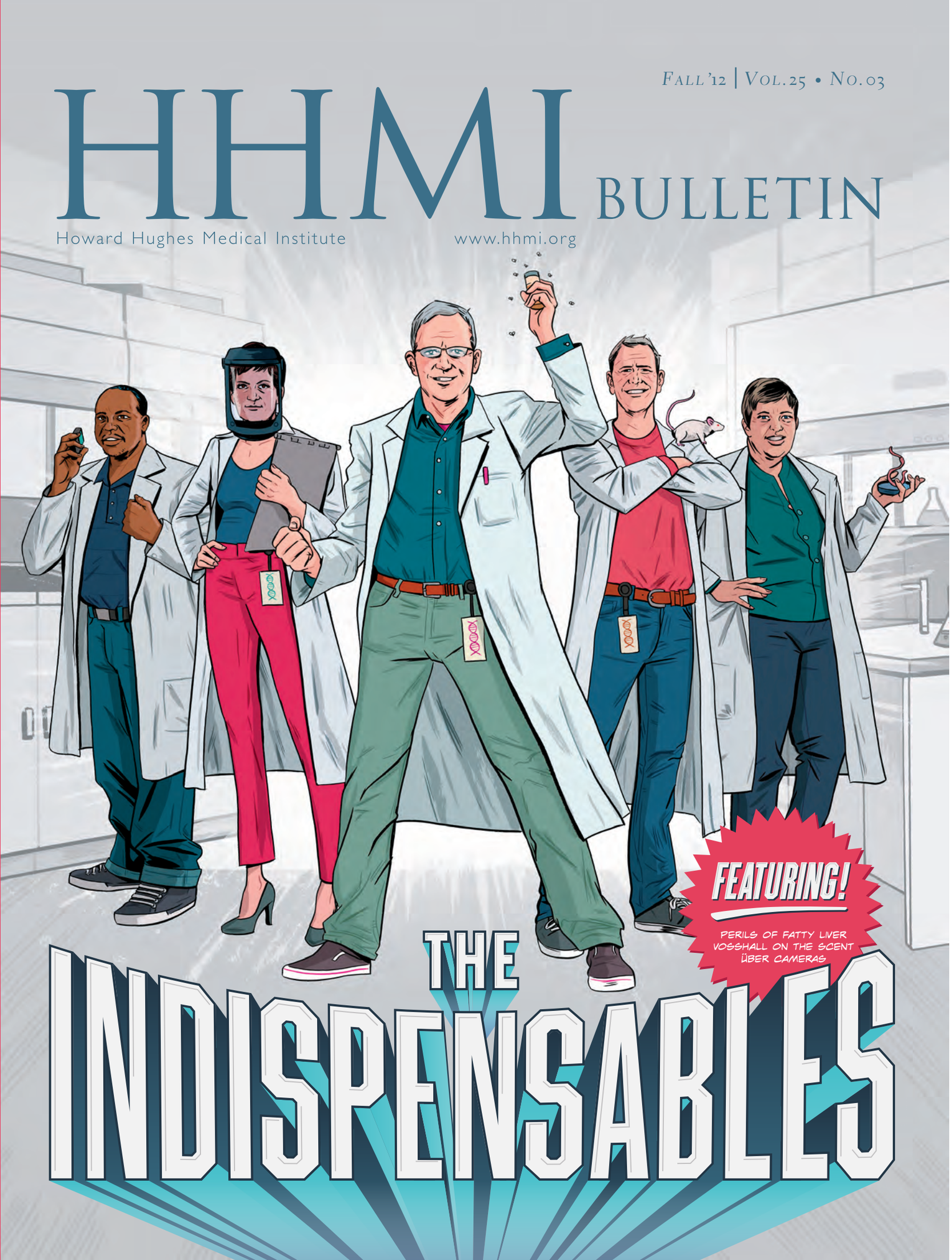
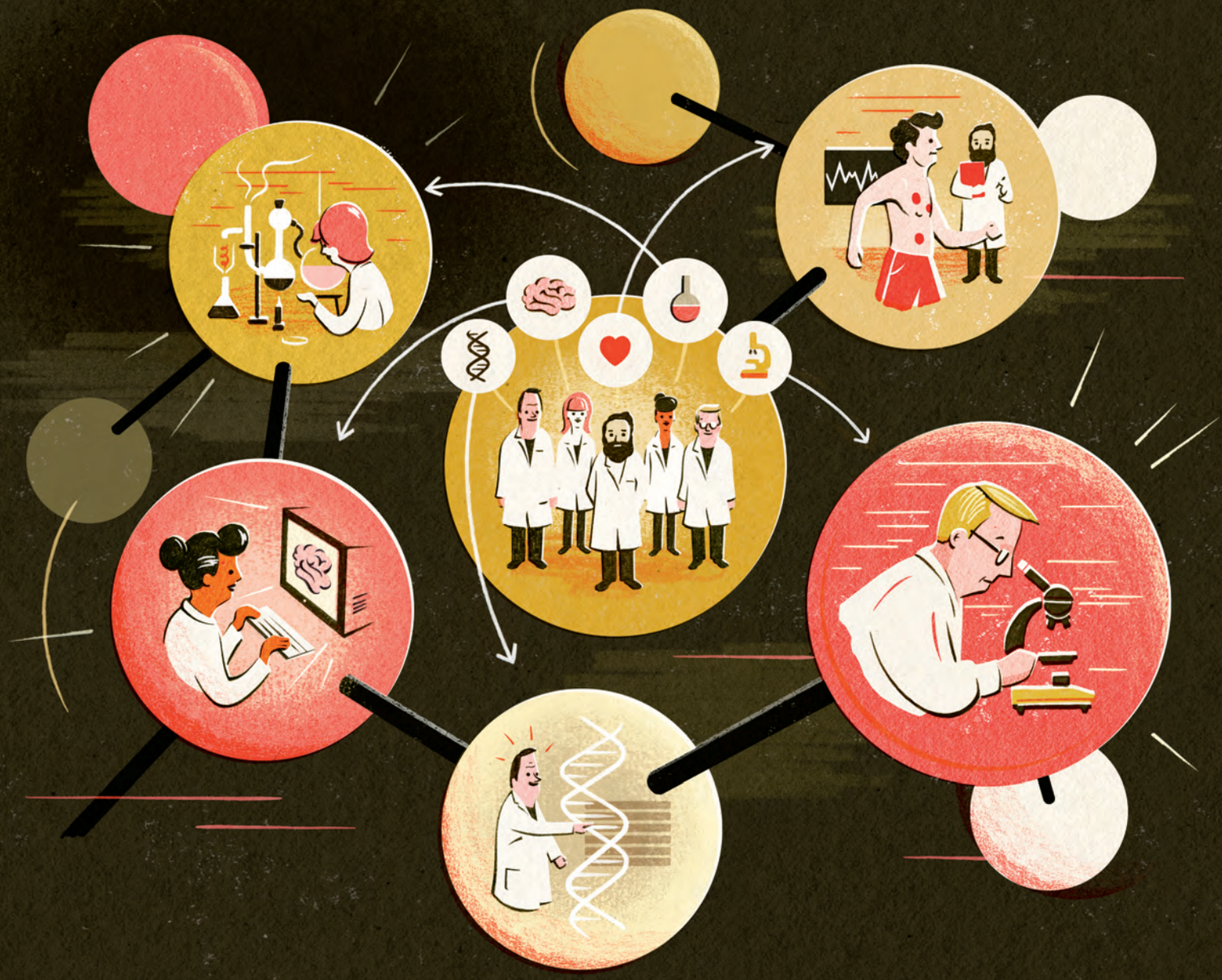
FALL '12 | VOL. 25 • NO. 03

HHMI BULLETIN

Howard Hughes Medical Institute www.hhmi.org





Owen Gatley

THE TRAINING PIPELINE

To prepare and retain a topnotch, diverse scientific workforce, trainees should be introduced to multiple career paths, according to a June report by the NIH Biomedical Workforce Working Group. One of those options—the staff scientist—could use a boost. Co-chaired by Princeton University President Shirley Tilghman (see "The Future of Science," *HHMI Bulletin*, May 2011) and Sally Rockey, NIH deputy director for extramural research, the working group notes a decline in biomedical Ph.D.s in tenure-track faculty positions, and suggests modifying training programs to include diverse experiences relevant to the careers they are likely to find. HHMI investigators on the 14-member committee included Leemor Joshua-Torr and Richard Lifton.

The typical academic laboratory consists of a PI and one or a small number of permanent technical staff, with the majority of the research carried out by trainees. This creates a system in which a large number of future scientists are being produced each year, well in excess of the number of research-oriented jobs in academia, government and industry. The working group believes that even a modest change in the ratio of permanent staff to trainees could have a beneficial effect on the system without reducing the productivity of the research enterprise. Staff scientists—individuals with master's or Ph.D. degrees—could play a more important role in biomedical research (one that may become increasingly necessary if the market for biomedical researchers strengthens outside of the United States in coming years).

Today, these scientists bring stability to many labs and provide important functions as part of institutional core facilities, but have a wide variety of titles and employment conditions. As an example, staff scientists constitute an essential part of the NIH intramural research program. In the extramural program, these scientists do not apply for their own grants, but are supported by research project, Center and Program Project grants. They should be differentiated from "soft money" scientists, whose employment depends upon their successful competition for research funds, a category that has been increasing over the last few years.

The working group encourages NIH study sections to be receptive to grant applications that include staff scientists and urges institutions to create position categories that reflect the value and stature of these researchers.

Excerpted from *Biomedical Workforce Working Group Report*, A Working Group of the Advisory Committee to the Director, National Institutes of Health, June 14, 2012.

FALL '12 VOL. 25 · NO. 03

12

18

24

30

Web-Only Content

- Meet some of the behind-the-scenes specialists who help modern science get done.
- Turtles and snakes and frogs, oh my! View the critters discovered during a herpetological survey at Janelia Farm.
- Read a Q&A with computer scientist Hanchuan Peng on developing sophisticated ways to make sense of biological images.
- Watch a fruit fly embryo develop and follow a dragonfly as it captures its prey, thanks to the latest developments in visualization techniques.
- Learn about the technology that lets researchers watch a heart grow, one vibrant cell at a time.

GO GREEN

Prefer to receive your *Bulletin* digitally? Go to hhmi.org/bulletin/subscribe.

Features

Departments

WHERE'S MY HHMI BULLETIN?

Something's different, all right. You didn't receive your August *HHMI Bulletin*. Not to worry; it just means that we're going to a new publication schedule. Beginning with this issue, the *Bulletin* will be published three times a year, rather than quarterly. The issues—called Fall, Winter, and Spring—will come out in September, January, and May, respectively.

Publication of the web and iPad editions of the *Bulletin* will follow the same schedule, with all the vibrant multimedia, interactive elements, and extra content you've come to expect. During the summer months, when many of you are busy with family and travel, we plan to make regular updates to our website with fresh content.

We hope the new publication schedule will suit you, our readers, as we move forward in an increasingly digital age. And we hope that with this change we are lessening the impact on the planet, in at least a small way. While many readers tell us they prefer the look and feel of the print *Bulletin*, others have wholly embraced the web or iPad editions. Whatever your preference, we are committed to continue to bring you stories of scientific discovery.

We hope, too, that you enjoy this issue, particularly our cover story on "the indispensables"—those research specialists who are critical to the success of myriad laboratory research programs. Having spent many years working at the bench, both in university medical centers and industry, I know just how essential these individuals can be. Though some shy away from the limelight, we take a moment to shine a light, however briefly, on the talents and contributions of a few of HHMI's lab heroes—in this print issue and on our website. We hope their stories will inspire future generations to follow in their footsteps.

HHMI BULLETIN STAFF

Mary Beth Gardiner / *Editor*
Cori Vanchieri / *Story Editor*
Jim Keeley / *Science Editor*
Nicole Kresge / *Assistant Editor*
Maya Pines / *Contributing Editor*

ADDITIONAL CONTRIBUTORS

Michelle Cissell, Mark Farrell, Lyn Garrity, Heather McDonald, Anne Sommer
VSA Partners, NYC / *Concept & Design*
Finlay Printing / *Printing & Binding*

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

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

Paul Fetters

Stabilizing Forces

James Kegley

WALK INTO ANY HIGH-LEVEL RESEARCH LAB TODAY AND YOU WILL find a few special individuals who are critical to that lab's success. Chances are they've been a fixture in the lab for a while. Some of the very best research associates, research specialists, and lab managers are in permanent jobs. When you land a really good one—we refer to them as "the indispensables" in this issue of the *Bulletin*—you feel very lucky because they are hard to find. And they are often the glue that holds the lab together.

In research labs, graduate students and postdocs are transient. It's the nature of the system. They float in an out at a pretty rapid clip, moving on after about four to six years. On one hand, having that ebb and flow of trainees is good—essential even—as it's a driving force for innovation and creativity. But at the same time, you need a core group to serve as the institutional memory of the lab, people who understand how we got where we are and why we are doing things a certain way—the progression, if you will.

If a postdoc who develops a new methodology leaves, the lab head can feel like he or she has to start over again. That information can get lost if there is a gap between postdocs. With a few key long-term people in place, however, the postdoc can transfer that knowledge and expertise to them. And these staff scientists are very high level, many with a master's, a Ph.D., and often even postdoctoral experience themselves. So they are extremely important for the continuity of the lab.

In my own lab at the University of California, Berkeley, there are a handful of people without whom the lab would not function. One is my lab manager, Mallory Haggart. Mallory has kept the lab running for over 12 years. She coordinates all lab activities and takes care of all the supplies, equipment, and chemicals, as well as repairs. A little older than the students and postdocs, she's the "mother hen" of the lab who helps establish the lab's culture and atmosphere.

Then there are our experts in specific technical areas. Carla Inouye, a biochemist, practices what is becoming a dying art: when a postdoc or student comes in and has to purify a protein, she's the one who shows them how to do it. She's been with me since 1998 and is fantastically valuable as the lab's memory. Same for Shuang Zheng, who is in charge of cell culture—everyone in the lab relies on his skills in that arena. And Gina Dailey is our expert in the complicated molecular genetics, all the cloning and sequencing.

These extremely skilled specialists are the stabilizing forces of the lab. Because graduate students and postdocs are trying to climb the academic ladder, they can be competitive, with sometimes-sharp elbows. You really need a small group of stalwarts to manage the whole thing.

Relative to 25 or 30 years ago, the composition of a high-performing research lab has shifted. There are more of these backbone employees on staff these days. At HHMI's Janelia Farm Research Campus, the ratio of permanent staff is somewhat higher than in most organizations. Janelia has certain project teams where a scientist is not, classically speaking, a principal investigator. Instead, that person is there to provide very high-level service, such as creating transgenic animal models or performing cryo-electron microscopy. It's a different model. And those scientists don't feel at all that they are not appreciated. They know the work they are doing is highly regarded and valued.

> "Recognizing the role of research professionals in today's laboratory organizations is important not only to the individuals who contribute their services but also to the research enterprise as a whole."
>
> ROBERT TJIAN

Recognizing the role of research professionals in today's laboratory organizations is important not only to the individuals who contribute their services but also to the research enterprise as a whole. To that end, it is notable that the National Institutes of Health (NIH) has just released its "Biomedical Research Workforce Working Group Draft Report," which makes recommendations for actions that NIH should take to support a future sustainable biomedical research infrastructure (see Observations, inside back cover). The report draws attention to the increasingly important and beneficial effect that staff scientists have on the system as a whole.

The message is clear: these professional scientists are remarkably valuable to the progress of research. They are not looking for a fancy title or a bookshelf of awards—that's not their motivation. They are no less effective as scientists; they're just a different kind of scientist. They have our support, and they have our respect and appreciation.

Good Stewards

Spring is lively in the woods surrounding HHMI's Janelia Farm Research Campus in Ashburn, Virginia. White-tailed deer rustle fallen leaves, pileated woodpeckers drum persistently overhead, and gray tree frogs trill in the treetops. But on one cloudy May afternoon, the sounds of nature are muffled by the cries of discovery. "Hey, Larry," comes the call, "I found something!"

A team of more than 20 explorers—Janelia Farm employees and their nature-loving recruits—are combing the terrain: rolling aside logs, prodding leaf piles, and gently displacing stones. The reptiles and amphibians they seek out do little to announce themselves, but this is an observant group, and the finds come quickly.

"Hey, Larry, is this another worm snake?" someone asks as an energetic wriggle of pink and gray catches his eye. "It's amazing what you find if you just flip things over." Another participant announces he's found a frog—a pickerel, he guesses, judging by the spotty skin. Moments later, a salamander sighting evokes a flurry of activity as a group peers into the stream waiting for its yellow tail to reappear.

Larry Mendoza, Janelia Farm's biosafety and laser safety officer, scurries to each find. Mendoza is president of the Virginia Herpetological Society and a co-founder of Janelia's Nature Club, through which he has gathered this crew for the second in a series of herpetological surveys of the Janelia Farm grounds.

Reptiles and amphibians are particularly sensitive to environmental pressures, so monitoring the snakes, lizards, frogs, salamanders, and turtles that inhabit this land helps reveal the health of the local ecosystem, Mendoza explains. As future surveyors begin to track population changes over time, the data will become even more valuable. Just as important, adds Mendoza, is ensuring the event is educational and fun.

Many of the scientists on the excursion have come recently to Janelia Farm and want to learn more about the local ecology. "We had very few reptiles of any kind in Seattle," observes Matthew Iadanza, a member of Tamir Gonen's structural biology lab, which relocated from the University of Washington last year. Others are here hoping to foster their children's curiosity, and some of the day's best finds—a slender water snake, an unusually vibrant box turtle—come from the group's youngest naturalists. "I walked right by that turtle," Mendoza says. "That's why you've always got to bring kids!"

After three and a half hours, the team has located 33 animals representing 11 different species, and Mendoza declares the survey a success. He is particularly excited by the 15 eastern box turtles. The species may be threatened in Virginia because of loss of habitat, and Mendoza says their prevalence indicates good stewardship of this land.

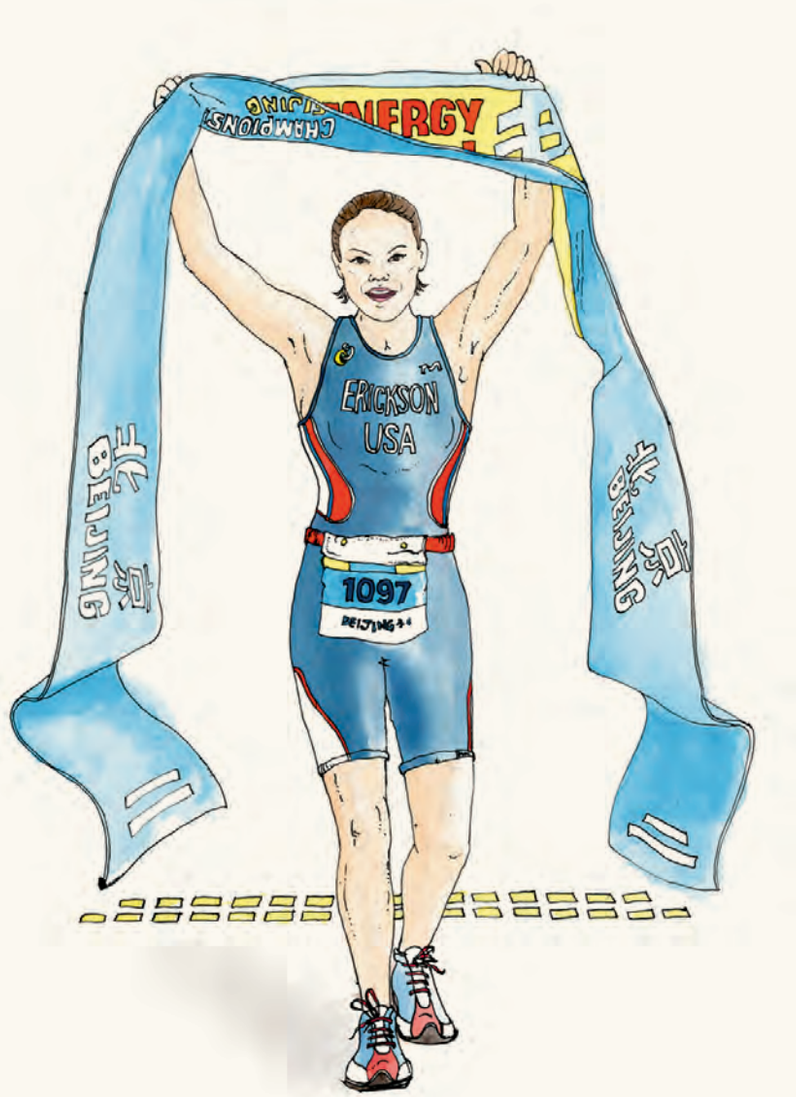
Mendoza has recorded the location, time, and discoverer of each find for inclusion in the Herpetological Society's biannual publication, *Catesbeiana*. All participants will be asked to review the manuscript, he tells the group. As they disband, he reminds the team of upcoming Nature Club events—more herpetological surveys, bird walks, and perhaps a tour of insect life led by a resident entomologist. "We have 689 acres here at Janelia," he says. "That's a lot of land—a lot of opportunity."

