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hhmi bulletin

in this issue

Super Resolution

Nobel-winning work provides a stellar view of cellular detail

12

Immune cells will do anything within their power to do their jobs. Some, like this neutrophil, are driven to crawl, swim, or ooze through our bodies' tissues and fluids to be a first responder at an acute infection or inflammation. Until recently, we couldn't have known what these cells look like in action, as they morph from a sphere into a shape-shifting tangle of appendages and ruffled edges. But now, remarkable advances in imaging live cells offer a whole new view of biology.

Lillian Fritz-Laylin, Graham Johnson,
Megan Riel-Mehan, and R. Dyche Mullins

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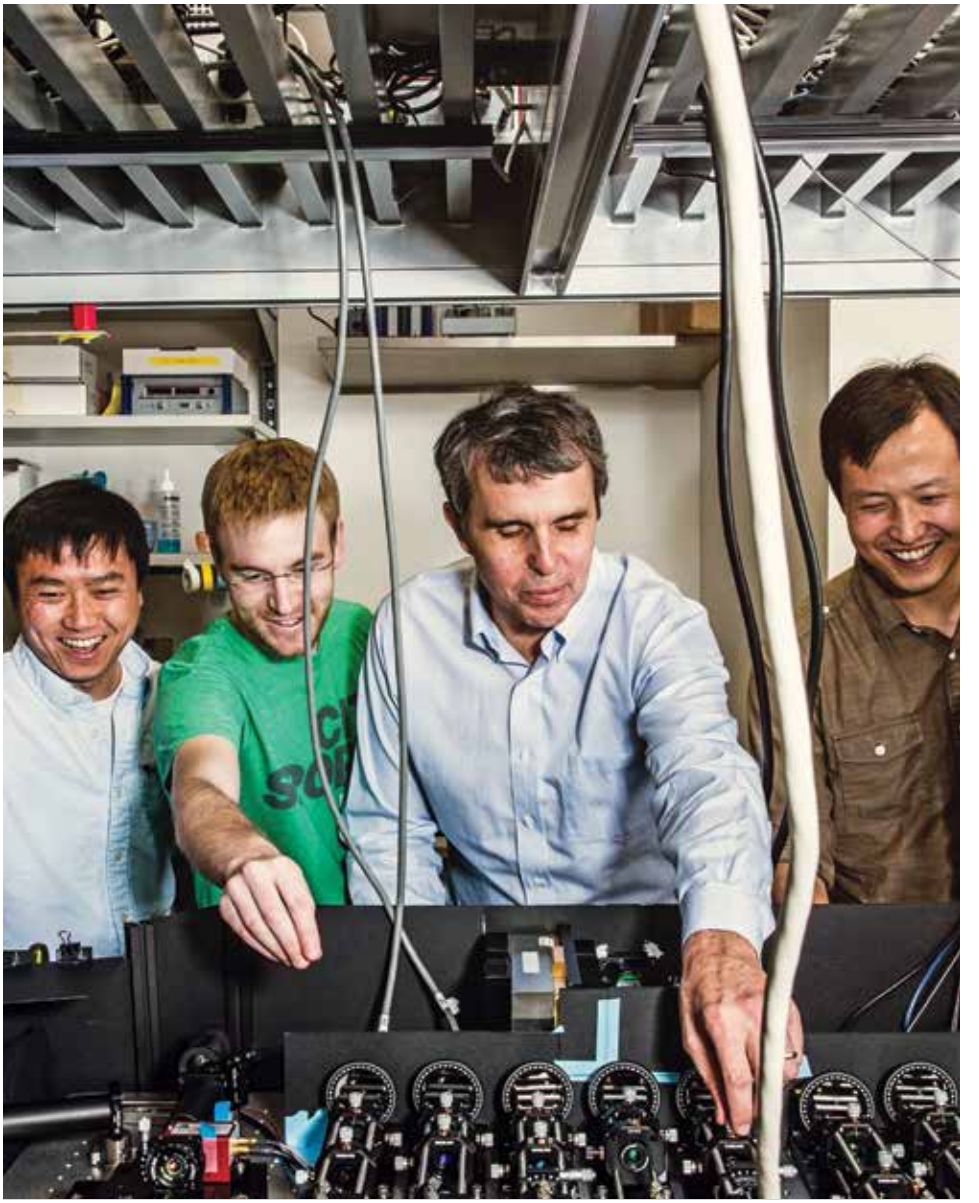

