sherwood High School in Sandy Spring, MD, the app offers an easy and effective way to dispel common misperceptions that students have about climate change. "Students who start the unit [convinced] that our current warming trend is normal and cyclical can immediately discover the cycles of carbon and temperature through time and note the current irregularity in the rate of warming," she says. "Because the app lets students discover and analyze independently, it prepares them to make the leap to 'what's next' without a political or moral agenda."

Based on early feedback from teachers, the team is updating the app and adding new features. Nielsen is developing a virtual scavenger hunt so that students can explore the different features of the app, and the team may add new data so that students can take a closer look at sea level change over time. HHMI is also considering adapting the app so it can be used on the Web. "This has been so popular, we're starting to think about other apps in completely different areas," Amagai says.
–Erin Peterson

Chronicle / *Lab Book*

Fly Brain Filters

A cluster of neurons help flies make sense of a visual scene.

WHAT GOES ON in a fly's brain as it buzzes around a room? Does it recognize every object, or does it take cues from general lines and shapes? Using two-photon laser scanning microscopy and calcium imaging, Vivek Jayaraman, a lab head at Janelia Farm Research Campus, probed the brains of fruit flies to find out.

An area deep in the fly brain, called the central complex, lets flies recognize visual landmarks while they're moving and use that information to orient themselves, locate safe places, and avoid not-so-safe ones. Until recently, however, scientists didn't know how the fly central complex takes in and processes visual information. Fly brains are very tiny, and the only way to study them was by immobilizing the flies, which prevents any sort of mobility study. A few years ago, however, Vivek's team figured out how to immobilize a fly's head in a two-photon microscope, while its wings and legs move freely.

Vivek's postdoctoral researcher Johannes Seelig used the technique to look at a cluster of neurons in the central complex called ring neurons. When flies were placed in a small virtual-reality arena and presented with simple patterns of light, their ring neurons responded more strongly to vertical bars than horizontal bars projected on the walls. This made sense, since flies have an innate tendency to walk or fly toward vertically oriented stimuli. The neurons were, in effect, extracting, or filtering out, visual information.

"These input neurons seem to help break down the visual scene around the fly into particular features that flies care about," Vivek says. "Later, neurons in the central complex presumably use these features to decide what to do in their surroundings." As the duo reported in *Nature* on November 14, 2013, this orientation preference mirrors what scientists have found in mammals—that certain neurons in the visual cortex tune in to an object's orientation.

Next, Vivek plans to look deeper into the central complex. "By marching through these networks, we hope to begin to understand how sensory information is integrated to make motor decisions," he says. – *Nicole Kresge*

The letters "HHMI" might look like this to a fly after being filtered through its brain.

Igor Siwanowicz, Johannes Seelig, and Vivek Jayaraman Illustration: Leif Parsons

IN BRIEF

A CREAM FOR PARKINSON'S

An ingredient in anti-wrinkle cream may soon be a potent weapon in the fight against Parkinson's disease. HHMI Investigator Kevan Shokat has shown that kinetin, a plant hormone with anti-aging properties, stops the nerve cell death associated with the disease.

Some cases of inherited Parkinson's are linked to mutations in a protein called PINK1. Normally, when the mitochondria that power nerve cells become damaged, PINK1 comes to the site and recruits other proteins to remove the mitochondria before they release toxic compounds. Mutated PINK1, however, is inactive and unable to signal its helper proteins. As a result, the damaged mitochondria are never removed, and the nerve cell dies.

Shokat and his colleagues at the University of California, San Francisco, wanted a way to ramp up PINK1 activity. As they reported August 15, 2013, in *Cell*, they found that kinetin activates both normal and mutant PINK1 and decreases nerve cell death. Because it also affected normal PINK1, the researchers hope kinetin may slow disease progression in those without a family history of the disease as well.

The researchers are testing kinetin in animal models of Parkinson's. "[It's] a great molecule to pursue because it's already sold in drugstores as a topical anti-wrinkle cream," says Shokat. "So it's a drug we know has been in people and is safe."