—Jennifer Michalowski

WEB EXTRA: For a sampling of what the naturalists found during their outing, see the audio slideshow at www.hhmi.org/bulletin/fall2012.

Photo of Larry Mendoza by Adam Auel

Undercover Triathlete



Emma Kelly

When Erika Erickson's California lab mates heard that she was just back from a triathlon competition in Beijing—where she earned gold in her age group—most were shocked. They knew she worked unusual hours for a graduate student, with lots of very early mornings and late nights, but they had no idea how she was spending her daytime hours.

Her graduate adviser, HHMI-GBMF investigator Krishna K. Niyogi, knew what she was up to but can see how it might have been a surprise to others. "She's pretty low-key about it," he says. "She'll really only tell you how it went if you ask her."

Erickson ran track and swam competitively as an undergraduate at the Massachusetts Institute of Technology but hadn't tried triathlons until she moved to the University of California, Berkeley, for graduate study in plant biology.

Two and a half years later, she has earned professional ranking in the sport, which means she can compete for prizes of $5,000 or more in events sanctioned by the sport's main association, USA Triathlon. She trains 15 to 25 hours per week and fits in her research around workouts. "I don't keep standard hours," she laughs, saying it's understandable that her lab mates were mystified by her trek to China. "And I don't talk about my personal life too often in lab; I don't want to give the impression that science is my second priority."

Along with training and travel to races, Erickson makes sure that her research on the structure and function of proteins involved in photosynthesis progresses at a reasonable pace. She says the dual focus gives her a bit of stability. "If I have a bad week in lab, my training can balance that out. Or if I have a bad week racing, a good week in lab can make up for it."

In her first professional race, the June 2 Pan American Cup in Dallas, her focus paid off. She placed 8th on the 1,500-meter swim, 40-kilometer bike, 10-kilometer run. Her time of 2:19:03 was just 8 minutes, 41 seconds behind that of the leader, Laura Bennett, who represented the U.S. at the Olympics this summer.

"The race went well overall, but the conditions were incredibly challenging—95 degrees and windy," she says. And her competitors were very different from her usual running mates.

"People weren't at the Dallas race just to see if they could finish or just to have fun." They were there for national pride, to win money and points in the ranking system. Those goals, she says, "bring an entirely different set of emotional responses to a race. I was really intimidated by my competitors. But I had fun and learned a lot."

As for her lab mates, their only first-hand experience with Erickson's racing talents came from a 2-mile run around the Lawrence Berkeley National Laboratory. Niyogi offered to buy lunch for anyone in the lab who would participate with him or watch the race. "Several of us went, and Erika was just way, way out in front of all of us," he says laughing. "She won the women's division. I don't think anybody was too surprised."

—Rabiya Tuma

Stepping into Sustainability

Peter Baumann will not go hungry if the local supermarket shuts down tomorrow. In fact, if all food production in the United States ground to a halt, he would probably be just fine.

Baumann is an HHMI early career scientist at the Stowers Institute for Medical Research in Kansas City, Missouri. In his spare time he hunts, grows, raises, and gathers almost 80 percent of the food that he and his wife, Diana, and their two dogs consume.

The couple lives on 5 acres of land scattered with fruit trees, berry bushes, a large vegetable garden, and a pond stocked with bass and catfish. They have three freezers crammed with venison and chicken; a cellar overflowing with squash, potatoes, jams, and canned vegetables; and a coop of egg-laying chickens.

"It sort of happened gradually," says Baumann of his family's foray into sustainable living.

Baumann was lured by the memory of the taste of fresh vegetables grown in his mother's extensive garden at their home in Germany. Diana's childhood gardening memories, on the other hand, are limited to the few radishes her brother managed to coax from the ground in England. When the couple moved to a house in the countryside six years ago, they planted a few tomato seeds in pots on the patio. Unfortunately, raccoons got to the harvest before they could.

The next year the Baumanns planted a small garden in their yard and adopted a dog to keep the raccoons away. It worked, and a year later they expanded: the garden got bigger and a second dog joined the family. "It really took off," Baumann says. Today, the garden occupies a little more than half an acre and is home to a variety of beans, tomatoes, peppers, greens, squash, and herbs.

What Baumann doesn't grow he gathers, which he learned to do during weeks-long hikes in Europe and America. He's been known to supplement his diet with unconventional items, such as the inner bark of a birch tree, which makes a tasty pasta substitute. His current foraging, however, is limited to mushrooms, cattails, pawpaws, and berries. "The inner shoots of the cattails are very similar to bamboo shoots, so you can put them in stir-fries or salads," he says.

For meat, Baumann kills about five deer each fall, which he and Diana butcher together. They use all the meat, keeping the good cuts for roasts and stews and grinding up the rest for the dogs, who also get the hoofs for chew toys.

Chickens were a late addition to Baumann's small-scale farm. It took some time for Diana to agree, but she came around and they mail-ordered 27 chicks in February 2011. This year, they added 47 more chicks and 16 ducks, which roam around a half-acre enclosure. Some of the chickens end up on the dinner table and the rest produce about 40 to 50 eggs per week.

Baumann is ready to add goat meat and milk to the family's diet. "We have the space and the ideal habitat for them," he explains. Since Diana is now a pro at butchering deer and plucking chickens, he expects that something as innocuous as a few goats wandering the property won't faze her at all.

—Nicole Kresge

WEB EXTRA: View a slideshow of Peter Baumann's small-scale farm at www.hhmi.org/bulletin/Fall2012.

Emma Kelly

upfront

WEB ONLY CONTENT

SAME BUT NOT EQUAL
A code within the genetic code explains why identical proteins are produced at varying speeds.

NERVE TONIC
Axon degeneration—an active process after injury—can be delayed.

Find these stories at *www.hhmi.org/bulletin/fall2012*.

To work on broad and basic problems that touch on many different fields has been Jonathan Weissman's goal. He made a recent leap to understand the nuances of protein production with encouragement from two stellar scientists. It's a gift when one generation of researcher can lean on the one that came before—perhaps through their papers and lab notes or, even better, by chatting over a meal. Go to the online *Bulletin* to learn about Weissman's inspirations.

Push and Pull

What appears chaotic is actually a well-orchestrated process for embryonic head-to-tail elongation.

FEW SIGHTS ARE AS CAPTIVATING AS THE MOVEMENTS OF A DEVELOPING embryo. Shapeless tissue remodels itself as cells migrate en masse to form elongated structures that, depending on the species, will become a frog spinal cord, a human gut, or the main body of a fly. ¶ Jennifer Zallen, an HHMI early career scientist at Memorial Sloan-Kettering Cancer Center, studies how embryonic tissues stretch along an anterior-posterior axis using the fruit fly *Drosophila melanogaster* as a model system. Shortly after formation of the embryo, the mass of fruit fly cells dramatically elongates over about a two-hour period and establishes a head at one end and a tail at the other, a process known as convergent extension.

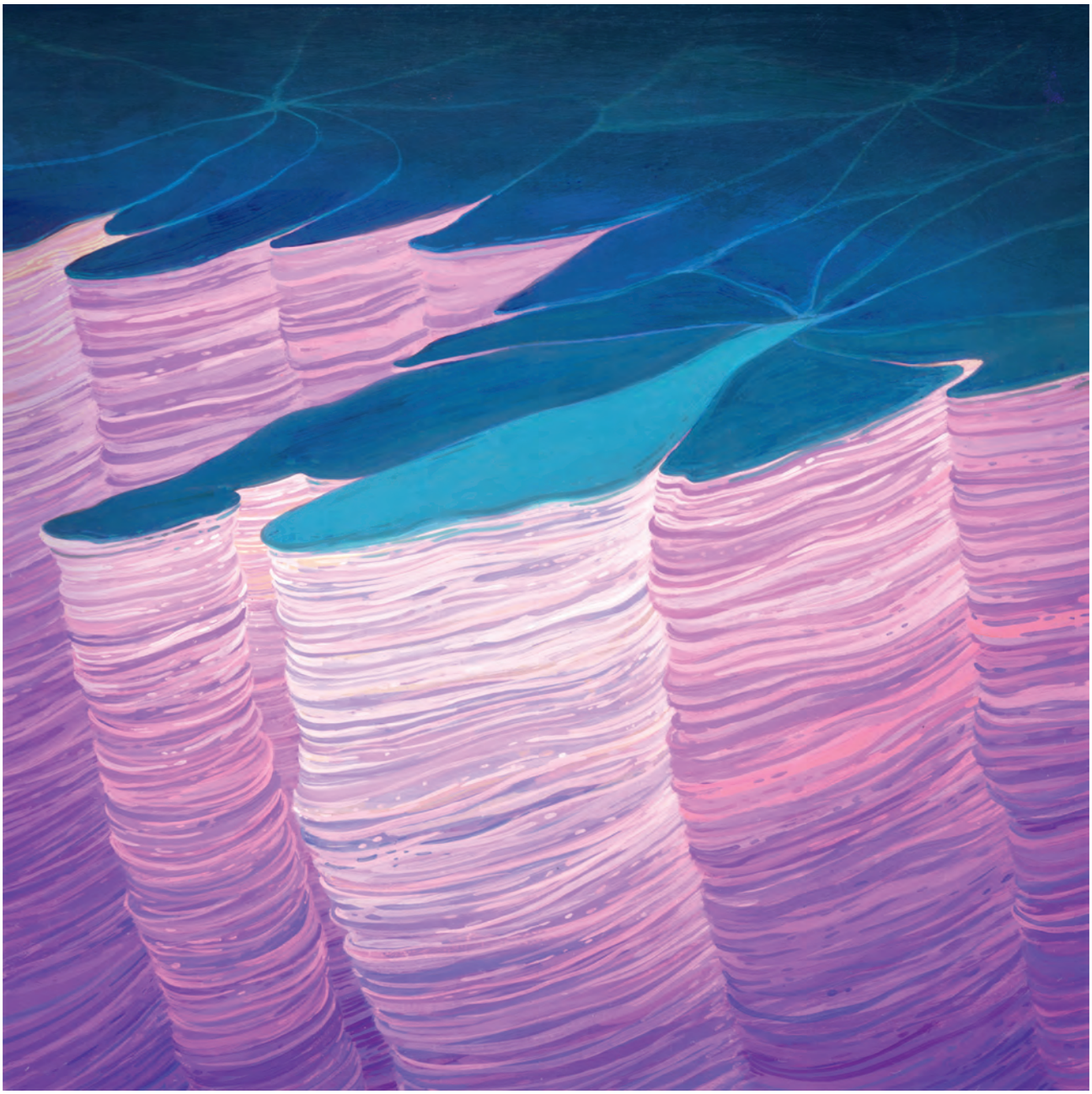
Over the past seven years, Zallen's lab has used high-resolution, time-lapse imaging to track embryonic cell migration during that two-hour window. She has found that what looks like chaotic pushing and shoving between neighboring cells in elongating tissue is actually a highly cooperative and orderly process.

Zallen's group established the ground rules of the game in a 2006 *Developmental Cell* paper. Tracking single cells in a living embryo, they showed that cells consistently join and then exit pinwheel-like structures known as rosettes as a fly embryo becomes longer and thinner. Computational analysis indicated that most cells repeatedly move in and out of multiple 5-8 cell clusters as the embryo stretches outward. Each group of cells reorganizes to become longer and narrower, suggesting that rosette formation could drive morphological change.

Since then, her laboratory has focused on understanding the multiple signals that encourage cells to move in and out of transient groups. "It is forces generated at the contacts between cells that cause the cells to rearrange and the tissue to elongate," says Zallen. "These forces place special demands on the cell junctions, which must be dynamic enough to dismantle individual contacts but strong enough to prevent the group from ripping apart under tension."

Zallen's lab group recently combined molecular techniques with *Drosophila* genetics to characterize one facet of that junctional regulation. In work published February 14, 2012, in *Developmental Cell*, they identified a biochemical cue that allows cells to disengage from some neighbors in a rosette so they can find new ones. They found that a signaling factor, the tyrosine kinase protein Abl, makes the contact points (known as adherens junctions) between cells in a rosette more dynamic or fluid—in other words, it allows cells to glide more smoothly against one another. Fruit fly embryos genetically engineered to lack Abl show poor rosette formation and impaired elongation. Surprisingly, the more dynamic junctions in normal embryos are also stronger, resisting apparent breaks or tears seen in embryos lacking Abl.

Equally essential for axis formation are mechanical signals that rope cells into a rosette. In work published in *Developmental Cell* in 2009, Zallen reported that cells perceive physical tension exerted by their



Brendan Monroe

neighbors. These mechanical signals promote rosette cohesion by prompting the motor protein myosin to join long cables that extend across multiple cells and contract like a drawstring, pulling cells together to form a rosette.

One way her group showed this experimentally was by literally tugging on a *Drosophila* embryo with a glass needle and then using live imaging to watch as fluorescently labeled myosin was recruited to the needle. This experiment helped explain one purpose that mechanical forces serve, namely, to bring cells together into multicellular gatherings.

Whether the same mechanisms that push and pull cells in a simple organism like *Drosophila* drive convergent extension in vertebrate embryos remains unknown. "Right now, people have seen snapshots of something that looks like rosettes in other animals," she says, noting that the vertebrate neural tube (the embryonic structure that gives rise to the central nervous system) exhibits pinwheel-like swirls of cells. "But rosette formation is a dynamic behavior that is difficult to assess in a static picture. Live imaging of vertebrate cells is needed to see if these cells move in a way that leads to elongation."

Even if she and others discover that vertebrates evolved a different way to form tubular organs, Zallen has an ambitious long-term goal: to figure out how large populations of cells act collectively. "Over the past 20 years, people have learned a lot about factors that determine cell identity," she says. "But we know much less about how cells get to the right place to build a three-dimensional animal. This is the big unsolved question in developmental biology." ■ – ELISE LAMAR

WEB EXTRA: See a video of rosette formation in *Drosophila* at www.hhmi.org/bulletin/Fall2012.

Cancer's Dead End

There is a way to restore p53's tumor-suppressing prowess.

AT FIRST GLANCE, THE TUMOR SUPPRESSOR GENE *P53* WOULD SEEM like an ideal weapon against cancer. It puts the brakes on cancer progression and is impaired in about half of human tumors. ¶ In mouse models of cancer, HHMI investigators Tyler Jacks and Scott Lowe restored p53 activity in tumors and the tumors regressed.

However, p53 activation kills some cancer cells, but not others, and no one knows why. HHMI early career scientist Joaquín Espinosa has set his sights on finding an answer, and with it, a strategy for making p53-based therapies effective. It's a goal he's pursued with relentless passion.

"He's an extremely creative thinker," says former HHMI President Thomas Cech, who is an HHMI investigator and colleague of Espinosa's at the University of Colorado at Boulder. Where a traditional approach might focus entirely on the *p53* gene itself, Espinosa has adopted a broader and far more ambitious target—he aims to inventory all the genes and pathways that govern how cells react to p53 activation.

Espinosa's approach grew from his realization that if you want to harness p53's tumor-squelching effects, you also need to block the myriad pathways that tumors use to circumvent p53's killing orders. Espinosa sought to identify all of the p53-disarming pathways and the genes that control them with a technique called functional genomics. "This is a very new technology which allows us to interrogate the function of every gene in the human genome as it relates to p53," Espinosa says. "With functional genomics, we can ask—which genes influence the programmed cell death mediated by p53?"

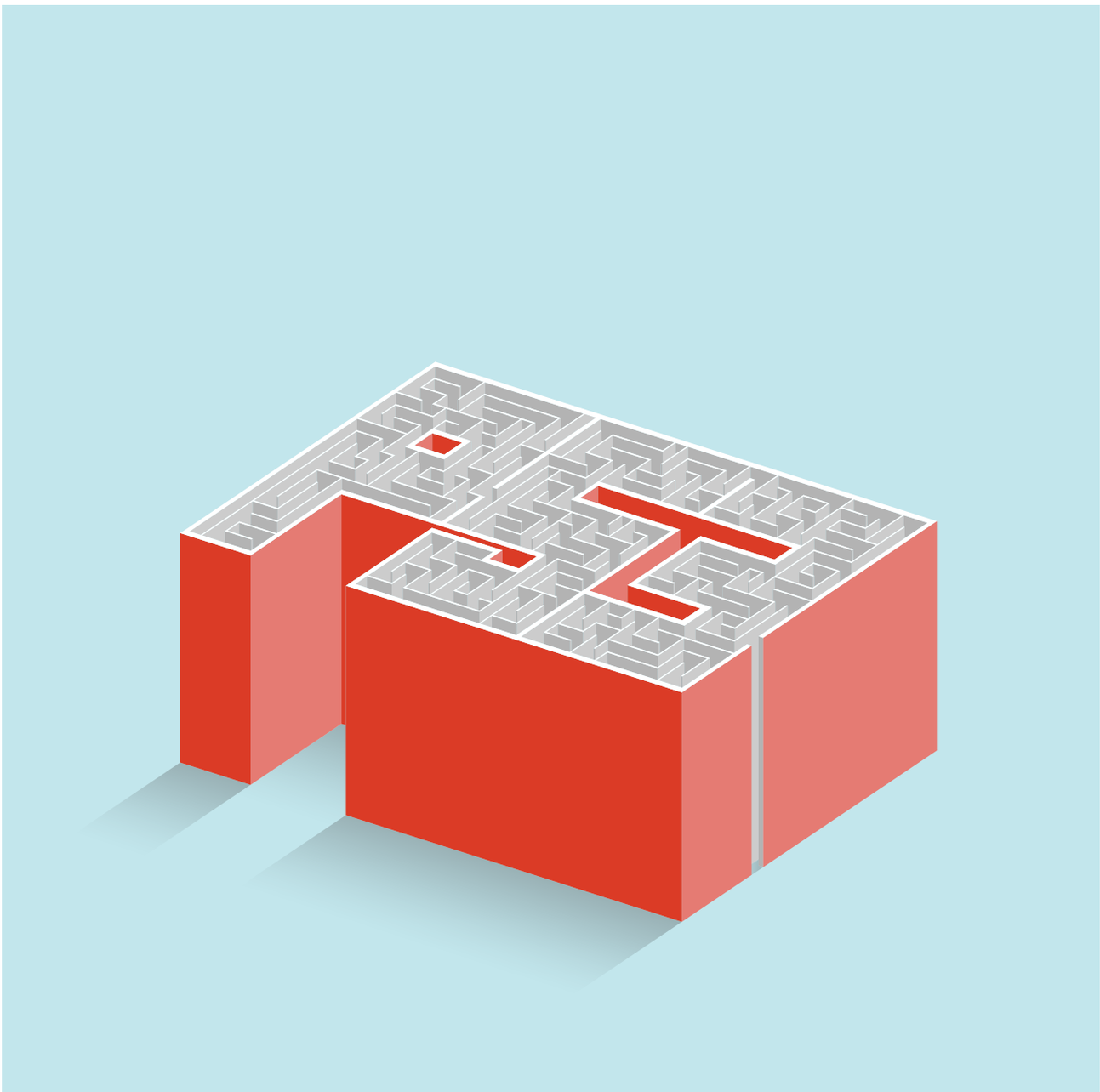
To do functional genomics requires expensive equipment and huge libraries of costly reagents, and only a few such facilities exist in the world. Espinosa wanted one at the University of Colorado at Boulder. He had a vision for a functional genomics facility in Colorado that would not only advance his own research, but also put his colleagues at the forefront of biomedical research. "Joaquín enjoys doing things for the benefit of the scientific community," Cech says. "He's more than a team player, he's an instigator of teamwork."