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- **Follow a group of Michigan scientists as they face flaming logs and pits of mud in a Warrior Dash.**
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Cover illustration: Markos Kay

Contributors

Henry McCausland ("Warrior for a Day," page 4) lives in London and works as an international illustrator. He enjoys pens, his health, sunshine, and being asked to draw people running.

A California-based photographer, **Leslie Williamson** ("Rethinking GMO Regulation," page 30) is best known for her portraits of people and their spaces, and for her two books, *Handcrafted Modern: At Home with Midcentury Designers* and *Modern Originals: At Home with Midcentury European Designers*. She travels the world in search of creative people working on exciting projects.

Connecticut-based writer **Cathy Shufro** ("Immersion in the Lab," page 34) also tutors writing at Yale College. A 2012 International Reporting Project fellow, she never considered visiting Russia until writing her story for this issue. Now she's reading the satirical novel that Russian scientist Mikhail Gelfand recommended– *Heart of a Dog*, by Mikhail Bulgakov.

Markos Kay (cover and "Defying Limits," page 12) is a digital artist, illustrator, and lecturer based in London. His work explores the visualization and abstraction of the microscopic world through computer simulations. He is a keen collaborator, always on the lookout for scientists interested in visualizing their data.

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Markos Kay, Leslie Williamson, Henry McCausland, Cathy Shufro

President's Letter

James Kegley

Discovery and Perseverance

LAST MONTH, ERIC BETZIG, a group leader at HHMI's Janelia Research Campus, traveled to Sweden to accept the Nobel Prize in Chemistry. Eric shared the award with two other scientists, Stefan Hell, of the Max Planck Institute, and William Moerner, of Stanford University, for their remarkable contributions to push beyond the limits of light microscopy. It was a watershed moment for Eric and for all of us at HHMI. After all, Janelia was created with people like Eric in mind—scientists who are intellectually daring and committed to discovery, productivity, and perseverance.

Eric joined HHMI in October 2005, a year before the doors opened at Janelia. He shared the Institute's vision for the campus: a place where small, interdisciplinary groups work collaboratively to build powerful tools and pursue some of science's most profound questions. And he helped recruit other talented scientists, including his close friend and PALM microscope collaborator Harald Hess.

As they and other Janelians built their labs in those early days, they were also helping to establish the principles and culture that now characterize this unique community. From the outset, sharing Janelia's resources and tools with the broader scientific community was part of our plan. One example of that is our visiting scientist program. We also recently partnered with the Gordon and Betty Moore Foundation to establish an Advanced Imaging Center, where select scientists from around the world can access potentially transformative imaging tools developed at Janelia, including those developed by Eric.

"From the outset, sharing Janelia's resources and tools with the broader scientific community was part of our plan."
—ROBERT TJIAN

My own work has benefited greatly from super-resolution imaging systems like PALM and Eric's more recent lattice light sheet microscope, which captures three-dimensional images of living cells with exceptional clarity and detail. These tools have allowed my team to track the movement and binding activity of individual transcription factors, which help transcribe DNA into RNA, in living human or animal cells. We can determine how long a protein needs to find a target site, how much time it spends at that site, and in what order the multiple proteins that make up the transcription machine complex are assembled.