STOPPING SYSTEMIC SCLEROSIS

Systemic sclerosis is a slow and painful hardening of the body's tissue. People with a mild form of the disease develop thick patches on their skin. More serious cases involve trouble breathing and swallowing, and can lead to death. "[It's] a very mysterious disorder," says HHMI Investigator Harry Dietz of the Johns Hopkins University. "There's been a lot of descriptive work on what happens to patients, but very little information about what causes it." Until now.

Dietz and his colleagues had traced a less severe form of scleroderma, called stiff skin syndrome, to mutations in the protein fibrillin-1. When they engineered mice with the same gene mutation, however, the mice showed symptoms of the more aggressive systemic sclerosis. As Dietz reported November 7, 2013, in *Nature*, the symptoms were caused by an immune reaction triggered by the protein's inability to do its job. As part of the extracellular matrix—the material that exists between cells—fibrillin-1 provides structural support and helps cells communicate with the matrix via molecules called integrins. Mutant fibrillin-1 can't interact with integrins. To compensate, more integrins are produced, causing an autoimmune reaction and fibrosis—the excessive production of connective tissue that results in scleroderma.

By interfering with the immune response, Dietz's team prevented fibrosis and even reversed the disease in mice. "This is one of the first, and the most dramatic, illustrations that fibrosis can be reversed," says Dietz. "I was quite surprised and quite thrilled."

WHEN CANCER SPREADS TO BONE

Tumors constantly shed cancer cells, yet only a few of these stray cells manage to survive and colonize distant organs. The rest succumb to the stress of the journey. HHMI Investigator Joan Massagué of Memorial Sloan-Kettering Cancer Center has found evidence that some breast cancer cells can turn on genes that increase their chances of

Halting Heart Damage

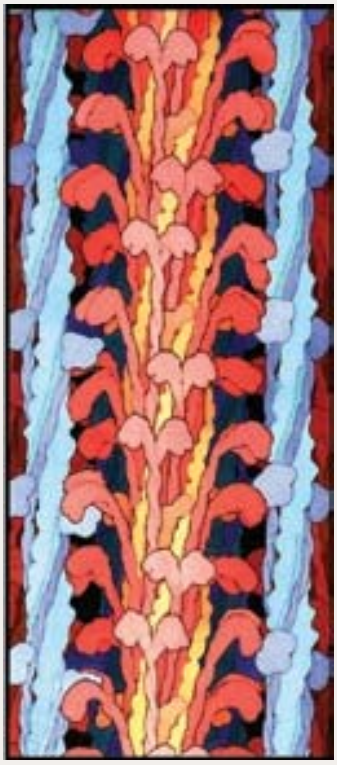
Small targeted RNA turns off mutant genes that impair heart muscles.

THERE ARE MORE than 1,000 ways in which genes can be mutated to cause hypertrophic cardiomyopathy (HCM). But any one of the mutations has the same result: the heart thickens, it has trouble pumping blood, and eventually up to 1 in 500 people with the condition dies. There is no cure, but HHMI Investigator Christine Seidman, at Brigham and Women's Hospital, has figured out how to prevent HCM symptoms from worsening in mice by shutting off some of the mutant genes that cause the disease.

Many of the mutations linked to HCM can be found in the genes that encode the heart muscle protein myosin. To develop new treatment strategies, Seidman focused on mutations in the mouse gene *Myh6* that encodes myosin; HCM myosin mutations alter the heart's ability to contract and relax. Her team designed small pieces of RNA, called RNAi (for RNA interference), that prevent the mutant gene from producing proteins. They used a virus that homes in on heart cells to deliver the RNAi to the correct location in the mice.