Espinosa's first proposals to fund his functional genomics experiments were rejected by the National Institutes of Health and private foundations. But he persisted, eventually securing support from HHMI, the BioFrontiers Institute, and the University of Colorado Cancer Center. The Functional Genomics Facility at the University of Colorado at Boulder began operations in May 2010 with Espinosa at its helm. "We could finally do our dream experiment," Espinosa says.

In a series of investigations published online June 3, 2012, in *Nature Chemical Biology*, Espinosa's group identified genes that, when inhibited, allow p53 to kill cancer cell types that wouldn't otherwise respond. They accomplished this using an experimental drug called Nutlin-3, which activates the p53 protein, yet fails to induce cell death in most tumors. Using functional genomics, Espinosa's team screened thousands of genes and identified a few hundred whose inhibition made Nutlin-3 effective at killing cancer cells. To their delight, they discovered that compounds to inhibit the protein products of two of these genes, *ATM* and *MET*, were already available.

When they combined the ATM or MET inhibitors with Nutlin-3, the treatment destroyed cancer cells that didn't respond to any of the drugs individually. "If you treat cancer cells with one of the drugs, nothing happens, but if you treat them with both drugs, the drugs kill them," Espinosa says. The drug combination delivered a one-two punch—Nutlin-3 turned on *p53*, then the ATM or MET inhibitor blocked

VSA Partners



the proteins that would allow the cancer cell to thrive despite *p53* activation. "With one genetic screen, we identified a combination of drugs that will kill tumors that otherwise resist them," Espinosa says. His team is collaborating with clinical investigators to test these drug combinations in animal models and human tumors grown in mice.

By hitting multiple genetic targets, Espinosa hopes to drive cancer cells into a dead end. "You can block resistance genes before they ever have a chance," he says. With this strategy, new treatments can be designed to overcome a tumor's natural work arounds. "The future is not in isolated drugs, but rather in combinations of drugs," Espinosa says.

Clinical trials are costly and time-consuming, and Espinosa hopes his studies can help drug researchers predict and anticipate how their p53-related treatments will work in the clinic, long before they've spent millions on trials. "These are very basic experiments we can do in the lab to get ahead of problems we'll see in clinic."

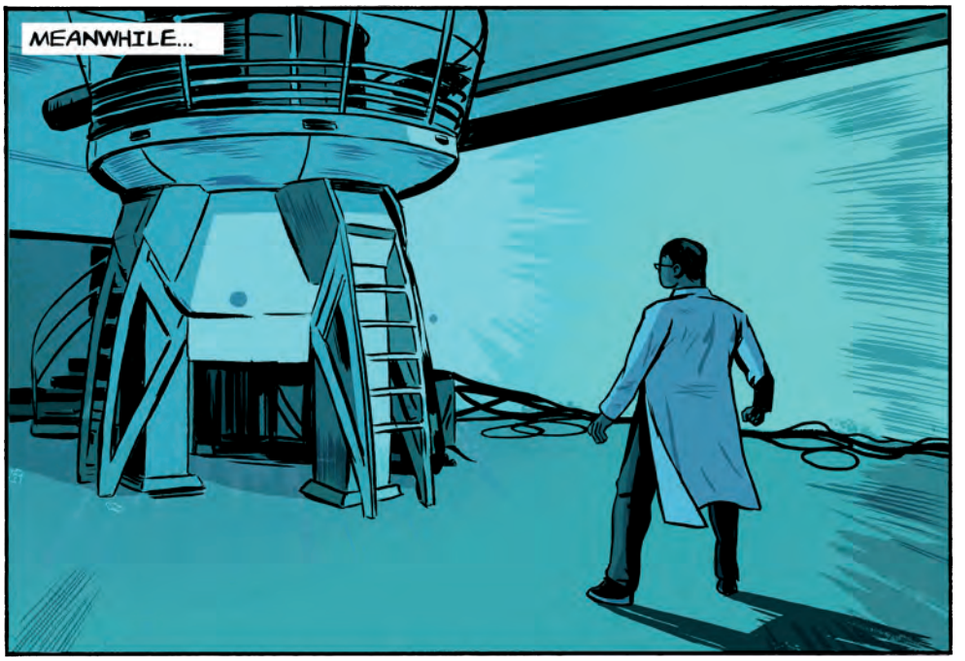
■ –CHRISTIE ASCHWANDEN

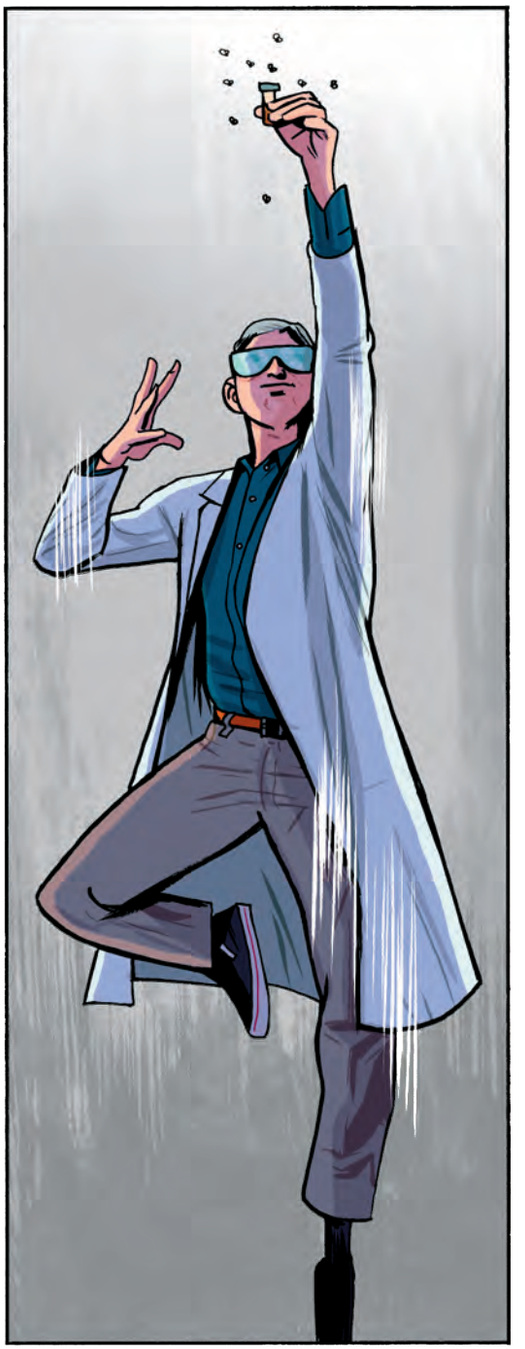
THE INDISPENSABLES

THE BACKBONE OF MOST LABS, THESE QUIET HEROES DO IT ALL AND THEN SOME.

WRITTEN BY
MADELINE DREXLER

ILLUSTRATED BY
CHRIS KING


MEANWHILE...



PROBLEM SOLVERS, SHELF STOCKERS, BENCH SCIENTISTS, RECORD KEEPERS, MACHINE FIXERS, WEEKEND WARRIORS, DEN MOTHERS, OLD HANDS, FRESH EYES, MENTORS, MANAGERS. EVERY RESEARCH LAB HAS BEHIND-THE-SCENES SPECIALISTS WITHOUT WHOM MODERN SCIENCE COULD NOT GET DONE.

Their bland bureaucratic titles—research technician, resource manager—belie their vital contributions. These women and men are not wannabe principal investigators (PIs) or discouraged academics. Some have specialized expertise; others are jacks-of-all-trades. They are ambitious, not for fame, but for personal excellence and the chance to make a lasting contribution, however unheralded, to science.

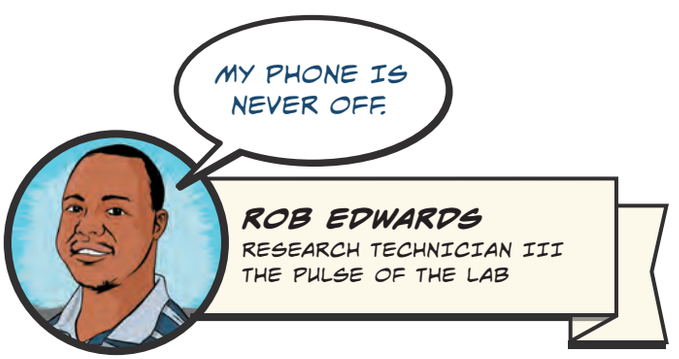
"There are very few laboratories that do not have at least one technician who often plays the role of lab manager, making sure the trains run on time, the supplies are ordered, the equipment gets repaired. It would be very hard to run a lab without such a person," says Shirley Tilghman, a molecular geneticist and president of Princeton University.

It's a career option that needs serious consideration, according to Tilghman, who cochaired the National Institutes of Health Biomedical Research Workforce Working Group. The group's June report calls for professionalizing the crucial research-affiliated roles of lab specialists and paying them accordingly. "Make them a professional category that academic medical centers and research institutes and universities recognize as valuable—in fact, invaluable—parts of the biomedical workforce."

A little recognition wouldn't hurt, either. "As in most areas, credit flows up the hierarchy," explains Gerry Rubin, director of HHMI's Janelia Farm Research Campus, in Ashburn, Virginia. "The people at the top get way too much credit. And the technicians and managers don't get enough. Academia very much rewards individual achievement—less so, team achievement."

Adds Rubin, "Labs are full of students and postdocs who come and go every three to four years. It's important to have somebody with a sense of continuity and context."

Here are the stories of five indispensable lab team members, among many acknowledged by grateful HHMI investigators.



Seventeen years ago, Rob Edwards noticed an ad for a radio frequency engineer in the HIV-focused laboratory of HHMI investigator Michael Summers. On paper, Edwards may have

seemed an odd fit for the lab: no academic experience, just lots of work for defense contractors. "I don't want to say this, but my old job depended on war—and sometimes, on destroying people's lives," he says. Today, after nearly two decades working alongside Summers, Edwards' career has been recast. "What we do here saves people's lives. It has a purpose."

At the University of Maryland, Baltimore County, Summers and his team are studying the architecture of HIV to understand how it and other retroviruses assemble, and how the viruses package their genetic material to infect other cells. This scientific pursuit hinges on nuclear magnetic resonance (NMR) spectroscopy—which is where Edwards enters the picture. He is responsible for two 600-megahertz NMR machines and a mammoth 800-megahertz instrument with a cryogenic probe. "There are so many things that can go wrong," he says. "The magnet, the probe, the platform, the software, the console, the amplifiers."

But NMR machines are just part of Edwards' job. He designs the electrical and mechanical components of laboratory upgrades and ensures the continuous operation of centrifuges, water purification systems, electrical generators, and other critical research tools. "I keep everything up and running, because you never know when a grad student or a postdoc will have that sample—the protein they've been working on for a year," Edwards says. "When they're ready to roll, there can't be any obstacles on the instrument side."

Edwards also mentors young scientists in Summers' lab—including a large number of minority grad students and postdocs, who may not have received ample encouragement in the past. "He has served as a positive force for about 50 high-achieving minority students who have worked in my laboratory," says Summers, "often privately enforcing his own interventions to challenge the students and support them during times of academic struggles."

As Edwards explains, "I'm a minority also; I know you can get down on yourself. So I always say, 'I believe in you.' That's what Mike said to me when I first started here: 'You can do it, I hired you for a reason, just be confident.' Now that I have that confidence, when I see someone without it, I try to instill it."

Edwards brings this sense of purpose to every facet of his job. "My phone is never off. My vacation time is maxed," he says with a laugh.

"Embryonic stem cells have to be tended and fed every day. They don't know about weekends or holidays," says Phil Smallwood, a research technician for 18 years in the laboratory of Jeremy Nathans, an HHMI investigator at the Johns Hopkins University School of Medicine who studies the mammalian visual system.

"When I'm actively working with the cell cultures, I'm here seven days a week. I have been here Christmas Day, New Year's Eve, New Year's Day," says Smallwood. "This is why Jeremy trusts me: he knows that it will get done and it will get done correctly. These cells are going to be turned into a mouse. I won't cut corners, because Jeremy is basing his reputation on the conclusions that he will draw from these mice."

"Extraordinary experimentalists" is how Nathans describes Smallwood and research specialist Yanshu Wang, another essential lab team member (see Web Extra, Lab Heroes). "They have the same relationship to doing experiments that Itzhak Perlman has to playing the violin."

For Smallwood, who has created 40–50 mouse lines in Nathan's lab—transgenics, knock-ins, knockouts—the musical analogy is apt: away from work, he is an accomplished flutist. "You always have to practice—you can't rest on your laurels. It's the same in science. You always have to keep learning new techniques, advancing with technology, or you'll get left behind."

Smallwood also draws on another analogy to explain the craft of science: cooking. "The first time you make a cake, you follow the recipe. It's the same with an experiment: you can't change a variable right away. But after doing it for many years, you get a feel for how the cell behaves, for what works and what doesn't. Take something as simple as pipetting an enzyme back and forth, such as in trypsinization, a process to chemically separate cells. I was originally taught that you add the trypsin once and place the colonies in the incubator. But the colonies don't completely break up. I found that if you pipette the trypsin back and forth a few times and then put the colonies in the incubator, it's like night and day. It's a little trick that goes a long way."

The co-inventor of 10 patents—beneficiary of Nathan's uncommon largesse in sharing professional recognition—Smallwood doesn't feel like an unsung hero. Yet while he appreciates the recognition, he doesn't crave it. "The students, they come in, find a project, get published, go on for a postdoc, look for a job. I'm content to be in the background. Jeremy arrives in the morning and there I am at my bench. I'm a constant. I'm with Jeremy for the long haul."

Among the many niche communities in the giant enterprise of science, worm experts are a special cadre. *Caenorhabditis elegans*—the slender and sinuous nematode—may be a model organism, but it also requires model experimental techniques. And in the small guild of *C. elegans* specialists, Peggy Kroll-Conner is a star.

Judith Kimble, an HHMI investigator at the University of Wisconsin–Madison who studies the fundamental controls of animal development, has for 16 years relied on Kroll-Conner's finesse. "Peggy is the heart and soul of all our genetics," says Kimble. "She has become the person who generates some of the most finicky strains in the lab. In the worm community, she is known as being exceptionally fastidious—her strains always have the correct genotype and, just as important, are not contaminated."

According to Kroll-Conner, the key to ensuring that frozen worm stocks are free of mold and bacteria is good sterile technique. She vigilantly labels items to prevent strain mix-ups. Once the worms are frozen, she thaws a portion to make sure the animals are viable and the stock pristine. She returns test thaws to the scientists who created the worms, so they can validate the strains. She also revalidates her own strains. "In other words," says Kroll-Conner, "we do a lot of quality control."

Alongside the ever-present threat of contamination is the challenge of fashioning strains with multiple mutations. "Mother Nature doesn't give up her secrets easily," says Kroll-Conner. "I try to figure out a way to balance the mutations in a user-friendly way, to minimize the work needed to maintain the worms." Certain mutations are more challenging than others, she says. In one experiment, she had to set up a mating with a strain of worms whose reproductive systems quickly developed tumors. "I set up numerous mating plates with large numbers of worms and ended up with only three cross-progeny. Only one of those had the genotype that I was looking for."

Such perseverance and attention to detail is essential in her role. It's also about mutual support, she says, a concept that seems to have been bred into the *C. elegans* world by Janelia Farm senior fellow Sydney Brenner, a molecular biologist who shared the 2002 Nobel Prize in Physiology or Medicine. "When Brenner started this science in the 1970s, he said that the worm community should work together in a spirit of cooperation. He felt that science would advance much faster that way," she says. "I like that philosophy."

"A lot of things go on behind the scenes to make science happen," says Todd Laverty, who for 29 years has supported the work of Gerry Rubin, director of HHMI's Janelia Farm Research Campus. For 23 of those years, Laverty managed Rubin's lab at the University of California, Berkeley. For the last six, he has overseen the *Drosophila* and Media Prep shared resources at Janelia. His specialty is feeding, sheltering, moving, breeding, and otherwise nurturing fruit flies, as well as preparing meals for other laboratory animals. "It was obvious, right from the beginning, that I had found my niche."

At Janelia, Laverty manages some 18,000 fly stocks—that is, 36,000 vials of flies, 50 to 100 flies per vial, each a unique genetic line. He shepherds the stocks through a 28-day lifecycle: egg, three larval stages, pupa, and hatched fly. Each morning, peering through a microscope at flies dozing on a pad suffused with carbon dioxide, he selects virgin females for genetic crosses. Each week, he starts new generations in fresh vials, using a duck feather to gently transfer the prospective parents from one plastic abode to the next. And each month, he manages the production of 130,000 vials of fly chow: a mixture of cornmeal, molasses, agar, and yeast—and gets four-star reviews from lab managers for quality and consistency.

"I can remember telling people, when I started out, 'What I'm going to be doing 20 or 30 years from now, I don't know. But I'm happy now doing what I'm doing,'" Laverty says. After he'd worked for Gerry four or five years, he remembers riding in an elevator with a gray-haired co-worker. "I teased him: 'Who gets gray hair working in a lab?' Funny thing: now *I'm* that gray-haired guy!"

Laverty is the only person Gerry Rubin brought from his Berkeley lab to Janelia. "There was no one better than Todd to set up a facility to support all the *Drosophila* labs here. He is very calm, and he's very good at managing people and building a team," says Rubin. "In my Berkeley lab, at times he had to deal with as many as 15 postdoctoral fellows, all with different needs and demands. He does a remarkable job of keeping everybody happy." In a high-pressure laboratory setting, Rubin adds, "That's a very unusual ability."

Laverty describes himself as "a pleaser by nature. I get satisfaction out of starting a task and following through. PIs get the big ideas: 'This is really cool, let's figure this out. I'm not going to worry about the little steps right now.' It's those little steps that I worry about."

In Rubin's lab at Berkeley, Laverty played a supporting role in more than 100 different research projects, including sequencing the *Drosophila* genome. Published in 2000, the sequence marked a scientific milestone because of the fruit fly's pivotal role as a model organism in research, in areas ranging from aging and cancer to learning and memory. "There were hundreds of people in the field who could do the same thing that I was doing. But I had the opportunity to do it every day in the Rubin lab—and it was the Rubin lab that was sequencing the fly genome."

In 1989, Bruce Walker, an infectious disease specialist at Massachusetts General Hospital, sifted through a tall stack of applications for a technician's job in his lab. He immediately noticed the application from Alicja Trocha, a recent immigrant from Poland who had joined the anti-Communist Solidarity movement as a student in the 1980s. Trocha had worked in a lab on the rabies virus—like HIV, a deadly pathogen. She had earned a veterinary degree. And she had done field work with farmers, suggesting a level of real-world maturity not always seen in hothoused science majors.

Walker interviewed Trocha in German, which she had picked up in an internment camp in Munich after fleeing Poland in 1986. Immediately impressed, he offered her the job. But Trocha, overwhelmed by Walker's laboratory operation and by the technical terms she would need to master, initially declined. He persisted, and the result has been a professional match made in heaven.

> "LABS ARE FULL OF STUDENTS AND POSTDOCS WHO COME AND GO EVERY THREE TO FOUR YEARS. IT'S IMPORTANT TO HAVE SOMEBODY WITH A SENSE OF CONTINUITY AND CONTEXT."
>
> GERRY RUBIN

"Alicja has been invaluable to my career," says Walker. "There is nobody in the world who can clone T cells better, and as my lab manager she has not only set the highest standards for performance and integrity but has done it in the most collegial way." Today, Walker leads an international research effort to understand how some rare individuals, known as long-term nonprogressors—infected with HIV but never treated—can fight off the virus with their own immune systems. Insights into their biology could lead to a vaccine or new treatments for the disease.

Trocha supports this effort at the lab bench. "Tissue cultures are like animals or kids," she says. "You have to tend to them, not when you want, but all the time. My colleagues tease me that, since I was a veterinarian, I think of the cultures as my sheep. You may feed them twice a week, but some of them like to eat more, some less. I look at each flask and each culture individually, because they differ in their ability to proliferate. That variability in proliferation might be a clue to why some T cells are effective in inhibiting HIV in the body—and some are not."