My lab has long hoped to "watch" transcription factors in action in normal and diseased cells. We are still early on in this exciting journey, but we know that single-molecule, single-cell imaging will significantly impact studies of transcription mechanisms, which will in turn help us understand how genes are expressed during an organism's development and how disease disrupts this process.

It's worth noting that the 2006 publication on PALM was the first journal article to come out of Janelia. Today, as Janelia approaches its 10th anniversary, the campus includes some 800 people, including scientists in fields ranging from computation to engineering to neural theory. We have much work to do—and more discoveries ahead.

For a GoPro video of the race, visit www.hhmi.org/bulletin/winter-2015.

Warrior for a Day

HOT BUT NOT yet sweaty, the small group of scientists had ditched their lab coats for a motley assortment of spandex and microfiber athletic gear (save one outlier in an orange jumpsuit). They stood in a vast field among thousands of similarly clad individuals, stretching and jogging in place, exchanging nervous banter, and eyeing the other competitors. Over the next hour or so, they'd test their mettle not by trying to design the best experiments and publish the most impressive findings, but by leaping over flaming logs, wading through pits of mud, and scaling 20-foot-tall cargo nets.

The harrowing feats were part of an event called the Warrior Dash. "You run like crazy and go as fast as you can," says molecular biologist James Bardwell, an HHMI investigator at the University of Michigan. Bardwell and biochemist Ursula Jakob, also a member of the Michigan faculty, recruited 11 members of their labs—two postdocs, four graduate students, and five undergrads—to enter the event with them. None of their labmates had ever done an obstacle race before, but all were game. "It sounded like a really fun, healthy, team-building thing to do," recalls Adam Krieger, an undergrad in Jakob's lab.

"Our labs have done things together before this," says Jakob. "But I have to say, the Warrior Dash was definitely the best experience because we were all in it together, just struggling to get through."

Billed as the "world's largest obstacle race series," Warrior Dashes are held year-round and worldwide; the Michigan scientists took part in a late July event in Mount Morris, Michigan. A Warrior Dash sends participants—aka, warriors—on a grueling 5K course through a "battleground" strewn with obstacles bearing formidable names like Muddy Mayhem and Diesel Dome. Afterward, they're rewarded with a massive party replete with live music, food, and plenty of beer.

The event also has a serious side. Warriors can raise money for St. Jude Children's Research Hospital. And many hundreds of muddy running shoes discarded by participants after the race are ground into playground surfacing material by a company called USAgain. Since 2009, warriors at 200-plus races have raised more than $10 million for St. Jude and donated some 70,000 pairs of shoes. They've also devoured 35,000 pounds of turkey legs.

Bardwell and Jakob believe events like the Warrior Dash help build a camaraderie that carries over to their labs. At the race, the 13 members of their group split into smaller squads that helped each other navigate the challenging terrain. "You really have to work together," says Jakob. "Science is all about communication and collaboration, and not much different from an obstacle course."

By the time they crossed the finish line, the warriors were bruised, tired, and grateful for the massive outdoor showers erected by race organizers. "You're totally covered in mud," says Bardwell. "Everybody's taking off shoes because they're all muddy. And everyone is feeling really good.

"The experience brought the lab together—there's no question," he adds. "When you go through some event that's tough, you pull together as a team."

–Nicole Kresge

Henry McCausland

To see more of McConnell's photos, go to www.hhmi.org/bulletin/winter-2015.

Second Calling

IT HAPPENED NEARLY a decade ago, but Susan McConnell remembers the moment with telephoto clarity: she was standing on the deck of a ship in the Svalbard archipelago, halfway between mainland Norway and the North Pole, watching a polar bear leap from one ice floe to another.

She pressed her camera to her eye, trying to anticipate each jump. Her biceps ached. Her fingers were so numb she could no longer feel the shutter button. "I was cold to the core," says McConnell, a neuroscientist at Stanford University, "and I realized that I'd never been happier in my life."