The experiment worked. For five months, mice with an HCM mutation in *Myh6* showed no thickening or other changes in their hearts, according to her team's report in *Science* on October 4, 2013. Although HCM could be prevented, existing damage wasn't reversed; but it didn't get worse, which is a benefit. Unfortunately, each RNAi targets only a single mutation. "There are nearly 1,000 human HCM mutations," acknowledges Seidman, "and it would be an extraordinary effort to make an RNAi that was specific for each one." As an alternative, her team also created an RNAi that targets common genetic variants that are tightly linked to a broad spectrum of mutations. Like the mutation-specific RNAi, this one worked for five months, making it a very promising method for targeting HCM mutations.

Next, Seidman wants to figure out why the RNAi becomes ineffective after five months. She suspects it's getting used up and that a booster of inhibitor could extend its effectiveness. – *Nicole Kresge*



Mutations in the heart protein myosin (red molecules) can cause hypertrophic cardiomyopathy.

David S. Goodsell and the RCSB PDB Illustration: Leif Parsons

survival, specifically in bone.

Massagué and his colleagues previously discovered that breast cancer cells expressing a set of genes called the *Src* response signature (SRS) were more likely to metastasize to the bone. Cells that expressed those genes were more sensitive to cell growth-promoting molecules—called cytokines—that are expressed by bone cells, the researchers reported August 29, 2013, in *Cell*.

"For any cancer cell, it's dreadfully rough to survive in the body after leaving a tumor," says Massagué. "These cells selected for being more responsive to cytokines might just have this tiny extra chance of surviving in bone. But when you're talking about tens of thousands of cancer cells circulating in the body per day, that tiny extra chance is enough to change the odds of a metastatic tumor forming."

Massagué is now testing drugs that affect the SRS pathway to see if they can block cancers from spreading to the bone.

CREATING FALSE MEMORIES

Many a science fiction movie is based on a person getting "brainwashed" into remembering something that never occurred. But is it really possible to create a false memory? For mice, the answer is yes. HHMI Investigator Susumu Tonegawa made rodents, after being placed in a certain location, recall receiving a mild shock there when, in reality, the event happened in a completely different place.

Memories cause lasting physical and chemical changes in brain cells. A few years ago, Tonegawa and his colleagues used these changes to pinpoint which nerve cells were activated in response to different situations. As a follow-up, they decided to see if they could use this information to create a false fear association in mice.

First, Tonegawa and colleagues at the Massachusetts Institute of Technology identified the nerve cells triggered in mice while they were exploring a new cage. Next, they put the mice in a different cage and applied a mild shock to their paws while stimulating the cells that contained memories of the previous cage. Finally, the mice were placed in the first cage again. They froze in place.

"We got the animal to be scared of an environment where, technically, nothing bad had ever happened to it," explains Steve Ramirez, a graduate student in the Tonegawa lab. The results were published July 26, 2013, in *Science*.

Next, the team would like to see if they can introduce pleasurable memories in mice, or memories of objects and other mice.

A CALCIUM SUPER SENSOR

When a nerve cell receives a message from a neighboring neuron, a wave of calcium ions rushes into the cell to keep the signal moving. Scientists at Janelia Farm Research Campus have created a new molecular sensor that glows each time it detects one of these calcium waves. By following the flashes of light, researchers can watch as a message gets passed from neuron to neuron throughout the brain.

Calcium sensors for brain activity have been around for about two decades, but earlier versions were less accurate or more cumbersome to use. "You can think of the brain as an orchestra with each different neuron type playing a different part," says Janelia Lab Head Karel Svoboda. "Previous methods only let us hear a tiny fraction of the melodies. Now we can hear more of the symphony at once."

Svoboda, along with Janelia Lab Heads Loren Looger, Vivek Jayaraman,

Chronicle / *Lab Book*

Regenerate or Mate

In zebrafish, spiky structures hinder fin regrowth.