As a lab manager, Trocha also deals with the minutiae of administration, regulatory requirements, and the exhaustive task of tracking a large repository of biological samples with bar codes. She documents growth and maintenance of the T cell clones (T cells derived from one "mother" cell that are 100 percent genetically identical), and she teaches fellows and postdocs how to do the same. She also catalogs the clones and the details of how they were established, so that when clinical questions arise years after, she has the answers.

"I believe in myself because Bruce gave me that chance. I would have never known what I could do if I hadn't come here," she says. That brimming confidence extends to the science. "It is bigger than life to be with a group of people who have such bold plans and high aspirations. But this is what I like: trying to make the impossible possible. An AIDS vaccine would change the world."

■ –MADELINE DREXLER

WEB EXTRA: Read about other indispensable lab heroes from HHMI labs by visiting www.hhmi.org/bulletin/Fall2012.

With an outsider's perspective, Leslie Vosshall has changed thinking about the meaning of olfaction—for humans and insects.

AVANT GARDE SCIENTIST

by Robin Marantz Henig
photography by Jon Moe

When Leslie Vosshall opens the door to her sprawling Manhattan apartment, I notice a child's drawing on the front door asking visitors to take off their shoes. Vosshall would never ask me outright, but this crayoned request from her 10-year-old daughter Ophelia is too charming to ignore. I do as directed, and we conduct the interview in our socks.

As it happens, Vosshall is wearing socks all over her body—thin nylon socks, one pair under her regular socks, and another pair with the feet cut off on her arms. The extra layer of clothing is all in the interest of science, specifically Vosshall's decades-long fascination with the mechanics of the olfactory system. To generate enough samples of human scent for their insect experiments, the people in Vosshall's lab occasionally wear socks on their arms and legs, while working up a sweat and foregoing deodorant or showers. After 24 hours the socks are removed, rolled up, and thrown into a freezer until needed. Her team uses the aromatic power balls to investigate how human scent attracts female mosquitoes.

Other scientists might have left such vaguely unpleasant tasks to their assistants. But Vosshall, an HHMI investigator at Rockefeller University, engages in the stinky socks enterprise the way she engages in so many things—with a spirited and contagious enthusiasm. After our chat in her apartment, which includes a tour of the artwork she collects from contemporary painters and photographers (as well as a quick hello to Ophelia's pet tarantula), we head crosstown to her lab. There she opens the freezer to show me where the socks are stored, and laughs at how many of the labeled specimens are hers.

The science of smell has intrigued Vosshall for years, and wearing odiferous nylons is just a small piece of it. In her office at Rockefeller, where she is head of the Laboratory of Neurogenetics and Behavior, several small refrigerators house rows of another kind of art she collects, high-end perfume (her personal favorites are two scents from the Parisian house of Frédéric Malle: Lys Méditerranée for summer, Dans tes Bras for winter). She says refrigeration keeps the scents fresher. Another fridge contains a strictly scientific collection of small vials of smell samples, part of Vosshall's attempt to categorize scents according to their chemical structure, a kind of periodic table of smell. Eventually, she hopes—but this is a long way off—scientists will be able to do for smell what is possible for vision and sound: provide an objective measurement (like a wavelength for a particular color or musical note) from which to infer a particular smell.

Her work on the mechanics of mosquito olfaction carries implications for global public health. Because mosquitoes detect their human targets by smelling them, her research might one day reduce the incidence of common and deadly mosquito-borne infections, such as malaria and yellow fever.

AN INSECTS-ONLY SMELL SYSTEM

Vosshall has been working in olfactory science since 1993, when she became a postdoc in the laboratory of neuroscientist and HHMI investigator Richard Axel of Columbia University. Her first discovery was controversial, since it described an olfactory receptor system that was unique to insects. This went against the conventional wisdom that most receptor systems are conserved from worms to humans. Her work on the insect olfactory receptor, called Orco, was originally done in the fruit fly *Drosophila melanogaster*. Later, Vosshall and her colleagues decoded an olfactory sensory map in the fruit fly brain, through which the activation of a particular fly odorant receptor leads to a particular behavior.

In 2008, Vosshall's lab group, which consists of six to nine postdocs and about five graduate students, began to shift its attention to mosquitoes—which explains the socks in the freezer. In 2008 and in subsequent studies in 2011, her group elucidated the first modern scientific explanation for how the popular anti-mosquito compound DEET works—it confuses the insects by jamming their odor receptors. Ultimately, Vosshall says, she hopes to help develop more targeted, more efficient insect repellants, a crucial weapon against mosquito-borne diseases like malaria.

ENTICING AROMAS

Reaching into her office fridge, Vosshall opens a vial and smells it, then passes it to me. "This is violet," she says, and I sniff. The smell is luscious and a little heady, almost more food than flower. Reluctantly, I give it back to her. (I probably couldn't have continued smelling with such pleasure even if I had held on to it; even a strong scent will pretty much disappear when you try to inhale it a second time.)

She opens another vial, sniffs, and visibly shudders. "Gaaaah," she says. "I didn't realize what this was. Here, smell." Foolishly, I sniff the vial that had thoroughly disgusted her. But I can't smell anything at all. Vosshall seems delighted.

"That's androstenone," she tells me. "It's a component of male sweat, and a small percentage of people are unable to smell it." Most people are totally grossed out by the scent, she says. Another 15 percent or so find it less disgusting—some even like it, describing it as smelling sort of like vanilla. And within that group is an unknown minority of people who, like me, can't smell androstenone at all. The key is the gene for the olfactory receptor OR7D4. Vosshall probably has two good copies. I probably have two faulty

copies. She's doing some studies now to test the sensitivity of young women to these odors at the most fertile point in the menstrual cycle. The women sniff from two vials of androstenone in different concentrations, as well as two control vials, while their sweat response and cortisol response are measured. Her "long-term dream," she says, is to study the effect of OR7D4 and other receptors on general sociability or sexual responsiveness, but these studies are especially difficult to conduct.

"The only available experiments are very indirect," she says. Asking a person to sit in a laboratory and smell a sex-related odor and give a rating of how pleasant or unpleasant the scent is, she says, "is very far abstracted from human sexuality and does not really capture the biology well."

Vosshall discovered OR7D4 with her Rockefeller collaborator Andreas Keller and a team led by Hiroaki Matsunami at Duke University. When they announced their finding of the androstenone-sensing receptor in 2007, there was a little ripple of amazement in the olfactory science world. A *Newsweek* report referred to some good-natured jealousy among other smell investigators.

WORKING AT THE FRINGES

"There are some people you meet and you know that they will do something very meaningful in science," Vosshall's postdoc advisor Axel told me in a phone conversation laden with superlatives. "She's really strikingly dynamic, and that's coupled with experimental fearlessness and thoughtfulness."

Axel said he is particularly impressed by Vosshall's perseverance, no matter how many wrong turns she takes along the way. "Leslie is able to enter into new and difficult experimental arenas with the knowledge that it's going to take a depth of understanding and time to make a meaningful contribution."

Vosshall's discovery of a unique olfactory system for insects was a bonanza, she says. "The fact that the target proteins are only present in insects is a huge convenience." It means scientists will be able to prevent and reduce mosquito biting in a way that should have no effect on humans. To move in that direction, she is collaborating with Bayer CropScience in Germany to devise an insect repellant that blocks the insect's ability to smell. The goal is to find a product as effective as DEET that is longer lasting, less oily, less toxic, and safe enough to use on infants.

Vosshall says she has always preferred working at the fringes of science, where the questions are most interesting and least explored. Her rule of thumb for whether to pursue a specific scientific inquiry: unless precisely three laboratories are already working on it, forget it. More than three and the topic is already too popular for her. Fewer and there aren't enough colleagues who are knowledgeable about the subject with whom she can exchange ideas.

When she studied the function of insect odorant receptors, for instance, the only other people she says were working on the problem were Kazushige Touhara in Tokyo and Bill Hansson in

With an eye for the uncommon, Leslie Vosshall's life is imbued with art, enterprising research, and community engagement—which feeds back into her science.

Germany, both of whom she eventually collaborated with. "When the field gets very large," she says, "collaborations become harder to arrange, and secrecy is more of a barrier."

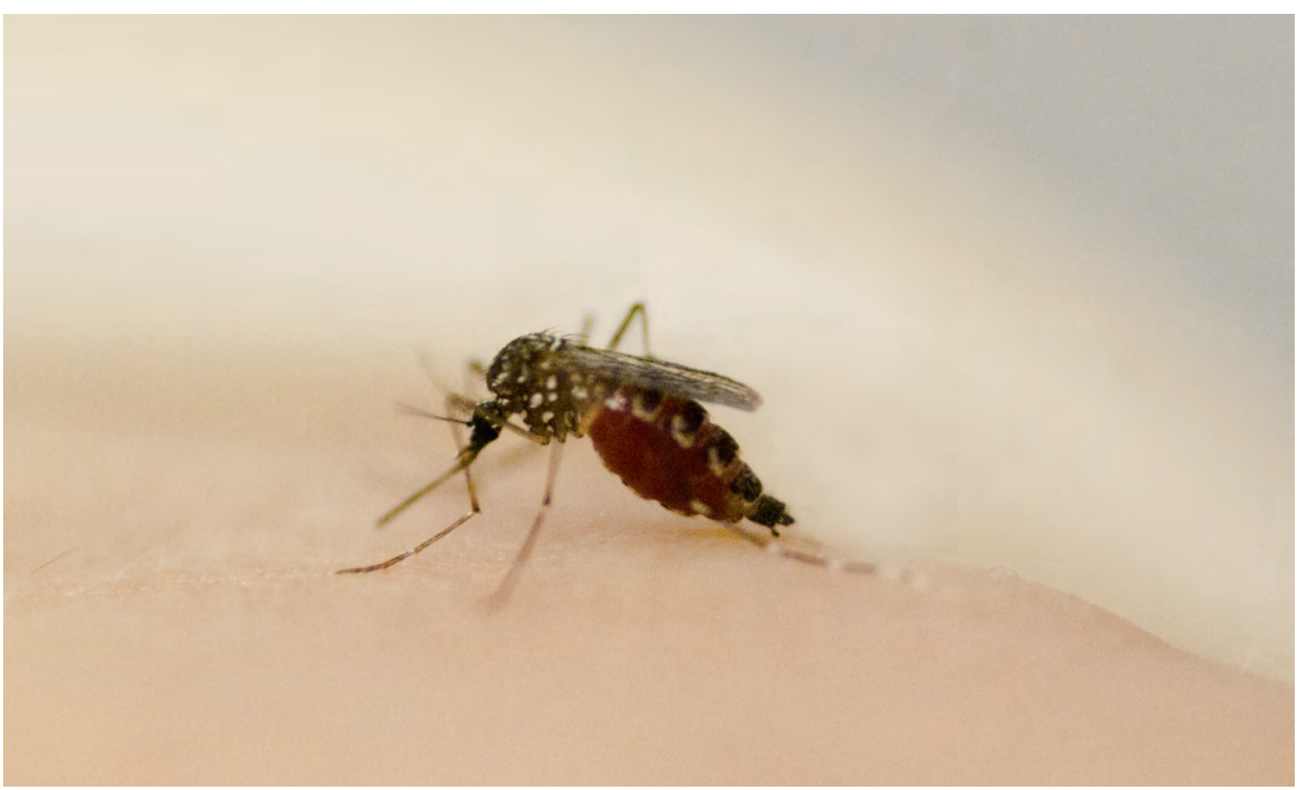
Vosshall's outsider status goes back to childhood. She was born in Lausanne, Switzerland, the eldest of three children of nomadic, adventurous parents. Before she was eight years old, she had moved six times to four countries, going back to Switzerland every summer for family vacations crammed into a bare-bones shack high in the Swiss Alps. On the first day of third grade in suburban New Jersey, where the Vosshalls finally settled, young Leslie didn't speak a word of English. She picked it up quickly—she's pure New Yorker now—but she always held on to that feeling of being just outside the normal flow of things.

"Even in high school she was fairly counterculture," says her sister, Nicki Dugan, a public relations executive in San Francisco. "We spent a lot of time poring through the Salvation Army men's department looking for ties and David Byrne–style big jackets. She tended to want to stand out."

Stylistically, Dugan says, Vosshall was into angular haircuts, multiple ear piercings, and hair dyed colors that nature never intended. As a young teenager she spent weekend afternoons exploring Manhattan on her own, and took solo summer trips, first to Greece and then to Cape Cod. It was during two summers at the Marine Biological Laboratory in Woods Hole, Massachusetts, where she worked for her uncle, Philip Dunham of Syracuse University, that she fell in love with science.

"Mainstream was just taboo for Leslie," says her sister. But even though Vosshall favored the punk scene and avant-garde music, she was an attentive student who graduated in 1983 as valedictorian at Kinnelon High School. She chose Columbia over the other Ivy League schools she could have attended, partly because she liked being in its pioneering first coed class, and partly because it was in New York, a city she always wanted to live in and now can't imagine leaving.

From Columbia, where she majored in biochemistry, Vosshall went to Rockefeller University for her doctorate, where she studied circadian rhythms in the fruit fly. "Even back then she was fearless," says Marina Picciotto, a close friend from graduate school who is now a professor of psychiatry, neurobiology, and pharmacology at Yale School of Medicine. "In every interaction you ever have with Leslie, she's always herself, always speaks her mind." Picciotto and Vosshall have remained close friends, both married to neuroscientists and with children about the same age. Sometimes the families attend scientific conferences together, so one of the parents can take the kids to the zoo while the other three attend the scientific sessions.



Vosshall's arm offers a pleasing scent to this mosquito, which detects its prey through smell. She aims to block that olfactory system to stop diseases like malaria, which kills up to 1 million people each year.

Leslie Vosshall lab

In grad school, Vosshall held on to a separate, bohemian life in addition to her workaday scientific world. She tried her hand at experimental filmmaking. "I made lots of really bad Super 8 nonnarrative films," she says. "I lived downtown at the time—I was married to a musician and I had a lot of friends who were filmmakers. I wanted to get into the action, too."

Picciotto remembers being cast in one of Vosshall's films as Julia Child in the laboratory, whipping up a preparation of brain specimen in a Sunbeam blender. That artsy phase was short-lived, Vosshall says. "You have to know your limits. Now I just buy a lot of art."

THE WOMAN'S ROLE

Vosshall takes her role as a woman in science seriously, but she doesn't put it at the forefront of her activities as some woman scientists do. "She's not afraid of taking risks or of being perceived as exactly who she is," Picciotto says, "which is an incredibly brilliant scientist with an extremely strong aesthetic sense—and also a woman in a world dominated by men."

At age 47, Vosshall says she is lucky to be part of the bridge generation, when it became much easier to be a woman in science. "I felt like, with a few exceptions, people expected me to be a scientist and encouraged me." She always had lots of female peers and colleagues, she says—that is, until recently. After their postdoctoral work, a "disappointing" number of women drop out of the tenure-track academic career path that she was on—meaning that now, as a full professor, she says she is "constantly the only woman in the room."

It's often the career-versus-family quandary that forces women to leave science, but Vosshall says she managed to have a child without breaking stride largely because Rockefeller made it easy. "There is terrific day care right here on campus," she says. And her husband, Kevin Lee, executive director of the Ellison Medical Foundation, is a hands-on father, taking Ophelia to fencing, art, and rock guitar lessons so Vosshall can spend time in the lab, or at home at her desk in a corner of the expansive living room.

PUBLIC TALKS AND SCHNOZMOPOLITANS

Vosshall takes her public role seriously and is committed to explaining her work to the public. She was featured last year at the World Science Festival, a three-day annual event in New York that brings together smart lay people and marquee-name scientists. At the session called "The Science of Smell," Vosshall entertained her audience with tales of "strip club science" (studies showing that female strippers get more tips when they're ovulating, possibly because they're sending off unconscious scent signals) and, as she put it, the "empirical" and "artistic" nature of perfumery.

She was also an early participant in the popular Secret Science Club, which meets monthly at a Brooklyn bar where people go to talk science, ask questions, and drink thematic cocktails. The one cooked up for Vosshall's lecture about smell was called the "Schnozmopolitan."

> MAINSTREAM WAS JUST TABOO FOR LESLIE.
>
> —NICKI DUGAN

Through these public appearances, Vosshall is not only disseminating scientific information to the public, she's also indirectly finding hundreds of volunteers for her smell studies. Her work on mosquitoes was detailed in *The New Yorker*, which brought forth hundreds of people willing to tell their mosquito-biting stories. Some people seem never to get bitten, others are mosquito magnets, and everyone has a pet theory about what distinguishes the two in terms of what they eat, what color clothing they wear, what their blood type is, or what cologne they use.

Vosshall is aiming at a more scientific analysis, bringing those volunteers to Rockefeller for a formal study involving blood analysis, detailed medical histories, and follow-up to see if she can find any genetic or other traits that make some people absolutely irresistible to mosquitoes.

She is also conducting a study of more than 1,000 volunteers who are brought in to give blood samples (for genetic analysis) and to describe a battery of smells according to 146 standard descriptors (such as smoke, soil, excrement, urine, gasoline, or rose). Her goal, she says, is to "try to correlate their olfactory idiosyncrasies to their DNA."

With so many different projects grabbing her interest at once, Vosshall is demonstrating the style and pacing that she's maintained her whole career. As her friend Picciotto says, "Leslie is always thinking of next steps rather than last steps." ■

FOR MORE INFORMATION: To see Vosshall speak at the 2011World Science Festival, visit www.worldsciencefestival.com/videos/how_do_we_smell.

THE FAT YOU CAN'T SEE

IN GROWING NUMBERS OF PEOPLE, THE LIVER HOLDS A HIDDEN, DANGEROUS STORE OF FAT. FINDING THE TRIGGERS IS STEP ONE.

written by Sarah C.P. Williams & illustrated by Graham Roumieu

A SHINY, PINKISH-BROWN TRIANGLE TUCKED UNDER THE RIGHT RIB CAGE, A HEALTHY LIVER IS A MARVEL. NUTRIENT-RICH BLOOD FROM THE INTESTINES PULSES INTO ONE SIDE, AND THE LIVER GOES TO WORK REMOVING TOXINS, CONVERTING DIGESTED FOOD TO ENERGY, STORING VITAMINS AND MINERALS, AND CONTROLLING HOW MUCH FAT AND SUGAR IS SENT BACK OUT TO THE REST OF THE BODY.

Without the liver acting as a filter and energy producer, a person can't survive, and no artificial organ can perform all of its duties. But in one in three Americans—and similar numbers in other developed nations—the liver has lost its luster. It is swollen, yellowish, congested with fat, and doesn't function up to par. Over time, this condition, called fatty liver disease, can lead to inflammation, scarring, and hardening of the organ, and eventually, liver failure. In some people, it causes liver cancer.

"Fatty liver disease is the number one liver disease in this country in both adults and children," says HHMI investigator Gerald Shulman of the Yale School of Medicine. Rates of fatty liver disease have risen dramatically over the past two decades. "Furthermore, it is strongly linked to hepatic insulin resistance and type 2 diabetes," he says. Understanding these triggers and how the disease progresses may stop the uptick in its occurrence.

Scientists know that fat buildup in the liver is more common in people who are overweight, have type 2 diabetes, or drink excessive amounts of alcohol. But beyond that, not much about fatty liver disease is well understood. Does the diabetes or fatty liver come first? Do certain genes predispose people to fatty liver disease? How can the disease be detected early and treated? Do other diseases contribute to fat in the liver? These are only some of the questions that Shulman and others are pondering as they study who gets fatty liver disease and why.

"This is a slowly progressing disease," says hepatologist Rohit Loomba of the University of California, San Diego, School of Medicine. "If we want to prevent liver disease down the road, we need to act now." According to a 2008 estimate, fatty liver disease will be the leading reason for liver transplants by 2020, overtaking hepatitis C.

With a sense of urgency, scientists are pushing forward with studies on the basic biochemistry behind fatty liver. They are coming at the problem from different disciplines—genetics, endocrinology, immunology, and biochemistry—and their findings show a complex, sometimes contradictory story of what pollutes the liver with fat.

FROM FOOD AND GENES TO FAT

Fatty liver disease is rarely detected because of symptoms. Most often, a patient gets a routine blood test and the doctor notices altered levels of proteins made by the liver. Even then, there's no one test to give a definitive diagnosis of fatty liver disease. By ruling out other causes of abnormal liver proteins in the blood—such as a hepatitis infection or tumor—a doctor may conclude that the patient has fatty liver disease. If the patient is not a heavy drinker, then the diagnosis is nonalcoholic fatty liver disease (NAFLD). Today, more than three-quarters of fatty liver cases are NAFLD.