Though McConnell had traveled to Svalbard seeking just such a sight, she wasn't an especially serious photographer. In fact, she was ambivalent about photography. Looking through a lens, worried McConnell, would disconnect her from the moment.

Instead, the opposite proved true. The challenge of composing a shot deepened McConnell's experience, making her aware of each subtle shift in the bear's posture, each change in light and mood. She returned from Svalbard with the fires of a new passion stoked.

Her passion for wildlife, though, had been there from the start. McConnell was an "animal-obsessed kid," she says, the kind who kept hamsters and rabbits and parakeets, who spent days watching a colony of wood rats beside her family's Pennsylvania home. She idolized Jane Goodall and dreamed of someday following the great primatologist's footsteps into an African jungle.

As an undergraduate in biology in the late 1970s, however, McConnell found behavioral studies unsatisfying. Sure, she could describe a monkey yawning or courting a mate—but what was happening in its brain to produce the behavior?

So began a neurobiology career devoted to understanding the dynamics of neurons in the cerebral cortex. McConnell's focus shifted from wild animals to laboratory animals, where it would remain for two decades. Then came the trip to Svalbard.

Wildlife photography turned into a second vocation, and McConnell quickly graduated from capturing pretty pictures to telling stories with her photographs: about a meerkat family staring down a marabou stork or about elephants rescuing a baby stuck in an abandoned water trough, as well as about the larger themes of conservation.

On a planet where so many creatures are imperiled by the rapacity of a certain bipedal primate, McConnell's new mission is to produce "powerful images that connect us with our natural heritage and stimulate a commitment to conservation," she says. Her work has been published in *Smithsonian* and *National Geographic* magazines and displayed in galleries.

Yet she doesn't consider taking pictures to be an escape from the laboratory. Rather, she says, conservation photography and neurobiology are two trunks that have grown from the same root.

Both can be traced back to the experiences of that animal-loving girl, and McConnell, who this year received funding as an HHMI professor to help life science students communicate research through art, wants to pass that love along.

At Stanford, she teaches conservation photography to undergraduates, helping them learn to tell stories with a purpose. "Just as much as I'm excited by my own pictures," says McConnell, "I'm excited by the photographs my students take." *–Brandon Keim*

Susan McConnell

Memory Swap

Researchers gain insight into how emotion-tinged memories are stored in the brain.

IMAGINE YOURSELF ON the warm, white sand of a tropical beach. As you listen to the lapping waves and inhale the salt-scented air, you form a memory that can summon a feeling of contentment for years to come. But if a destructive hurricane roars in and ruins your vacation, remembering that same beach in the future might evoke anxiety or fear. Can you rewire your memory post-hurricane, ensuring that white sand and salt air will continue to evoke positive feelings?

Perhaps. "Emotion is intimately associated with memory of past events, but the [positive or negative] valence of memories is actually quite malleable," says HHMI Investigator Susumu Tonegawa. He explains that the brain stores a memory's contextual information—details about where and when an event occurred—separately from its emotional content. The brain circuit connecting those two components can be rewired, giving the memory of an event a new emotional association, he says.

There's evidence of that malleability in the success of psychotherapies that help people overcome anxiety or depression by summoning positive thoughts to replace negative ones. Tonegawa, a neuroscientist at the Massachusetts Institute of Technology who studies memory in mice, wanted direct evidence of how the brain makes such a switch. So he and his colleagues, research specialist Roger Redondo and grad student Joshua Kim, devised a strategy to replace an animal's fear of a specific place with a positive memory of the very same location.

They succeeded. What they discovered helps explain the connections between different components of memory and how altering those connections might reassign a memory's emotional value. "We've provided some scientific basis for already ongoing psychotherapy and found a target in the brain for future therapies," he says.