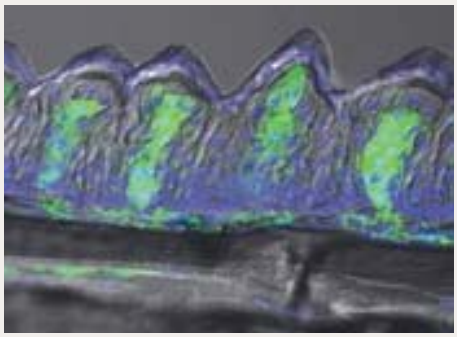
WHEN IT COMES to sex versus survival, most male zebrafish can't have it both ways. The fish is either good at mating or good at growing a new fin after an amputation injury. Scientists are now a little closer to understanding why.

A few years ago, Ken Poss, an HHMI early career scientist at Duke University, noticed that female zebrafish were better at regenerating their pectoral fins than male fish. "This type of sexually dimorphic regeneration is pretty unusual," explains Poss. "It suggests there's some sort of signaling malfunction in the males."

Poss and his colleagues discovered that the males had high levels of a protein called Dkk1b in their pectoral fins. Dkk1b is a potent inhibitor of Wnt—a signaling molecule that plays a big role in development, and thus regeneration. The researchers traced the source of Dkk1b to a cluster of needle-like structures, called epidermal tubercles, on the pectoral fins. Males use these spiky growths to grasp their partners when mating. "Normally, [Dkk1b] is regulated in a very precise way during fin regeneration," Poss says. But when the fins have tubercles, this changes completely.

Poss realized that the production of Dkk1b from epidermal tubercles was short-circuiting the regeneration of pectoral fins. The findings, published in *Developmental Cell* on October 14, 2013, suggest that zebrafish traded an ancient ability to regenerate tissue for a new way to enhance reproductive success. The good news for zebrafish is that it's not all or nothing. A certain percentage of males can recover from amputation.

The discovery of this level of control by a signaling inhibitor during tissue regeneration is big news. "It says that these inhibitors are important too," says Poss. "Putting them in the right places at the right time should be part of making tissue artificially." – *Nicole Kresge*



Zebrafish use these spiky structures on their pectoral fins to grasp their partners when mating.

Ken Poss Illustration: Leif Parsons

IN BRIEF

and Rex Kerr, created a sensor named GCaMP6. It is the most sensitive calcium sensor ever developed, and the first sensor that can detect impulses in every neuron in the brain, according to their report in the July 18, 2013, issue of *Nature*. The team continues to tinker with the sensor and develop new versions for specific uses, including sensors that emit different wavelengths of light so they are easier to detect.

CAREFULLY CONCOCTED TALES

Every cell in the body has the same genes, yet many of them—for example, nerve cells and skin cells—have very different jobs. This specialization is due in part to a cell's ability to turn genes on and off using short stretches of DNA known as enhancers. David Stern, a lab head at Janelia Farm Research Campus, has found a way to learn more about these regions by targeting them with particular DNA-binding proteins.

Stern and Justin Crocker, a researcher in his lab, used a group of proteins called transcription activator-like effectors, or TALEs. Originally isolated from bacteria, TALEs can be altered to bind to a specific sequence of DNA. Stern and Crocker created TALEs that bound to the enhancer for a gene called *eve*. They then attached part of a protein known to stop gene expression to those TALEs. As they reported in the August 2013 issue of *Nature Methods*, their engineered TALEs were able to halt *eve* expression in developing fly embryos.

Scientists will be able to use the technique to engineer their own TALEs and learn more about enhancer function and gene expression. "Not only does this let us understand enhancers in their native context," says Crocker, "it also lets us take the next step forward to controlling them."

ACCURATELY HITTING GENE TARGETS

Scientists have been manipulating genes in test tubes for years. Doing the same in living organisms, however, is a different story: It requires a way to precisely target that gene. A mismatch could mean the difference between effectively treating a disease and triggering the development of cancer. Two HHMI investigators recently teamed up to test the accuracy of a molecule called Cas9 that can be instructed to bind to and modify specific genes.

Cas9 is a naturally occurring bacterial protein with a bright future in genome engineering and gene therapy. It can be programmed with a short piece of RNA to cut specific DNA sequences. The RNA "guide" is usually about 20 base pairs long and is complementary to the target DNA. David Liu of Harvard University, Jennifer Doudna of the University of California, Berkeley, and their students evaluated Cas9's ability, when paired with guide RNAs of different lengths, to cut one trillion different DNA sequences.