"It's an incredibly broad diagnosis," says HHMI investigator Helen Hobbs of the University of Texas Southwestern Medical Center. "It can mean anything from mild fatty liver to severe inflammation."

As recently as a decade ago, not much more was known about the prevalence of NAFLD, says Hobbs. Clinicians had a poor grasp of the overall incidence, how it progressed, or how to identify patients at risk for developing it in the first place. So when Hobbs launched the Dallas Heart Study, tracking the health of 6,000 individuals in the Dallas area, she and her colleagues included questions about all aspects of cardiovascular and metabolic health. Among topics such as cholesterol and diabetes, they also homed in on NAFLD.

"One thing we were very curious about was whether nonalcoholic fatty liver disease has a genetic underpinning," says Hobbs. As part of the study, Hobbs and her colleagues used a special type of magnetic resonance imaging (MRI) scan to quantify liver

fat in 2,349 participants. If the liver fat content was greater than 5.5 percent, the participant was classified as having NAFLD; about one-third of the total population fit the diagnosis.

"The biggest surprise for us wasn't the incidence—we had suspected it would be high," says Hobbs. "It was the differences among races."

Among Hispanics, her team found, 45 percent had NAFLD. But only 33 percent of European Americans met the diagnosis criteria, and only 24 percent of African Americans had NAFLD. Even when they factored in obesity and diabetes, there were still major differences in the rate of NAFLD among ethnic groups.

Hobbs and her colleagues delved into genetic data from the patients and tested whether any genetic mutations could explain the different frequencies of NAFLD. They discovered that one variant of a gene called *PNPLA3* seemed to predispose people to fatty liver. Moreover, the gene variant was most common in Hispanics, and least common in African Americans. It explained more than 70 percent of the differences in NAFLD incidence between races.

"Within all populations studied to date, the variation has been associated with fatty liver," Hobbs says. "If you have the risk allele of the gene, you tend to have higher triglyceride content in the liver."

In the liver, the PNPLA3 protein is responsible for breaking down triglycerides, the main building block of fats. So Hobbs suspected that the variant of *PNPLA3* stopped the protein from working, or from being expressed at all in the liver, which would lead to an accumulation of fats. In mouse studies, however, her team has shown that an excess of the variant PNPLA3 protein causes fatty liver. Hobbs' team is working to flesh out the mechanism involved. Solving the mystery will help uncover the biochemical pathways involved in fatty liver disease progression, but it's unlikely to explain the full story of fatty liver.

"This is a gene-environment interaction," says Hobbs. "If you have this gene and you're thin, you won't have fatty liver disease. But if you have this gene and you're obese, it is very likely you will."

THE DIABETES LINK

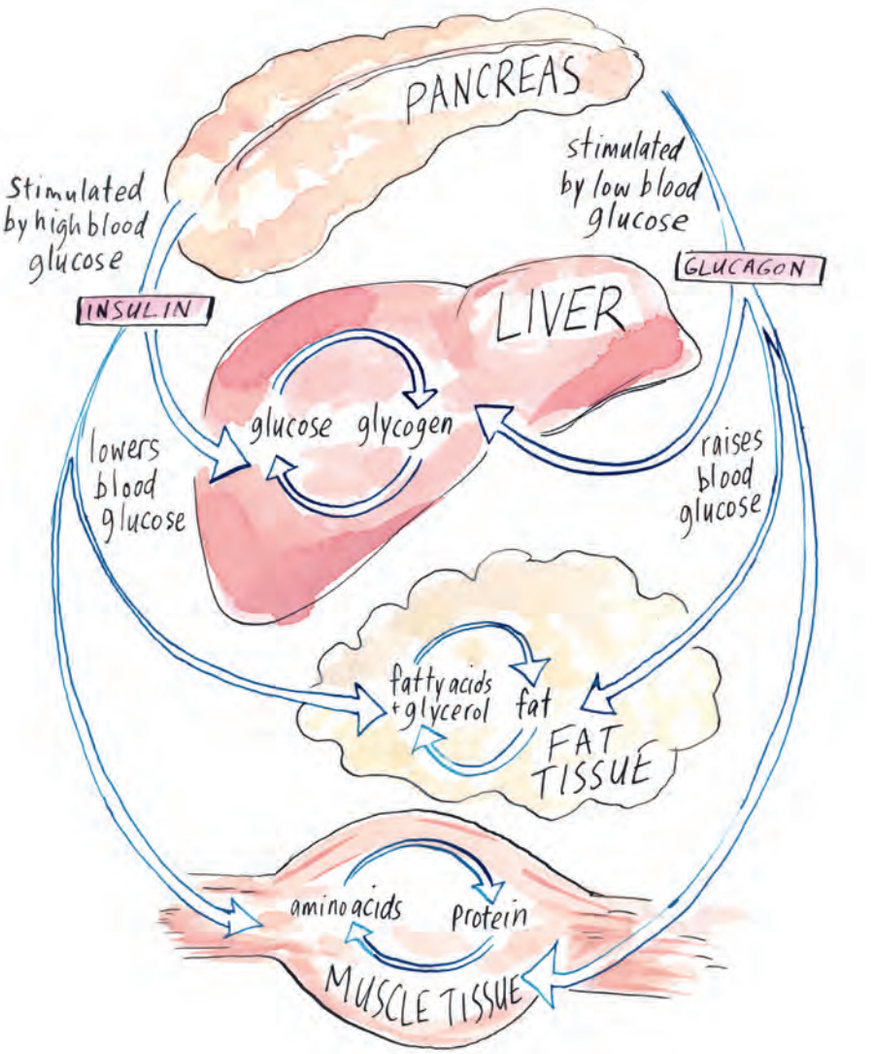
Even if there's a genetic component to NAFLD, that doesn't explain the increase in rates. The change in incidence can most likely be tracked back to changes in people's diets—particularly an increase in sugar consumption—over the past few decades, says Loomba, whose San Diego clinic sees hundreds of NAFLD patients a year.

"If you take any normal, healthy person and do an MRI of their liver, and then start giving them three cans of soda a day, you can scan their liver again two weeks later and see liver fat already going up."

Soda doesn't have fat in it, but a diet high in sugar changes the way the body deals with nutrients, including fat. Normally, the hormone insulin produced by the pancreas after eating a meal causes the liver to store sugars—taken up from the blood

Shulman: Brian Park Hobbs: David Cresham / UT Southwestern Medical Center Flavell: Yale University

HHMI investigators Gerald Shulman, Helen Hobbs, and Richard Flavell are each studying fatty liver disease from different perspectives and finding unique roads into the complex disease and its causes.



Normal Energy Storage & Use

WHEN YOU EAT, INSULIN PRODUCED BY THE PANCREAS PUTS YOUR BODY INTO STORAGE MODE TO SAVE ALL THE ENERGY YOU'VE INGESTED (LEFT SIDE OF DIAGRAM). IN THE LIVER, GLUCOSE IS CONVERTED TO GLYCOGEN FOR LONG-TERM STORAGE. IN FAT DEPOSITS AROUND THE BODY, MOLECULES COME TOGETHER TO FORM FATS. AND IN MUSCLES, THE BUILDING BLOCKS OF PROTEINS ASSEMBLE. ONCE YOU'VE DIGESTED THE FOOD, GLUCOSE AND INSULIN LEVELS DROP AND THE MOLECULES THAT HAVE BEEN ASSEMBLED FOR ENERGY STORAGE START BEING BROKEN DOWN AS YOUR BODY NEEDS THEM.

in the form of glucose and fructose—for later (see diagram). And some of the glucose is repackaged into fat molecules. But a diet high in sugar can lead to insulin insensitivity, or insulin resistance. The body, including the liver, becomes less efficient at responding to insulin's signals. Eventually, it stops responding at all. And rather than send the fat molecules into the blood, the liver retains the fat it produces.

"The liver is clearly central to normal glucose homeostasis," says Shulman, who is determined to sort out the intricate interplay between sugar and fat metabolism in the liver. The critical questions, he says, are what factors lead to the development of NAFLD and how do alterations in the liver's metabolism of fat contribute to insulin resistance in the liver.

Shulman's lab group has been studying the development of insulin resistance in muscle and the liver for nearly three decades. They want to know the precise sequence of events that occur when patients develop muscle insulin resistance, liver insulin resistance, NAFLD, and type 2 diabetes. So they have developed

novel magnetic-resonance scanning techniques to noninvasively measure the concentrations of metabolites within liver and skeletal muscle in patients who are prone to develop type 2 diabetes and in those with well-established type 2 diabetes. Using this approach, they have found that healthy, young, lean, insulin-resistant offspring of parents with type 2 diabetes, who have a high likelihood of developing type 2 diabetes, have insulin resistance only in skeletal muscle and not in the liver.

"We have shown that selective insulin resistance in skeletal muscle—the earliest defect we can observe in these otherwise young, healthy, lean individuals—can predispose them to hyperlipidemia, NAFLD, and liver insulin resistance by diverting ingested carbohydrate away from muscle, where it is normally stored as glycogen, to the liver, where it is converted to fat," says Shulman. "By screening patients for muscle insulin resistance, and understanding the progression to fatty liver and diabetes, researchers may be able to uncover new ways to stop these diseases in their early stages," he says.

In a separate study, Shulman and his colleagues studied healthy, young, lean individuals in the New Haven, Connecticut, community from five different ethnic groups and found a striking increase in the prevalence of NAFLD associated with insulin resistance in the lean Asian-Indian male volunteers. "Just about everyone will develop NAFLD if they become obese, independent of ethnicity," says Shulman. "But there appears to be something going on in the Asian-Indian men that predisposes them to develop NAFLD and hepatic insulin resistance at a much-lower body mass index."

To further investigate this question, Shulman's group teamed up with HHMI investigator Richard Lifton at Yale and found a different mutation than Hobbs' team had uncovered. They pinpointed mutations in a gene called *APOC3*. While Hobbs' PNPLA3 is involved in triglyceride breakdown within the liver, the APOC3 protein regulates the breakdown of triglycerides in the blood for storage in fat cells. Increased plasma concentrations of APOC3, which these gene variants have been shown to cause, will predispose these individuals to both NAFLD and increased triglyceride concentrations in the blood.

In transgenic mouse studies, Shulman's team found that mice overexpressing human *APOC3* developed both fatty liver and hepatic insulin resistance when fed a high-fat diet, supporting his theory that increased plasma concentrations of APOC3 predisposes an individual to the development of NAFLD, and that fatty liver can cause hepatic insulin resistance and contribute to the development of type 2 diabetes.

"I think the *APOC3* variants that we describe predispose lean individuals to NAFLD and hepatic insulin resistance reflecting a gene-environment interaction. Just having high plasma concentrations of APOC3 will not do anything in itself, as reflected by the lack of fatty liver and hepatic insulin resistance in the *APOC3* transgenic mice fed a regular chow diet," says Shulman. "But if you add a little bit of fat to their diet, they get fatty liver and hepatic insulin resistance." When the young, lean, Asian-Indian men with NAFLD lose a relatively small amount of weight, Shulman's team has found, their fatty liver and hepatic insulin resistance can be reversed.

Hobbs' and Shulman's evidence of two different genes with profound effects on NAFLD hints at the complexity of this metabolic disease. More research will be needed to sort out how these genes, and likely others, affect the development of this devastating syndrome.

BACTERIA'S ROLE

Doctors in the clinic need a way to identify patients who are at risk of developing worse forms of the disease—inflammation and scarring of the liver. After all, some people with fatty liver do just fine without treatment (although if Shulman is right, their fatty liver could be contributing to diabetes). In other patients, however, the liver becomes inflamed, clogged with immune molecules, and eventually begins to harden and stop working.

"We need to find out who is at risk for developing full-on liver disease and who isn't," says Loomba. "And then we need to focus our energy on stopping liver disease in those at risk."

Patients with more risk factors—diabetes, smoking, poor diet, and alcohol consumption—are more likely to develop worse stages of liver disease. But even without those risk factors, some patients end up worse off than others.

Now, a theory is emerging out of left field on why some patients may develop more severe inflammation in the liver. HHMI investigator Richard Flavell at Yale studies bacteria that inhabit people's guts. He recently discovered problems in the lining of the gut that lead to the body's inability to control these types of bacteria in mice with susceptibility to inflammatory bowel disease (IBD).

"Once we had found this problem in the gut, it occurred to us that it was unlikely to be limited to just the intestines," says Flavell. Researchers knew that the blood vessels between the intestines and liver of people with IBD often had leaks, letting unwanted material through.

"The function of these blood vessels is to carry food to the liver," says Flavell. "But we hypothesized that there could be a way in which the bad components of bacteria—or even whole bacteria—were traveling to the liver."

So he took mice with mutations that predispose them to IBD and put them on diets that usually lead to NAFLD. "We immediately saw that if the mice don't have this pathway in the intestines working, they get much worse fatty liver," he says.

The experiment, which included help from Shulman, was the first to suggest that NAFLD isn't just a metabolic disease, but could have a link to bacteria. The gut bacteria, while likely not causing fatty liver in the first place, could explain the inflammation that can lead—in a fraction of NAFLD patients—to more severe liver disease. Flavell plans to study how the bacterial populations in the intestines of people with NAFLD vary from those in healthy people. It's an angle of the disease that may someday lead

(continued on page 48)

THE VIEW FROM HERE

Unbounded creativity—and powerful computers—make possible the latest devices designed to peer into the deepest recesses of organs and cells.

WRITTEN BY IVAN AMATO
ILLUSTRATED BY MITCH BLUNT

PHOTOGRAPHY HAS COME A LONG WAY IN THE TWO DECADES SINCE DIGITAL CAMERAS ALL BUT RELEGATED PICTURE TAKING ON FILM TO A CHAPTER IN THE HISTORY BOOK OF TECHNOLOGY. IMAGE-PROCESSING TOOLS LIKE PHOTOSHOP AND MOBILE APPS LIKE INSTAGRAM HAVE TURNED ANYONE WITH aspirations into a postproduction touch-up artist. Revolutionary, yes. But it's nothing compared to the evolutionary explosion in imaging techniques that the digital crossover has unleashed in laboratories.

Every major advance in imaging technology precipitates a new round of breakthroughs in cell biology," says structural biologist Grant Jensen, an HHMI investigator at the California Institute of Technology. Seeing is the quickest route to understanding, says Jensen, who has been using a technique similar to a computed tomography (CT) scan to render visible the molecular machinery inside cells in three-dimensional splendor.

By combining advanced microscopes, new-generation cameras, innovative methods of acquiring raw data, and computational processing of the data, HHMI scientists and others are creating unprecedented depictions of biology's magnificent marriage of form and function. Think of the leaps from still photography to movies, from black-and-white to color, and from silent to sound, and you begin to get a sense of how much more of life these pumped-up imaging tools are allowing researchers to see.

Neuroscientist Mark Schnitzer, an HHMI investigator at Stanford University, has been working with colleagues to extend the reach of their microscopes to watch ensembles of cells deep inside the brains of live, mobile animals. Schnitzer has been thinking hard about the evolution of imaging in biology. As he sees it, there has been a three-phase progression since the 17th century when the likes of Robert Hooke in England and Antonie van Leeuwenhoek in Holland first ushered microscopy into scientific investigations.

That first phase, based on what scientists could see in real time with their eyes peering through a microscope, lasted for centuries. First they recorded what they saw with hand drawings and then later with photographs. Their recorded images, says Schnitzer, were essentially data in pictorial form.

With phase 2 and the advent of digital cameras and laser-scanning techniques over the past few decades, "the image becomes a set of measurements," says Schnitzer. Each pixel has associated with it digital values of light intensity, color, or some other parameter. More than just a pictorial rendition of what is visible, he notes, "the image becomes a numerical representation of reality."

The camera's automatic digital accounting of the images' characteristics opened up a world of computer-assisted analyses of what once were only pictures. It also opened options for novel data syntheses, among them visual reconstructions of three-dimensional biological structures from two-dimensional images of consecutive "slices" of a biological sample.

"The third phase of biological imaging is where things have gotten really interesting," Schnitzer says, adding that the ready availability of powerful computers is as much the driver here as are the microscopes and cameras. Here, he notes, the meaning of the raw data is hard to interpret by eye until a computer processes and reconstructs it into, say, a two- or three-dimensional view with discernible cellular or molecular features. This is akin to the way today's high-end sonography equipment can reconstruct images of a fetus from sound echoing from it, or the way magnetic resonance imaging machines convert electromagnetic signals from molecules in the body into portraits of internal anatomy. It takes a lot of computing power to turn those raw signals into medically useful pictures.

THE THIRD PHASE

Jensen has been pushing this third phase of imaging into the molecular domain. He combines a freeze-prep step—designed to keep water inside cells from evaporating when inside an electron microscope's vacuum sample chamber—with a multiple-view reconstruction process. The process yields enough 3D clarity, he says, that the resulting images alone can provide sufficient evidence

to choose between competing theories about roles played by otherwise mysterious molecular complexes. One example he likes to talk about is the syringe-like mechanism of a molecular defense complex in bacteria whose action had been a matter of speculation. (See Web Extra sidebar, "A CT Scan for Protein Complexes.")

At HHMI's Janelia Farm Research Campus, developmental biologist Philipp Keller has been pushing a different multiview, computer-intensive technique into new biological territory. Called simultaneous multiview light-sheet microscopy, the technique has allowed Keller, a Janelia Farm fellow, to acquire breathtaking movies—they literally evoke gasps—of the daylong embryonic development of fruit flies. He combines computerized tracking of cell lineage, proliferation, and migration with color-coding techniques to make movies of some of biology's most spectacular performances. (See Web Extra sidebar, "Embryogenesis in Motion.")

Meanwhile, Anthony Leonardo, a group leader at Janelia Farm, has been applying a variety of sometimes Hollywood-esque techniques to help answer questions about whole organisms. Here's one of his questions: How do dragonflies deploy brain-encoded "guidance rules" to execute the acrobatic, high-speed task of capturing prey during flight? In time, he will map the "microstructure of behavior"—among them, details of how the hunting dragonfly orients its head and body—captured in the data from video recordings of the insects' less-visible neurophysiological and muscular control system. (See Web Extra sidebar, "Eye on the Fly.")

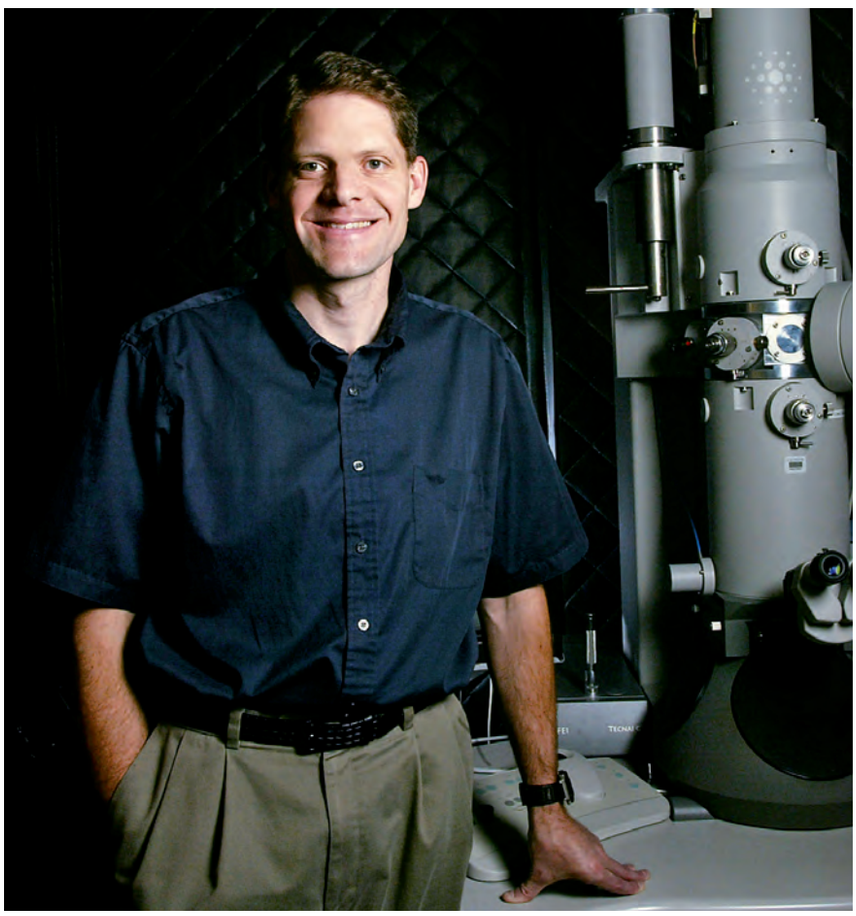
In a nearby lab, Janelia Farm fellow Kristin Branson has been ramping up camera- and computer-based observations of fly behavior to an industrial scale. Putting her computer science background to work, she transformed what amounts to a surveillance camera system into an automatic behavior detector that she and co-workers have used to spy on 300,000 genetically diverse fruit flies. The scientists teach the computers to associate specific fly behaviors with particular patterns of data, which are then embedded within the image files recorded by the cameras. Rather than having someone perform the time-consuming task of making direct observations of flies, one by one, this automatic machine-based approach sums high-throughput observations on aggression and courtship, among other behaviors. The hope is that the quantitative leap in data gathering and analysis will lead to a qualitative leap in understanding how genes orchestrate nervous systems and related behaviors. (See Web Extra sidebar, "Capturing Behavior.")