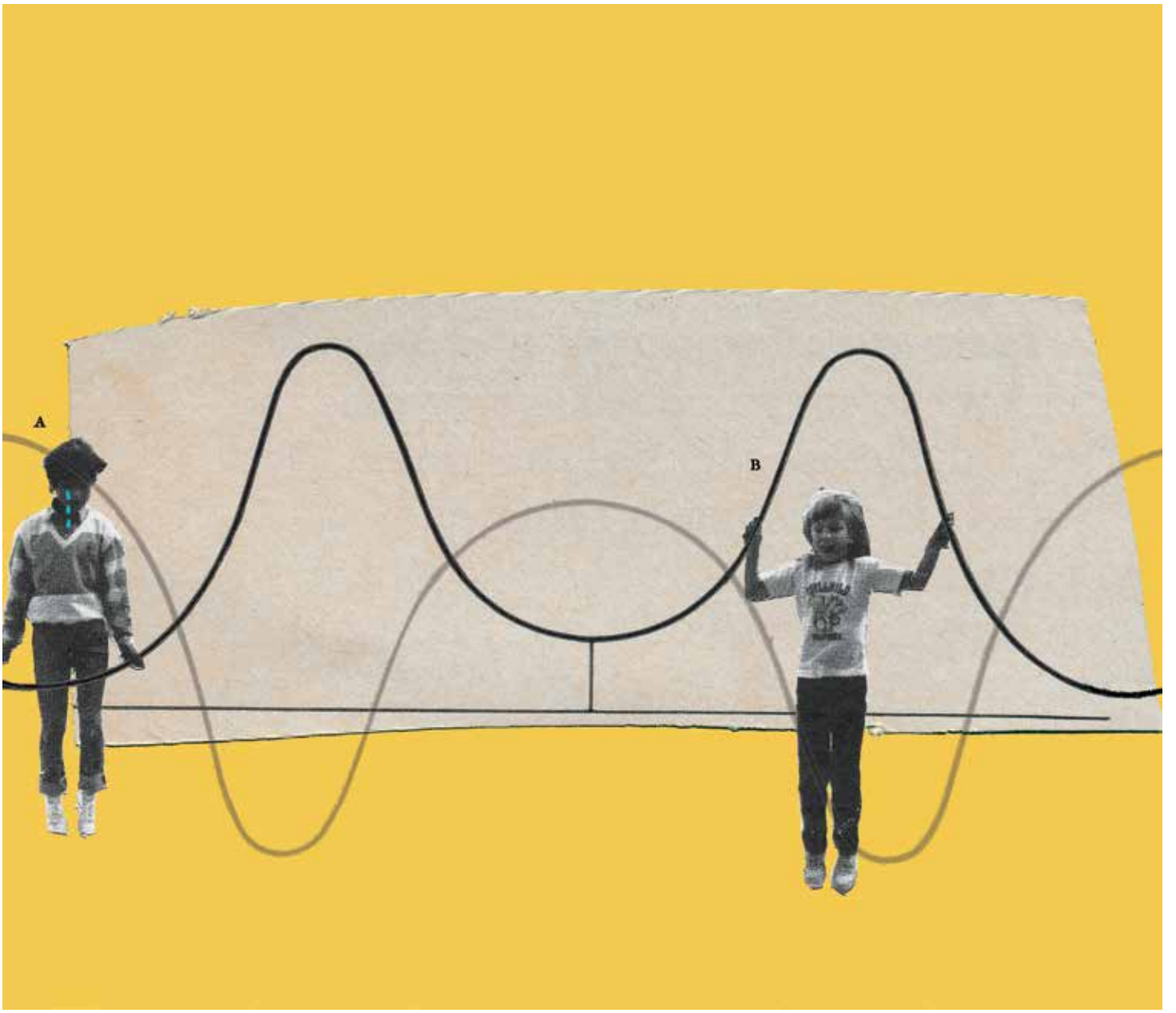
Tonegawa and his colleagues first created false memories in mice in 2013. They placed mice in a safe environment, then made the brain cells storing the memory of that place sensitive to light, a feature the researchers could use to activate the cells. When they switched on those cells in a mouse exploring a perilous new space, it caused the mouse to fear the original environment. So they knew they could attach emotion to a memory that previously evoked neither fear nor pleasure. But once an animal had developed fear of a place, could its memory of that place be made pleasurable instead?