They found that while the entire guide RNA sequence can program Cas9 specificity, some genome sequences that do not exactly match the guide RNA can nevertheless be cleaved, according to the report in the September 2013 issue of *Nature Biotechnology*. The tolerance for mismatches depends on what site in the genome was targeted and where the mismatches occurred in the guide RNA sequence. The team also found a trade-off between activity and specificity. Shorter, less active guide RNAs are more specific than longer, more active ones. Doudna and Liu are continuing their collaboration, which is now aimed at improving Cas9 specificity. They believe that, with future improvements, the molecule should be able to home in on any single site in the human genome, with potential therapeutic implications.

Observations

Elisabeth Moch

Kindred Spirits

Fledgling scientist Jacques Monod and aspiring writer Albert Camus didn't know each other as active members of the French Resistance, in the early 1940s. But each man shared a fierce determination to thwart the oppression and devastation of their country by Hitler's army. The friendship they forged after the war became a lifelong bond, inspiring further political activism and extraordinary creativity that eventually resulted in a Nobel Prize for each man in his respective field.

Monod and Camus were introduced at the meeting of a human-rights group and hit it off immediately. Their attraction to each other was deep. Although the two men had nothing in common in terms of their upbringing or professions, they were kindred spirits. Francis Crick described Monod in terms that applied equally well to his new friend Camus: "Never lacking in courage, he combined a debonair manner and an impish sense of humour with a deep moral commitment to any issue he regarded as fundamental." In addition to the special bond of former resistants, Monod and Camus discovered they shared many similar concerns. Over the course of their friendship, those concerns would encompass a broad spectrum of humanitarian issues, including the state of affairs in the USSR, human rights in Eastern bloc countries, and capital punishment in France.

Monod gave Camus further ammunition for his indictment of the Soviet Union, an indictment that terminated many of Camus's friendships with left-wing peers. Camus gave Monod access to his world of literature and philosophy.

Monod, too, was a conjunction of work and action. While Camus wrote "The Blood of the Hungarians" (1957) to arouse the world's conscience about the Soviets' crushing of the Hungarian revolution, Monod used his clandestine experience from the days of the Resistance to organize the escape of Hungarian scientists. As Monod's fame grew from his scientific achievements, he used his standing to advance many causes, including reproductive and human rights, and he was a prominent figure in the May 1968 unrest that nearly toppled the French government.

Camus had a profound influence on Monod and the philosophical ideas the biologist pursued in later years. After receiving his Nobel Prize, Monod turned to consider the implications of the discoveries of modern biology—how the answers to Schrödinger's question "What is life?" bore on the question of the meaning of life. He explained his impulse in Camusian terms: "The urge, the anguish to understand the meaning of his own existence, the demand to rationalize and justify it within some consistent framework has been, and still is, one of the most powerful motivations of the human mind." The opening epigraph of Monod's resulting, widely acclaimed, bestselling book, *Chance and Necessity*, was the closing passage from his friend's *The Myth of Sisyphus*.

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In-Flight Recorder

Dragonflies surpass all competitors when it comes to hunting on the fly. During their rapid flight forays, the multi-winged insects can predict the trajectory of their prey—a fruit fly, for example—and intercept it with apparent ease. Their predictions are so precise that dragonflies have a near-perfect capture rate—few of the flies they go after escape. To understand the neurobiology behind the dragonfly's aerial feats, Janelia Farm Research Campus Lab Head Anthony Leonardo and colleagues created an indoor flight arena and a recording device that is mounted on the dragonfly's back. This lightweight, wireless "backpack" probes select neurons and muscles, and broadcasts their electrical activity to a computer for later analysis. Read more about the team of engineers who enable this and other work at Janelia in "Technical Wizardry" on page 28.

Susana Raab