FLY ME THROUGH THE CELL

Schnitzer is interested in virtual tours of inaccessible places. His group has been developing techniques for simultaneously imaging hundreds of brain cells in live mice to tease out the neural circuitry in the cerebellar cortex that underlies learning and memory. He and co-workers also have developed a microendoscopy technique that enables them to apply fluorescence microscopy to deep-brain structures that had been inaccessible to conventional microscopy, at least in living and behaving mice. Schnitzer's team mounts tiny microscopes that weigh a fraction of an ounce onto the heads of mice. Central to these devices are micro-optical needles with a lens that can magnify like a typical microscope's much larger, curved lens—needles that can penetrate deep into the brain and image living cells and tissue there.

And in a project that complements Branson's, Schnitzer aims to develop instrumentation for massively parallel brain imaging of up to 100 fruit flies at once—both normal flies and those with

(continued on page 48)



Jill Connelly / AP

Grant Jensen flash freezes specimens to lock water in place then gets 3D images of cells in near-native state. He likens the result to a CT scan for protein complexes.

Dianne Newman

CONNECTING CULTURES

MEDICAL RESEARCHERS SHOULD SEIZE THE TOOLS OF GEOCHEMISTRY.

Darcy Hemley

Medical microbiologists, whether studying tuberculosis or cystic fibrosis, often ignore the chemical environment of the tissues in which microbes naturally thrive. Dianne Newman argues that geobiologists like herself—who study how microbes have coevolved with the earth—have tools that could be applied to the medical sciences.

During a sabbatical at the University of Oxford last fall, I spent hours in a beautiful 16th-century library. The shelves included headings in Latin denoting the subject of the volumes in each row. One day, I did a double take at a heading that paired two fields rarely discussed together: *MEDICINA & HIST. NAT.*, or medicine and natural history.

This pairing makes perfect sense. Both Earth and the human body are complex ecosystems that depend on coevolution, where life impacts the chemical and physical properties of the environment, and the environment, in turn, feeds back into life's evolutionary adaptations. And for both, it is impossible to do controlled experiments on the whole. Instead, we must perform creative but imperfect analog experiments or make careful measurements of the natural system.

Experimental Earth scientists, unable to reproduce the complexity of the natural world in a lab, have focused their efforts on developing analytically dazzling ways to measure dynamic environments. For example, geochemists have dated Earth's age by measuring radioactive isotopes of lead in meteorites and tracked the rise of carbon dioxide in the atmosphere by analyzing trapped bubbles in ancient ice cores. In my lab, we are collaborating with organic geochemists to make sense of molecular fossils from bacteria in billion-year-old sedimentary rocks.

Although they share an interest in microorganisms, medical and environmental microbiologists have separate scientific cultures: they don't typically attend the same conferences or publish in the same journals. As a consequence, we know shockingly little about the chemistry of the places in the body where microorganisms reside.

Yet, the same methods geochemists apply to sediments and ice cores can be tweaked for cells, tissues, and organs.

Take the area of infectious disease. Medical researchers typically culture bacteria after isolating them from their natural microenvironments. While these scientists are adept at identifying genes, proteins, and pathways involved in lab models of disease, whether their findings extend beyond their specific model system is not always clear. What's more, most pathogen studies have focused on the microbes' early rapid-growth stages, even though that represents only a fraction of their life in the human host. Partly due to these artificial experiments, antibiotics that work on organisms in the lab often fail in the clinic.

So, what can geobiology offer the study of infectious disease? To give just one example, my colleague Victoria Orphan combines fluorescence in situ hybridization and secondary ion mass spectrometry to study slow-growing bacterial populations in methane seeps on the ocean's floor. These populations cannot yet be cultured in the lab, so she learns about their metabolic activities by measuring their RNA and isotopic composition. The same approach could be used to study the metabolism of slow-growing pathogens inhabiting the mucus-filled lungs of patients with cystic fibrosis (CF). By understanding the chemical composition of the mucus in space and time, and how the organisms are coevolving with their environment, we could potentially devise better ways to disrupt the microbes in their natural niches.

Geobiologists have much to learn from the mechanistic approaches taken by medical microbiologists, as well. For example, a major question in Earth history is, when did oxygenic photosynthesis arise? Although geochemists have uncovered a wealth of molecular fossils spanning vast time periods, they are limited in their ability to interpret them. By studying the functions of their lipid counterparts in living cells using the tools of molecular and cellular biology, geobiologists could gain insight into the fossils' evolutionary significance.

Building bridges across these two scientific cultures will take more than the buy-in of a few research labs. These ideas should germinate in college and graduate school, when budding researchers are most open-minded. One of my graduate students, HHMI international student research fellow Sebastian Kopf, is using the isotope-measuring techniques he learned as a geology student in Germany and at Massachusetts Institute of Technology to measure bacterial growth rate in sputum samples from cystic fibrosis patients. While I encouraged Sebastian to pick the CF lung as his "field site," his strategy to study the microbial community in this environment is a product of his own creativity and training in the geosciences.

My ultimate hope is that future generations of scientists will feel comfortable pairing medicine and Earth science—just like the builders of that library in the 16th century.

INTERVIEW BY VIRGINIA HUGHES. *Dianne Newman is an HHMI investigator at the California Institute of Technology.*

Q&A

What part of your job would people find the most surprising?

They do everything from making fly food to fixing centrifuges. Here, a few lab managers and research specialists describe some of the more unusual aspects of their jobs.

—EDITED BY HALLEH B. BALCH

Heike Pelka

RESEARCH SPECIALIST III
DANNY REINBERG LAB
NEW YORK UNIVERSITY

"I think what people will find most surprising is that, as a lab manager who has worked at the bench for 33 years, I do a lot of architectural work. I even have my own architectural ruler now. The biochemistry wing at NYU was recently renovated, and I designed a section of the labs. Of course, people sometimes say, "That's not in your job description." Well, I don't believe in job descriptions. You do what you are capable of."

Matthew Adams

RESEARCH TECHNICIAN II
CHRISTOPHER PLOWE LAB
UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE

"The part of my job that I find most surprising is our challenge study. We give patients malaria to study the disease process and its symptoms, and then we cure them. It's humbling and interesting to me that people come to us to get malaria. They're sacrificing a little bit of their health so that we can learn something new to create a vaccine or to learn about the parasite. Our duty to them is to make sure they aren't sick for too long."

Sarah Sarsfield

LAB MANAGER
DAVID GINTY LAB
JOHNS HOPKINS UNIVERSITY

"Some of the procedures with mice—like checking plugs—would probably be among the weirdest stuff I've done. We do developmental neuroscience, which means that we study embryonic development. So we need to determine when mice have mated and then track the pregnant mice. The morning after you put a male and a female together, you check for a plug. If the mice mated, the female will have a sperm plug. As a scientist, I don't really think much of it, but when people ask what I do, I don't usually answer, 'Oh, I spent yesterday morning checking 80 female mice to see if they had sex the night before.'"

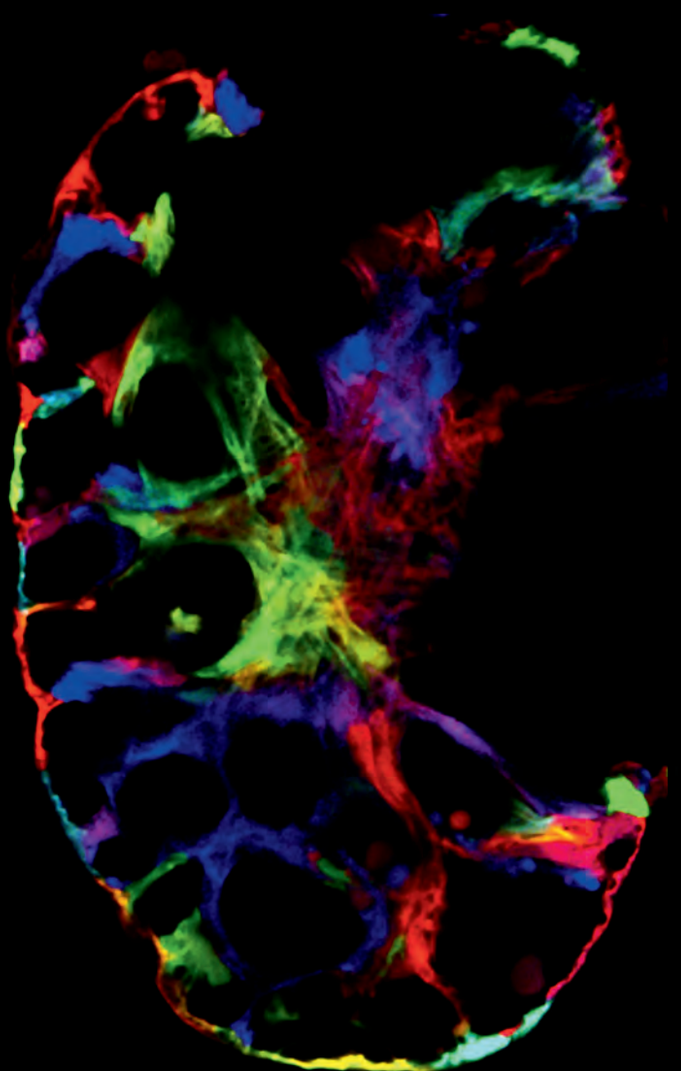
Frank Wilson

LAB MANAGER II
PIETRO DE CAMILLI LAB
YALE UNIVERSITY

"I'm responsible for maintaining the lab's infrastructure along with other operations-related duties. People are surprised to learn that, rather than call in a specialist, I often repair the lab's instrumentation myself. My father was adept at repairing and constructing electronic devices, and in my early years I picked up a lab by watching him at work in the basement. I'm not afraid to open up an instrument, even if I don't normally operate it, and I can usually diagnose the point of failure if a system is faulty. I find it a welcome change from tedious administrative tasks."

Heike Pelka, Matthew Adams, Sarah Sarsfield, Frank Wilson

chronicle



Vikas Gupta / Poss Lab

A tiny zebrafish has just hatched from its egg. Under a microscope, the slim, translucent fish lies motionless on its side, too young even to swim. The only movement is its beating heart. At this early stage of life, the single ventricle of the fish's heart is a hollow tube composed of only about 120 muscle cells. Within three months, those cells, called cardiomyocytes, will replicate, morph, and spread to form a full-sized adult heart. HHMI early career scientist Kenneth Poss recently visualized this dramatic transformation using a colorful new technique to label heart cells like the ones seen here. To read more about this research, visit www.hhmi.org/bulletin/fall2012.

Teaching Genomics, Plainly

DAY ONE OF NEUROCHEMISTRY CLASS LEFT JUNIOR NATHAN ACHILLY dumbfounded. He had sauntered in, confident that this course would follow the same path as his previous lab experiences at Franklin & Marshall College (F&M), in Lancaster, Pennsylvania: a predetermined project, a known outcome, a 100-point lab report.

But on that first day in 2010, professor Rob Jinks stood waving a list of seven mysterious genes. Researchers at the nearby Clinic for Special Children had recently linked mutations in those genes with diseases in Amish and Mennonite patients. The students' assignment was to find out if the genes were the culprits.

The clinic team, two physicians and a molecular geneticist, did not have the resources to determine whether the mutations actually caused the slew of neurological and other symptoms. That required "functional data," observations in cells and animals confirming that the mutations incited problems that could explain the diseases.

Jinks sent his class after that functional data. "It was like, OK, here are some genes and a list of symptoms. Pick one and go," says Achilly.

Aided by an HHMI grant and Jinks' steady encouragement, the students boldly advanced. Through a semester—followed by a year of independent study for some—pairs of students searched databases of gene sequences, engineered bacteria to shuttle mutated genes into cells, and captured images of how those cells reacted to the alterations. In December 2010, over shared pizza, the students handed their evidence to the clinicians.

"We just sat there with our mouths open," recalls clinic physician Kevin Strauss. Jinks, his 13 undergraduate students, and the clinic group published their results on January 17, 2012, in *PLoS ONE*, revealing the mechanisms behind seven perplexing genetic conditions plaguing the local community.

The audacious and controversial experiment was a far cry from the typical college "canned lab," in which students might count wrinkled peas or extract caffeine from coffee. With real-life gene sleuthing as the focus, students would need to find time to learn the neuroscience content necessary to pass a standardized exam. And their teacher would have to manage seven diverging projects.

Jinks wasn't worried. The students taking this upper-level course had already taken a prerequisite neurobiology course; they would simply be learning content to solve problems rather than to pass a

Naomi Wilkinson

test. And he had experience managing several students doing independent study projects at once.

Jinks knew he could do it—with HHMI support. Awarded in 2008, the $1.3 million HHMI grant was structured broadly to bolster genetic analysis and bioinformatics at F&M, allowing for the recruitment of instructors, the career development of current faculty, and outreach to high school teachers to help boost their understanding of bioinformatics. Jinks, who had long studied vision in horseshoe crabs, first tapped those HHMI funds in 2009, to embark on a sabbatical at the University of Pennsylvania. There he performed protein experiments, learned DNA sequencing, and analyzed genetics to better understand vision-related diseases in children.

When he returned to F&M, he approached the Lancaster clinic's research team to collaborate. Physicians Strauss and Holmes Morton, alongside molecular geneticist Erik Puffenberger, had already been teaching an F&M course, describing in colorful case studies their work with patients from the Amish and Mennonite communities.

These so-called Plain People are named for their eschewing of cars, televisions, and other modern conveniences. Because the 50,000 Amish in Lancaster County descended from only 50 to 200 original settlers, and because they tended to intermarry, they—along with the Mennonites—have a high rate of certain genetic disorders. The smaller gene pools and clear family histories make it easier for researchers to trace possible genetic causes. Since 2009, the clinic team has been discovering new genetic illnesses at a rate of 5 to 10 each year.

Their success fueled Jinks' bold and risky idea: to restructure his neurochemistry course to support the clinic team's genetic discoveries. "It was probably ambition coming off a really exciting sabbatical," he says. "But it is a very unique population of patients and a great group of students."

> "The critical thinking involved, thinking of these things as puzzles, has been captivating."
>
> NATHAN ACHILLY

Mutations and Behavior Changes

Jinks went forward with the restructured course, teaching undergraduates how to make strong connections between the bedside and bench. Some students did so literally, by going to the clinic to meet patients and taking that experience back to the lab. Independent study student Rebecca Willert, for example, studied a Mennonite family in which five members displayed a form of intellectual disability characterized by learning delays. Through gene mapping and sequencing of family members' genomes, the clinic team had come up with a single gene as the possible culprit. Puffenberger handed over that information to Willert, in addition to—with the patients' consent—medical records data detailing symptoms and onset.

By searching bioinformatic databases, Willert hypothesized that the normal gene, called *CRADD*, regulates how brain cells sprout and maintain proper connections. Her cellular studies showed that the mutated version of *CRADD* found in the Mennonite family alters the ability of the CRADD protein to interact with other proteins necessary to initiate programmed cell death. Thus, her findings offered a potential reason for the patients' cognitive and learning deficits.

With Strauss and team, Willert shared her scientific sleuthing story with the family and at the annual meeting of the Society for Neuroscience in Washington, D.C., in November 2011.

"We were looking for answers to whatever would cause this," says family member Geneva Martin, whose three sons, brother-in-law, and sister-in-law are affected. "It helps just to know."

Willert says that meeting the family helped her gain a better focus in the lab. "I could put a name and face on what I had been studying for so long," she says, "and actually see the kids and how they interacted."

Now she can imagine how a mutation in a nerve cell might translate into the behavioral changes that she saw. It also helped her better design the experiments that she conducted this past summer when she was hired by the clinic team to continue her work.

Shaking off those first-day jitters, Achilly took to the project as well. After two years uncovering how a mutation in an enzyme involved in protein synthesis might cause Usher syndrome type IIIB, characterized by hearing loss and progressive vision loss, Achilly, who graduated in May, has expanded his sights beyond a medical degree. He now plans to pursue an M.D., Ph.D. in translational neuroscience.

"The two years of experience that I gained from Rob's lab have changed my life in many ways," he says. "The critical thinking involved, thinking of these things as puzzles, has been captivating."

■ –TRISHA GURA

FOR MORE INFORMATION: Among other colleges and universities, Franklin & Marshall College recently won another HHMI grant to advance its work with the Clinic for Special Children (see Institute News).

HHMI Awards $50 Million to Colleges

FORTY-SEVEN SMALL COLLEGES AND universities have accepted a challenge: to create more engaging science classes, bring real-world research experiences to students, and increase the diversity of students who study science.

With HHMI grants totaling more than $50 million, these U.S. institutions will discuss strategies and share know-how with an eye toward eventually disseminating effective models more broadly.

The expectation is that the four-year grants, ranging from $800,000 to $1.5 million, will have a big impact on these small schools, which should be more nimble than larger research universities and better able to quickly develop and test new ideas.

Hope College in Holland, Michigan, plans to use some of its grant money to fund a research program for incoming freshmen the summer before they enroll. The grant will also provide continued funding for Hope's Fostering a Community of Excellence in Science program, which pairs freshman and upperclassman minority students to encourage them to stay in the sciences. The program has already supported nearly 40 students and will expand to help meet the needs of transfer students in the coming years.

"HHMI is investing in these schools because they have shown they are superb incubators of new ideas and models that might be replicated by other institutions to improve how science is taught in college," says Sean B. Carroll, vice president of science education at HHMI.

The Institute invited 215 schools to apply for the competition. Of those, 187 schools submitted proposals. After two rounds of peer review, a panel of 23 leading scientists recommended that 43 awards be made to 47 schools (a joint award went to five undergraduate schools in southern California known as the Claremont Colleges).

Each school chose to work on one of six overarching objectives (see box). "The strategic theme-based approach is a new opportunity that enables the grantees to organize into smaller groups so that faculty from schools can come together throughout the next four years to share ideas, challenges, solutions," said David J. Asai, director of HHMI's precollege and undergraduate program. "We anticipate that the theme-based programs will provide useful models that will inform other institutions, including larger research universities, about strategies that might be replicated." ■

FOR MORE INFORMATION: To see a list of the 2012 awardees, visit www.hhmi.org/news/hhmicolleges20120524b.html.

COLLEGE INITIATIVE STRATEGIC THEMES

- Preparing undergraduates to become K-12 teachers who understand inquiry-based learning.
- Creating curricula that emphasize learning competencies.
- Defining and assessing what it means for a student to be scientifically literate.
- Developing effective strategies that promote the persistence of all students in science.
- Creating course-based research experiences that will help students learn science by doing authentic research.
- Encouraging students to engage in research through one-on-one apprentice-based experiences.

Fifty International Students Get Support from HHMI

ELISA ARALDI IS STUDYING IN THE UNITED States to advance science in Italy. "The reason I came to the U.S. for my graduate education was to train in the best scientific environment in the world and bring my knowledge and expertise back to Italy," she says. Thanks to an HHMI fellowship, she's one step closer to her goal.

Araldi is one of 50 graduate students from 19 countries who were awarded HHMI International Student Research Fellowships. The program provides $43,000 a year to international science students studying in the United States. The fellowships fund the students during their third, fourth, and fifth years of graduate school—a pivotal point in their studies when they must delve into intense laboratory research for their doctoral dissertations.

"Now in its second year, HHMI's International Student Research Fellowships Program is supporting nearly 100 outstanding future scientists from 28 different countries," says William R. Galey, director of HHMI's graduate and medical education programs. "We anticipate that the program will eventually support 150 outstanding biomedical science graduate students in the crucial years of their Ph.D. work." Funding for the program this year is $2.15 million, up from $2 million last year.

Sixty-three Ph.D.-granting institutions were eligible to nominate their graduate students for the fellowships. Of the 19 countries represented in this year's slate of awardees, six—Australia, Bulgaria, Chile, Italy, Latvia, and Switzerland—are new to the program.

HHMI started the program because it recognized that international students in the U.S. often have difficulty securing funding to support their graduate studies. "Being an international student, it is virtually impossible to find fellowships that fully support my stipend," says Araldi, who is studying macrophage biology at New York University's Sackler Institute. "Part of my project involves very sophisticated and expensive experiments, and this fellowship will allow me to perform more experiments."

And more experiments for Araldi means more scientific expertise for Italy. ■

FOR MORE INFORMATION: The names of the International Student Research fellows can be found at www.hhmi.org/news/popups/isrf2012.html.

Medical Fellows Get a Chance to Try Research

THIS PAST SUMMER, 70 MEDICAL, DENTAL, and veterinary students put their courses and rotations on hold to focus on laboratory research. The students, who came from 27 schools across the country, are part of HHMI's Medical Research Fellows Program, a $2.5 million annual initiative to increase the training of future physician-scientists.

The program gives the students the opportunity to spend a year doing biomedical research at laboratories across the United States. Started in 1989, the fellowship has enabled more than 1,400 students to participate in research.

When applying for the program, each student submits a research plan outlining the work they will do with a mentor of their choice. Dylan Wolman, a fellow from Tufts University School of Medicine, applied to work at HHMI's Janelia Farm Research Campus with JFRC fellow Davi Bock and group leader Karel Svoboda. Wolman will be spending his year using light and electron microscopy to study the neural connectivity between the mouse motor and barrel cortices.

"A year of research provides an avenue to practice what should be an essential skill in any scientific field: questioning 'why,'" says Wolman. "It is a thought exercise [that will teach] you to question why an unexplained symptom in a particular disease constellation occurs, and perhaps even help you develop the spark necessary to pursue that question to its answer."

In a first for the program, one of the medical fellows will be spending part of his year in Durban, South Africa, at the KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH). Eric Kalivoda, a student from the University of Vermont College of Medicine, will spend several months in Durban, assisting HHMI investigator William Jacobs with his research on tuberculosis. He will then spend the rest of the year in Jacobs' lab at the Albert Einstein College of Medicine in New York.

"K-RITH offers the unique perspective to study and address [the co-infection of HIV and drug-resistant TB] at its epicenter, and I look forward to working with my mentor and the K-RITH team of scientists to develop improved diagnostics for rapid TB drug-susceptibility testing," says Kalivoda.

This year HHMI also launched a Summer Medical Fellows Program, providing opportunities for 27 medical students to do research for 8 to 10 weeks in the labs of HHMI or Janelia Farm scientists.

The yearlong Medical Research Fellows Program has an important impact on the awardees. Seven of the 71 medical fellows involved in the 2011–2012 program are continuing for a second year, and ten students have decided to pursue a Ph.D. in addition to their medical degree. ■

FOR MORE INFORMATION: For more about the Medical Research Fellows Program and a list of the 2012–2013 fellows, go to hhmi.org/medfellowships.

WEB EXTRA: Go to www.hhmi.org/bulletin/fall2012 to hear from a medical research fellow alumnus.

2012 Holiday Lectures on Science—Changing Planet: Past, Present, Future

JUST OVER 4 AND A HALF BILLION YEARS ago, a cloud of gas and dust came together to form our planet. Since then, it has undergone massive change. In HHMI's 2012 Holiday Lectures on Science, three leading scientists will explore the history of life on Earth and the forces that have shaped, and will continue to shape, our ever-changing planet.

Andrew H. Knoll of Harvard University will use the fossil-record geochemistry to explain the history of life on Earth, as well as discuss his exploration of the geology and possibility of life on Mars. Naomi Oreskes of the University of California, San Diego, will present past scientific models of the forces responsible for shaping the physical features of our planet and describe the current understanding of plate tectonics. Daniel P. Schrag of Harvard University will discuss climate change over geologic time and explain how understanding the past can help us comprehend the impact of our activities on current and future climate.

The Holiday Lectures will take place November 15–16, 2012, at HHMI headquarters in Chevy Chase, Maryland. You can sign up to view a live webcast at www.holidaylectures.org. ■

The Yin and Yang of Plant Defense

A PLANT'S RESPONSE TO INFECTION IS CONTROLLED BY A CONCENTRATION GRADIENT OF SALICYCLIC ACID.

When a plant suffers a microbial infection, it produces salicylic acid. But the role this hormone plays in protecting plants has been somewhat of a mystery. A team of researchers led by HHMI-GBMF investigator Xinnian Dong has proposed a model to explain how salicylic acid controls both cell death at the site of infection and cell survival and immune activation in noninfected tissue.

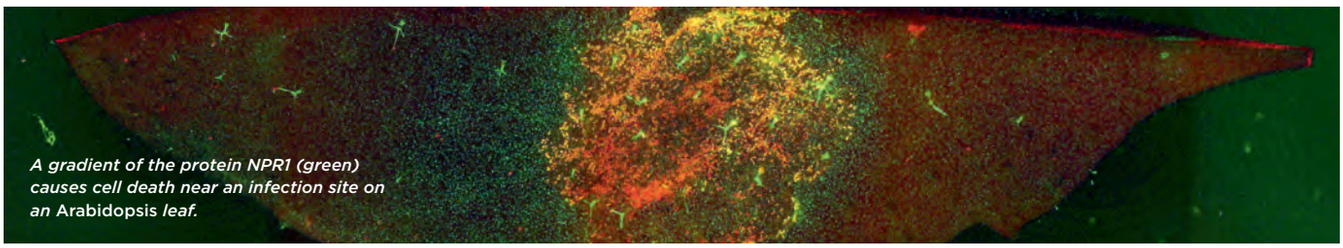
When exposed to infection, plant cells respond either by dying, to protect the rest of the plant, or by activating resistance measures to survive. Salicylic acid and its signal transducer, NPR1, both influence the outcome, but they do not interact directly. Dong and her research team at Duke University studied how two other proteins—NPR3 and NPR4—interact with salicylic acid and NPR1 before and after infection to modulate levels of plant defense.

Observing mutants of the model plant *Arabidopsis thaliana* that lacked NPR3, NPR4, or both, Dong's team concluded that NPR3 and NPR4 both promote degradation of NPR1, whose buildup prevents cells from dying. Next, the team found that salicylic acid had opposite effects on these two protein-protein interactions. It stimulates NPR3's degradation of NPR1—but only with high levels of salicylic acid—and prevents NPR4's degradation of NPR1 in the presence of salicylic acid.

Dong's group proposes that without infection, NPR1 levels are kept low by NPR4-mediated degradation. Upon infection, salicylic acid forms a concentration gradient with higher levels at the infection site and lower levels outward. A high level of salicylic acid in infected cells facilitates NPR3-based degradation of NPR1, and the infected cells die. In cells distant from the damage, the lower salicylic acid level limits the binding of salicylic acid to NPR3 and NPR4, blocking degradation of NPR1, and those distant cells survive.

The team's findings, published June 14, 2012, in *Nature*, may be applied to both agriculture and medicine. "But our dynamic model has to be tested first," Dong says. "That is not going to be easy."

■ –NORA TARANTO



A gradient of the protein NPR1 (green) causes cell death near an infection site on an Arabidopsis *leaf.*

Nadoka Oka and Yasuomi Tada / Kagawa University, Japan, and Xinnian Dong / Duke University

IN BRIEF

MEMORY PLAYS MUSE

An overactive enzyme called HDAC2 may be at the core of memory decline, according to HHMI investigator Li-Huei Tsai, whose lab team showed that blocking the enzyme restores memory in mice with Alzheimer's disease.

HDAC2 switches off genes implicated in learning and memory. When the enzyme was overexpressed in mice, memory function dropped and there was a significant loss of synapses—the information transfer stations between brain neurons. When the researchers reduced HDAC2 in neurons of mice exhibiting Alzheimer's disease, they found a dramatic increase in the number of active synapses. After only four weeks, the HDAC2-blocked mice were indistinguishable from healthy mice in memory tests.

The researchers, whose work was published February 29, 2012, in Nature, also discovered what might put HDAC2 into overdrive. Two known Alzheimer's indicators, the protein amyloid- and oxidative stress—an excess of a reactive form of oxygen—can activate the protein glucocorticoid receptor 1, which in turn switches on rampant production of HDAC2.

Tsai, who is at the Massachusetts Institute of Technology, is eager to see whether blocking HDAC2 is effective in humans. "We need to test this hypothesis in humans to see whether this concept holds up," she says. "While all the data look very promising in animal models, human studies are a completely different ball game."

FOLLOWING A FISH'S EVOLUTION

After the last ice age, when glaciers melted and fresh water streamed across land masses, many animals adapted to new habitats. HHMI investigator David Kingsley and a team of international scientists have followed a fish—the three-spine stickleback—to determine what genetic changes allowed it to thrive in new environments in North America, Europe, and Asia.

Kingsley's team at Stanford University School of Medicine and scientists from the Broad Institute of MIT and Harvard collaborated with international scientists to uncover the complete genome sequences of 21 members of the stickleback family collected from around the world. As described in *Nature*, on April 5, 2012, the scientists looked for DNA sequences that had changed as the fish adapted.

Amazingly, 147 regions in the genome were consistently altered across the 21 sticklebacks. The genetic regions are associated with metabolism, developmental processes, behavior, and body armor formation, identifying a suite of changes that enabled the formerly saltwater fish to survive in fresh water. More than 80 percent of the changes were found in regulatory sequences rather than protein-coding regions, helping to answer a long-standing debate about the relative importance of regulatory and coding changes during adaptive evolution. By studying the diverse stickleback genome, Kingsley says, "we can find the key genes that control evolutionary change."

THE AUTISM NETWORK

Scientists estimate that about one quarter of autism spectrum disorders (ASDs) may be caused by mutations in the eggs or sperm of parents who have no history of ASD. Tracking the origins of some of these so-called sporadic mutations, HHMI investigator Evan Eichler and Jay Shendure, at the University of Washington, found that many of the mutations affect a large protein network that functions in cell cycle regulation and neuronal development.

Like a Chinese Finger Trap

SCIENTISTS ZERO IN ON DISEASE-CAUSING AMYLOID STRUCTURE.

In neurodegenerative diseases like Alzheimer's, the needle-like fibers that accumulate in the brain are not the real damage-doers. The culprits are intermediate protein structures, called small amyloid oligomers, made of a few proteins that misfold and aggregate. But the oligomer's fleeting existence—sometimes lasting only minutes before forming the longer fibers—make them nearly impossible to study. HHMI investigator David Eisenberg has at last pinned down the structure of an amyloid oligomer.

"We wanted to find the toxic agent," Eisenberg, at the University of California, Los Angeles, says about his research published March 9, 2012, in *Science*. "You can't design drugs if you don't know the structure of the toxic agent."

Though the oligomers in major amyloid diseases such as Alzheimer's, Parkinson's, and even type 2 diabetes are short-lived, Eisenberg found one that lasts longer. The needle-like amyloid fibers in some cataracts take decades to form, so the oligomer state of these misfolded proteins can be easily trapped. Eisenberg and graduate student Arthur Laganowsky took advantage of these unhurried aggregates to study the cataract-forming protein, αß crystalline (ABC).

Lagonowsky used a computational algorithm to find the segments of the ABC protein responsible for forming the fibers. He then confirmed that the ABC oligomer had antibody affinities and toxicity patterns similar to those in the major amyloid diseases. Finally, using x-ray diffraction, Lagonowsky saw that the small oligomer consists of a cylinder of six protein chains. They dubbed the structure "cylindrin."

"This cylinder looks sort of like those toys you get in Chinatown, where you put your fingers in and realize they're stuck," says Eisenberg. "It has a structure unlike any of the 70,000 structures catalogued in the open-source Protein Data Bank."

The toxic agents in many neurodegenerative diseases are intermediate structures that form small cylindrical oligomers.

Eisenberg hopes that understanding the cylindrin structure may lead to new approaches to studying the structures of the more elusive oligomers associated with major diseases. "The fundamentals are absolutely critical to understanding medicine," he says. "Work in structure and computation of amyloid diseases is just starting." ■ –HALLEH B. BALCH

David Eisenberg / University of California, Los Angeles

IN BRIEF

Comparing autistic children's genes to those of their parents, the scientists found more than 250 mutations unique to the children. Some mutations had been linked to autism in past studies; others were strong new candidates.

Analyzing the data further, the researchers found that the number of mutations increased with the father's age, and that 39 percent of the mutations affected proteins within the same network.

"I was surprised to see so many of them as part of a highly interconnected set of proteins," Eichler says. Skeptical at first, the scientists sequenced the exomes (the protein-encoding genes) of 50 nonautistic siblings; none carried the mutations found in the network. The research was published April 4, 2012, in *Nature*.

"Understanding how the mutations affect cells will require further experiments," Eichler says, "but converging on a network is a step forward in the field of autism genetics."

IT DOES A BODY GOOD

Milk may contain the calcium that helps build strong bones, but we rely on subtle biological interactions to absorb and make use of the mineral. In one of these interactions, called phosphorylation, a kinase enzyme affixes phosphates to proteins. With correct phosphorylation, proteins are able to bind calcium to form bones and teeth.

For over a century, scientists have known that the milk protein casein contains phosphate. In a study published June 1, 2012, in *Science,* researchers led by Jack E. Dixon, HHMI vice president and chief scientific officer, found that Fam20C—a member of the seldom-studied Fam20 family of proteins—is responsible for the phosphorylation of casein.

Scouring old scientific reports, the researchers also discovered something unexpected: mutations in Fam20C had been reported in patients with Raine syndrome—a fatal disease in which bones become too dense. Dixon's team developed a cell line with the Raine syndrome mutations. "Every single one of these mutations inactivated the Fam20C kinase and prevented it from being secreted," says Dixon. The mutations affected phosphorylation of casein as well as several other proteins involved in enamel and bone formation.

"What we've discovered isn't just the casein kinase," Dixon says. "It's a whole new branch on the kinase tree that seems to play an important role in bone and teeth formation."

FOLLOWING CHI TO ITS PERFECT FORM

Joe Noel calls chalcone isomerase, or CHI, the "perfect enzyme" because of its extreme speed.

Noel, an HHMI investigator at the Salk Institute, wanted to know how CHI evolved to catalytic perfection. He discovered that CHI likely evolved from a noncatalytic protein that played a completely different role in plants, and that CHI and its noncatalytic relatives coexist today.

CHI is crucial to production of flavonoids, which customize plants to varied terrestrial environments. Flavonoids provide UV sunscreens, for example, and colors that lure pollinators.

Noel and colleagues combed through the genome of *Arabidopsis* and found that CHI shares genetic similarities with three members of the FAP protein family.

To learn how the two protein families were related, the scientists introduced *FAP* genes into *E. coli* bacteria, and then studied

Wiring the Brain

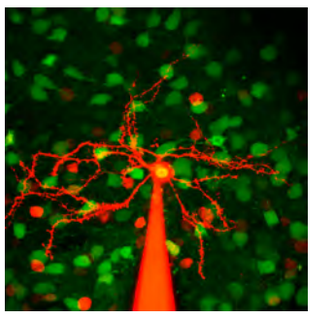
DEVELOPMENT IN SOME PARTS OF THE BRAIN IS CONTROLLED BY NEURAL ACTIVITY RATHER THAN EXPERIENCE.

A newborn's brain develops by forming and strengthening new synapses—connections between neurons—in response to sights, sounds, and experiences. But one part of the brain, the basal ganglia, doesn't receive inputs from the external world, so how does it build its wiring shortly after birth?

Research by HHMI investigator Bernardo Sabatini suggests that self-reinforcing loops of neural activity may drive the development of synapses in the basal ganglia, a region of the brain that uses sensory and social context to direct movement.

The basal ganglia's main input center—the striatum—orchestrates movement through two pathways: a direct pathway that stimulates movement and an indirect pathway that inhibits movement. To learn how striatal activity affects neuronal development, Sabatini and his colleagues at Harvard Medical School bred mice whose indirect or direct pathways were turned off because they were incapable of releasing the chemical messenger GABA.

The scientists expected that silencing these neurons would stop them from forming connections between the striatum and receiving neurons. Instead, even with the striatum's output signal silenced, the synapses grew normally. However, silencing the direct pathway prevented the formation of connections delivering input to the striatum, and silencing the indirect pathway increased the growth of these input synapses. The circuit was basically wiring itself—output controlled the development of input.



An electrode fills an indirect pathway neuron with a red fluorophore to help measure its growth.

In a follow-up experiment, the group reduced the activity in neurons providing input to the striatum during development. When these mice reached adulthood, their brains had fewer neuronal connections in their striatum than normal mice, suggesting that wiring changes in the basal ganglia during early development can have lasting effects.

This research, says Sabatini, reveals the existence in basal ganglia development "of a very important positive feedback loop where something can establish itself by driving its own maturation into the right state." Sabatini's team published its results May 31, 2012, in *Nature*.

"We went into the study with hypotheses that were completely disproven by the study," says Sabatini. Ready for more surprises, he will continue to engineer mice, activating and inactivating specific neurons to reveal how perturbations affect wiring later in life. ■ –NORA TARANTO

IN BRIEF

the expressed FAP proteins with x-ray crystallography. Unexpectedly, the researchers saw pockets in FAPs that held tightly to fatty-acid molecules from the bacteria. In a similar way, CHI grabs chalcone to turn it into plant flavonoid.

Tracking FAP proteins in *Arabidopsis*, the team found that they play a role in fatty acid biosynthesis and regulation in chloroplasts. Even algae, the modern-day example of early plant ancestors, rely on FAPs. Based on these findings, Noel posits that FAPs are the immediate precursor to CHI. The work was published May 13, 2012, in *Nature*.

WHAT MAKES YOUR CLOCK TICK?

Two proteins, CLOCK and BMAL1, are responsible for the circadian rhythms that regulate our sleep patterns, blood pressure, and metabolism on a 24-hour schedule. Now, HHMI investigator Joseph S. Takahashi has solved the three-dimensional structure of these proteins.

CLOCK and BMAL1 are "the batteries of the biological clock," says Takahashi, at the University of Texas Southwestern Medical Center.

Despite the proteins' important role, scientists had been unable to resolve their structures. Since the discovery of the *Clock* gene in 1997, researchers had tried in vain to isolate the CLOCK and BMAL1 proteins in the crystalline form required for x-ray crystallography. Takahashi's group succeeded by coexpressing the two proteins and then paring them down to isolate the areas that interact with each other. They discovered that CLOCK is entwined around BMAL1 and that tight interactions occur in three different regions. In one region, the proteins are interlocked, and in a second region, a single amino acid from BMAL1 fits snugly into a groove in CLOCK.

With the structure in hand, published May 31, 2012, in *Science*, scientists can delve into how other proteins vie for the binding spot on CLOCK, and how gene mutations of the protein-binding site alter circadian rhythms. Takahashi next hopes to solve the proteins' full-length structures and their complexes with other CLOCK proteins.

NOT YOUR PARENTS' HOME VIDEO

Suspended in liquid and illuminated by beams of light, thousands of cells divide and differentiate into the neurons, muscle, and skin of a fruit fly embryo. These are the kinds of movies scientists can capture with a new microscope designed by biophysicist Philipp Keller.

Named SiMView, for simultaneous multiview light sheet microscopy, the microscope captures millions of images, allowing scientists to watch developmental biology unfold with unprecedented detail. The images rely on two sheets of laser light that scan back and forth over the embryo for hours on end. The fluorescently labeled cells emit light in response to the laser illumination, allowing Keller and his colleagues at HHMI's Janelia Farm Research Campus to watch each cell move and divide throughout the embryo's development.

On a large table filled with optical lenses and mirrors angled to precision, the lasers are flawlessly aligned, and the two cameras straddling the sample chamber must be in perfect calibration to achieve the high-resolution videos. "Instead of looking at the sample from one direction" like most microscopes do, Keller says, "we look at it from four directions at the same time." The result is a view of the developmental process as it occurs—from every side of the embryo, start to finish.

The results were published June 3, 2012, in *Nature Methods*.

Bernardo Sabatini / Harvard Medical School

Q A

We all have foods we like and dislike, but from an evolutionary standpoint it seems like we shouldn't turn up our noses at anything that helps us survive, and we shouldn't have different tastes from other people living in the same environment. Why do we develop distastes for certain foods?

Asked by a college student from Maine

Our genes and the environment influence our food preferences. Our taste buds distinguish between bitter, sour, sweet, umami (savory), and salty flavors, and we may even have a "fat" taste receptor. Scientists have identified some of the genes responsible for these different receptors and believe that variations within these genes may affect our individual food preferences. Environmental pressures that are no longer an issue probably caused our taste buds to evolve, leading to preferences that may not make sense in the modern world.

For example, scientists believe that the ability to detect bitterness evolved to prevent consumption of poisonous—and often bitter—plants. Unfortunately, some healthy plant-based foods, such as broccoli, also trigger the same bitter taste perception and associated negative reaction. The ability to perceive the bitterness of a chemical called phenylthiocarbamide (PTC) reflects a particular genetic variation affecting the range of a person's bitter taste recognition. Interestingly, different populations have different sensitivities to PTC. One study showed that only 3 percent of West Africans do not perceive PTC as bitter, while about 30 percent of Caucasian North Americans do not (see Further Reading). One can imagine that populations in West Africa may have developed this advantage based on greater exposure to certain bitter-tasting poisonous plants.

Sour taste may also serve as a protective mechanism. Most animals reject sour-tasting foods. This reaction probably evolved to protect against eating spoiled food or unripe fruit.

Our preference for sweet foods may be due to the fact that sweet foods are usually high in energy, and our prehistoric ancestors would have needed high-energy meals to survive. The many generations of humans preceding us did not have the food luxuries we have today. Food was scarcer, and an active lifestyle kept them from developing obesity. While the genetic variations underlying the preference for sweetness are still being studied, one can imagine that these variations could have arisen based on the availability of food sources for a particular population. Overall, preference for sweetness is a complex trait—one influenced by genetics and the environment.

Umami food—that is, food with a savory, meaty flavor—is another taste that appeals to humans. Since the body uses similar molecular processes to detect sweet and umami foods, our affinity for this high-energy food group is not surprising.

Our preference for salt seems to be influenced most by environmental factors, mainly childhood exposure to salty foods. Studies suggest that people with lower sodium exposure as infants have less fondness for salty foods as adults, compared with those who had higher exposure to sodium in infancy.

Recent research indicates that mice recognize and prefer fatty foods. A receptor on the tongue called CD36 appears to control how mice perceive fat. Though humans have a version of CD36, its role in human fatty-taste perception is unknown. As with sweet and umami taste preferences, an attraction to fatty foods would have maximized survival chances of our ancestors by directing them to eat high-calorie foods.

We'd likely be better off if we were less quick to choose the sweet or meaty, high-calorie foods that supported our ancestors' survival and, instead, went for the bitter-tasting broccoli or mustard greens.

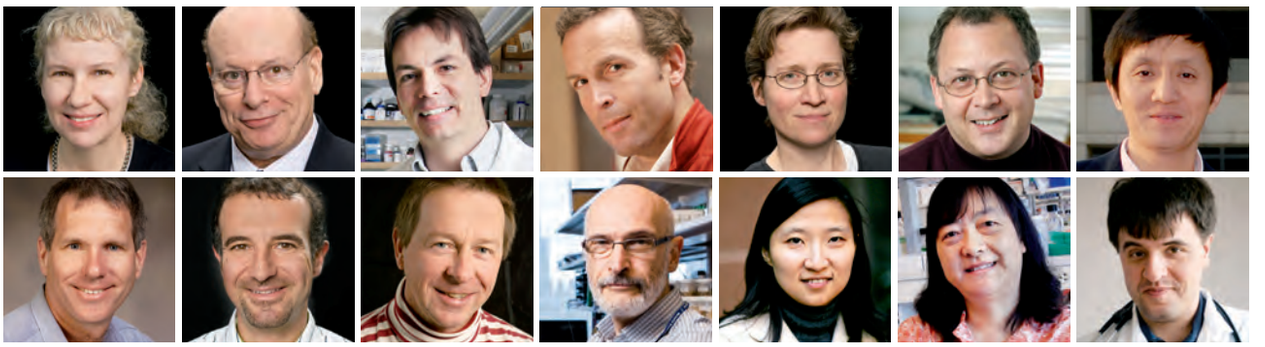
ANSWERED BY ROXANA DANESHJOU,
a 2011 HHMI medical fellow at Stanford University School of Medicine.

FURTHER READING:
Garcia Bailo B, Toguri C, Eny KM, El-Sohemy, A. Genetic variation in taste and its influence on food selection. *OMICS* 2009; 13:69–80.

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

SPOTLIGHT

Fourteen Elected to National Academy of Sciences



TOP ROW: NANCY M. BONINI, GIDEON DREYFUSS, EVAN E. EICHLER, K. CHRISTOPHER GARCIA, RACHEL GREEN, GREGORY J. HANNON, LIQUN LUO
BOTTOM ROW: ROY PARKER, NIKOLA P. PAVLETICH, LOUIS J. PTÁČEK, ALEXANDER Y. RUDENSKY, XIAOWEI ZHUANG, XINNIAN DONG, KARL DEISSEROTH

Twelve HHMI investigators, an HHMI-Gordon and Betty Moore Foundation investigator, and an HHMI early career scientist were elected to the National Academy of Sciences. The investigators are **Nancy M. Bonini**, University of Pennsylvania; **Gideon Dreyfuss**, University of Pennsylvania School of Medicine; **Evan E. Eichler,** University of Washington School of Medicine; **K. Christopher Garcia**, Stanford University School of Medicine; **Rachel Green**, Johns Hopkins School of Medicine; **Gregory J. Hannon**, Cold Spring Harbor Laboratory; **Liqun Luo**, Stanford University; **Roy Parker**, University of Arizona; **Nikola P. Pavletich**, Memorial Sloan-Kettering Cancer Center; **Louis J. Ptáček**, University of California, San Francisco; **Alexander Y. Rudensky**, Memorial Sloan-Kettering Cancer Center; and **Xiaowei Zhuang**, Harvard University. The HHMI-GMBF investigator is **Xinnian Dong**, Duke University, and the early career scientist is **Karl Deisseroth**, Stanford University.

Nine HHMI investigators and one HHMI professor are among 220 new members and 17 foreign honorary members elected to the American Academy of Arts and Sciences. HHMI's newly elected members are JAMES J. COLLINS, Boston University; BRIAN J. DRUKER, Oregon Health and Science University; SARAH C. R. ELGIN, HHMI professor, Washington University in St. Louis; SUSAN FERRO-NOVICK, University of California, San Diego; TYLER E. JACKS, Massachusetts Institute of Technology; RICHARD P. LIFTON, Yale School of Medicine; LIQUN LUO, Stanford University School of Medicine; DANNY F. REINBERG, New York University School of Medicine; BRENDA A. SCHULMAN, St. Jude Children's Research Hospital; and STEVEN A. SIEGELBAUM, Columbia University. DAVID W. OXTOBY, a member of the HHMI Science Education Advisory Board, was also elected to the academy.

Foldit, an online game created by HHMI investigator DAVID BAKER of the University of Washington, took first place in the interactive game category in the National Science Foundation's 2011 International Science and Engineering Visualization Challenge. Baker's free game allows players to fold proteins for fun while contributing to scientific research.

HHMI investigators CORNELIA I. BARGMANN of the Rockefeller University, BONNIE L. BASSLER of Princeton University, and JACK SZOSTAK of the Massachusetts General Hospital were elected to the American Philosophical Society. The APS is the oldest learned society in the United States; its members are selected from a variety of disciplines ranging from the arts to the physical sciences.

The Norwegian Academy of Sciences and Letters awarded its 2012 Kavli Prize in Neuroscience to CORNELIA I. BARGMANN, an HHMI investigator at the Rockefeller University; WINFRIED DENK, a senior fellow at Janelia Farm Research Campus; and Ann M. Graybiel of the Massachusetts Institute of Technology. The three scientists, who are recognized for elucidating basic neuronal mechanisms underlying perception

SPOTLIGHT

Quake Receives Lemelson-MIT Prize

STEPHEN QUAKE

HHMI investigator **Stephen Quake** of Stanford University was awarded the 2012 Lemelson-MIT Prize, which honors outstanding midcareer inventors dedicated to improving the world through technological invention. Quake is being recognized for his work in drug discovery, genome analysis, and personalized medicine. His group was the first to use microfluidic technology in the determination of protein structure through x-ray crystallography.

Bonini: Paul Fetters Dreyfuss: Paul Fetters Eichler: Ron Wurzer / AP Garcia: Barbara Ries Green: Paul Fetters Hannon: Zack Seckler / AP Luo: Charlene Liao Parker: Margaret Hartshorn Pavletich: Paul Fetters Ptáček: Paul Fetters Rudensky: Memorial Sloan-Kettering Cancer Center Zhuang: Cheryl Senter / AP Dong: Jim Bounds A/P Deisseroth: Darcy Padilla Quake: George Nikitin / AP

and decision, will share an award of one million dollars.

HHMI investigator **BONNIE L. BASSLER** of Princeton University and HHMI vice president and chief scientific officer **JACK E. DIXON** have been elected as foreign members of the Royal Society. Fellows and foreign members gain membership in the society for contributions to fundamental research and for leading scientific and technological progress in industry and research establishments.

HHMI investigator **JOANNE CHORY** of the Salk Institute for Biological Studies was awarded the 2012 Genetics Society of America Medal for outstanding contributions to genetics for the last 15 years. Chory studies the mechanisms plants use to alter their growth in response to changes in the environment.

The American Phytopathological Society presented **JEFFERY L. DANGL**, an HHMI-GBMF investigator at the University of North Carolina at Chapel Hill, with the 2012 Ruth Allen Award. Dangl was honored for his research on the molecular mechanisms underlying the plant immune system's ability to discriminate between friend and foe.

KARL DEISSEROTH, an HHMI early career scientist at Stanford University, was awarded the University of North Carolina's 12th Perl-UNC Neuroscience Prize. Deisseroth shares the prize with Edward Boyden and Feng Zhang of the Massachusetts Institute of Technology for their development and application of optogenetics to study neural circuit function. Deisseroth also received the 2012 Klaus Joachim Zülch Neuroscience Prize from the Gertrud Reemtsma Foundation in Germany and the 2012 Rush and Helen Record Neuroscience Award from the Baylor College of Medicine.

The **EDUCATIONAL RESOURCES GROUP** in the HHMI science education department recently won two Telly Awards for the short film *The Making of the Fittest: Natural Selection and Adaptation*. The awards are given to film and video productions, both broadcast and online.

STEPHEN J. ELLEDGE, an HHMI investigator at Brigham and Women's Hospital, has won the American-Italian Cancer Foundation's 2012 Prize for Scientific Excellence in Medicine. Elledge studies the regulation of the cell cycle and is also interested in the development of genetic technologies to aid in gene and drug discovery.

The National Hemophilia Foundation presented **KATHERINE A. HIGH**, an HHMI investigator at the Children's Hospital of Philadelphia, with its Leadership in Research Award. High studies the molecular basis of blood coagulation and the development of novel therapeutics for the treatment of bleeding disorders.

HHMI investigator **HELEN H. HOBBS** of the University of Texas Southwestern Medical Center received the International Atherosclerosis Society's inaugural Antonio M. Gotto Jr. Prize in Atherosclerosis Research. Hobbs' research focuses on defining genetic factors that contribute to variations in the levels of cholesterol in the blood.

The Gruber Foundation presented HHMI investigators **LILY Y. JAN** and **YUH NUNG JAN**, both of the University of California, San Francisco, with its 2012 Neuroscience Prize. The scientists were recognized for their work on how potassium channels control brain activity and how brain cells diversify and specialize during embryonic development.

ERIC R. KANDEL, an HHMI investigator at the Columbia University College of Physicians and Surgeons, received the American Psychiatric Association's 2012 Adolf Meyer Award. Kandel studies the basic molecular mechanisms underlying learning and memory in animals.

HHMI investigator **WILLIAM G. KAELIN JR.** of the Dana-Farber Cancer Institute is a recipient of the 2012 Lefoulon-Delalande Foundation's Scientific Grand Prize. He shares the prize with Peter J. Ratcliffe of the University of Oxford and Gregg L. Semenza of the Johns Hopkins University School of Medicine for research on how tissues and organs sense and adapt to changing oxygen levels.

JEREMY NATHANS, an HHMI investigator at the Johns Hopkins University School of Medicine, is the recipient of the 2012 Albert C. Muse Prize, given by the Eye and Ear Foundation of Pittsburgh. Nathans uses molecular genetic approaches to study the development of the mammalian retina and embryo.

HHMI investigator **CHARLES L. SAWYERS** of the Memorial Sloan-Kettering Cancer Center was elected president of the American Association for Cancer Research. In this role, Sawyers will work with the AACR membership and board of directors to further the association's mission to accelerate progress in the prevention and cure of cancer.

The 11th annual Wiley Prize in Biomedical Sciences was awarded to **RONALD D. VALE**, an HHMI investigator at the University of California, San Francisco. Vale shares the prize with Michael Sheetz at Columbia University and James Spudich at Stanford University for explaining how cargo is moved by molecular motors inside cells.

SPOTLIGHT

Horwich Awarded Shaw Prize

ARTHUR HORWICH

Arthur Horwich, an HHMI investigator at the Yale School of Medicine, received the 2012 Shaw Prize in Life Science and Medicine. He shares the $1 million award with Franz-Ulrich Hartl of the Max Planck Institute of Biochemistry for their contributions to understanding the molecular mechanism of protein folding. Horwich and Hartl determined that the protein chaperonin acts as a cage-like folding "machine" that provides a safe place for proteins to fold, away from outside interference.

Jerry Domian

CONTINUED FROM PAGE 29
(THE FAT YOU CAN'T SEE)

to ways to identify patients at risk for severe liver disease, or to distinct treatment methods from those targeting obesity and blood sugar.

For now, though, scientists agree on the best way to treat—and reverse—NAFLD: weight loss, exercise, and a balanced diet.

In the same way three cans of soda a day can quickly lead to fatty liver, Loomba says, weight loss and exercise can rapidly reverse it. He's seen patients, within weeks of beginning an exercise regimen, show improvement in both liver fat levels and insulin resistance in liver cells.

"If we get patients with type 2 diabetes to lose relatively small amounts of weight by diet alone, we can cure most cases of fatty liver, hepatic insulin resistance, and type 2 diabetes," says Shulman. "But unfortunately getting patients to lose weight and keep it off is one of the most challenging clinical endeavors."

There are multiple drugs in clinical trials for treating later stages of liver disease—the inflammation and scarring—but no pill to cure early NAFLD. And because of the complex interconnections between diabetes, weight, and liver fat, a single cure-all drug targeting only the liver is unlikely to be discovered. But with each discovery of genes, molecular pathways, and bacteria that contribute to the disease, scientists feel they are getting closer to being able to identify patients most at risk and help lessen the severity of their disease.

"I think we have probably just moved from the infancy to the adolescence of understanding this disease," says Loomba. "The hope is that our understanding of fatty liver is really going to explode in the next five years. I think we are on the cusp of something big." ■

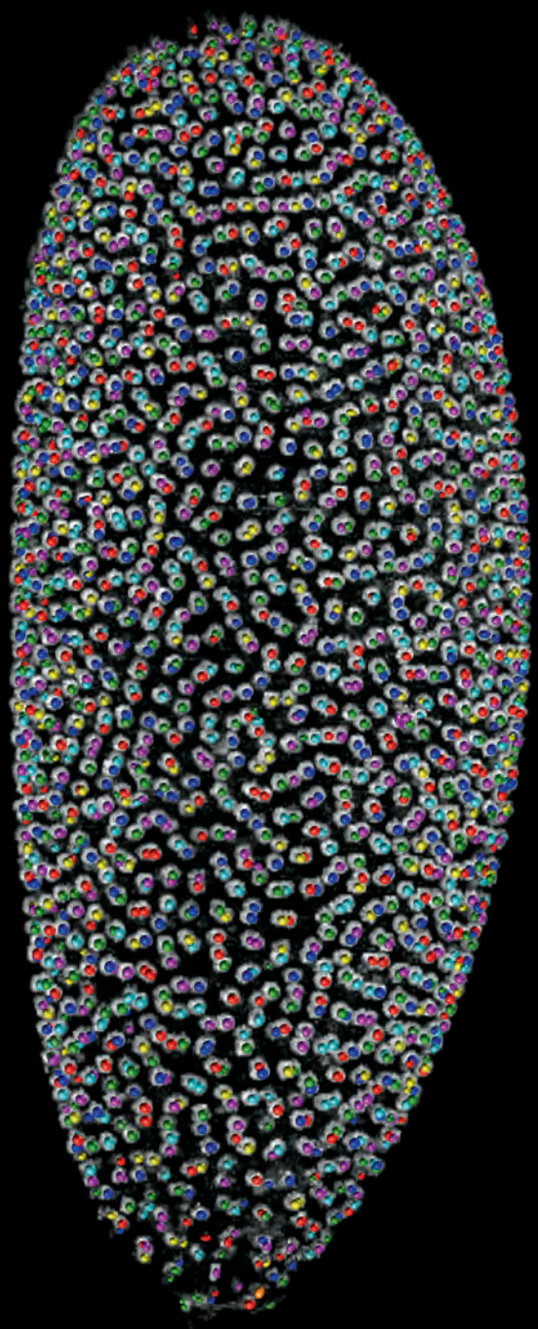
CONTINUED FROM PAGE 33
(THE VIEW FROM HERE)

genetically induced changes. He aims to learn how neural circuits associated with those genetic variations produce and control specific animal behaviors.

Jensen is pushing computational reconstruction of reality in pursuit of scientific understanding in yet another direction. He and visualization experts in his laboratory have been transforming their electron cryotomography data into mechanism-revealing animations. Says Jensen, "If a picture is worth a thousand words, then an animation is worth a million."

"We have done movies where we fly into cells and look at things from different points of view," says Jensen. "We have tried to illustrate mechanisms of how things work inside cells." He believes animations can be powerful educational tools. "Some people still don't believe that HIV is the causative agent of AIDS, for instance, so they decline treatments that could help them," says Jensen. "Our animations of HIV's molecular transformations will help them understand that we are not making this up."

As researchers move through the early part of Schnitzer's third phase of biological imaging, Jensen is already imagining what might be a fourth phase. "The ultimate would be a microscope where you could see down to individual atoms while they were being arranged and rearranged inside a cell in its living state," Jensen muses. "If you could develop an imaging tool like that, the study of cell biology would be all but over," Jensen says, noting, however, that it would take a century or more to digest such a comprehensive portrait of life. An outlandish vision for revealing biology's deepest structures and mechanisms, to be sure, but that is just the sort of thinking it takes to unveil molecular biology in all of its elaborate minutiae. ■



30

From small beginnings come great things. It's hard to believe that this brightly studded balloon will within hours become a flight-ready fruit fly. By following color-coded cells in a *Drosophila* embryo, such as the one seen here, Philipp Keller tracks the origin and movement of cells over time using a microscopy technique he developed at Janelia Farm Research Campus. See his video of the embryo's development from start to finish at www.hhmi.org/bulletin/fall2012.

Keller lab / Janelia Farm Research Campus

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

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

Out of Africa

This beauty is *Aedes aegypti*, a mosquito that can transmit dengue and yellow fever viruses, among others. Originally from Africa, it is now found in tropical and subtropical regions around the world. While this mosquito prefers to feast on animals over humans, it lives in close proximity to blood-hungry relatives that will choose a human meal every time. HHMI investigator Leslie Vosshall and her lab group are studying the evolutionary changes behind the insects' diverse dietary habits. Their research may help reduce mosquito-borne illnesses. Read about Vosshall and the arc of her scientific career in "Avant Garde Scientist," page 18.

Leslie Vosshall