To find out, Tonegawa's team placed male mice in a chamber whose floor delivered a mild shock. The scientists had previously introduced a light-sensitive protein into the rodents' brain cells that stored information about the dangerous place. As in their 2013 experiments, they did this by linking production of the protein to a gene that is switched on as memories are encoded, thus restricting light sensitivity to the precise cells that stored the newly formed memory. They further restricted the light-sensitive protein to one of two regions of the brain—the hippocampus, where contextual information about a memory is stored, or the amygdala, which houses a memory's emotional content. Then they removed the mice from the uncomfortable chamber.

A few days later, the scientists artificially reactivated the memory by shining a light on the animals' brains. The mice stopped their explorations, freezing in place—typical fear-based behavior.

To try to overwrite that fear, the scientists placed the mice in a new environment. Instead of electrical shocks, pleasure awaited: the new cage held female mice. As the mice mingled, the researchers reactivated the male rodents' memory-storing neurons. They switched on the cells responsible for the original fear memory one set at a time—in some mice, they activated cells in the context-storing hippocampus; in others, they triggered memory cells in the emotion-storing amygdala. Then they tested what emotions the animals now associated with the original chamber.

The results were striking. When the scientists reactivated the amygdala component of the original memory during the pleasurable encounter, the male mice retained their fear. But when the researchers reactivated the memory-storing hippocampal cells while the mice interacted with females, the memory of the original chamber acquired a new



Joanna Neborsky

"Emotion is intimately associated with memory of past events, but the [positive or negative] valence of memories is actually quite malleable."
—SUSUMU TONEGAWA

emotional association. Now the mice sought out environments that triggered the memory. When they put the animals back in the unpleasant environment, the mice explored without hesitation. "The original fear memory is very much lost," Tonegawa says.

The researchers found similar results when they switched the emotion of a memory in the opposite direction, replacing a pleasurable memory with fear.

By locating regions of activity in microscope images of the animals' brains, Tonegawa's team concluded that a set of hippocampal neurons stores contextual information about a memory and connects to either of two sets of amygdala neurons: one responsible for positive memory, the other for negative memory. The memory-altering experiments rewired those connections after they had been established.

The study, published September 18, 2014, in *Nature*, confirms that brain circuits that attach negative emotion to a memory are not permanent. Further, it identifies a specific part of the brain as a potential target for new treatments against depression and other mental illnesses.

"To target drugs or any other therapy to a particular area of the human brain is a challenge," Tonegawa acknowledges. "But the technology develops so fast. It's quite possible that there's a way of noninvasively manipulating cells' activity in humans. These kinds of studies with animal models are providing information about how to target those procedures." –*Jennifer Michalowski*

Bench Report

Immunity Meets Metabolism

Long associated with allergies and parasitic infections, a rare immune cell now appears to have a role in other bodily processes as well.

WHEN RICHARD LOCKSLEY attended grade school in the 1950s, peanut butter sandwiches were a lunchtime staple. Yet "by the time my kids went to school, they were outlawed," says the HHMI investigator at the University of California, San Francisco.

Allergies to nuts and other foods soared 50 percent between 1997 and 2011. The symptoms—rashes, breathing trouble, and even life-threatening anaphylaxis—are due to overblown inflammatory responses. Asthma, another condition traced to hyperactive immune cells, is also on the rise.

That poses a conundrum. "Why would we evolve an immune response to torture ourselves?" Locksley wondered.

Recent work by the physician-turned-scientist suggests the answer may lie in unusual lymphoid cells that sense nutrient intake in the gut. According to conventional wisdom, immune responses linked to allergic inflammation serve to protect us from intestinal worms. After all, the branch of the immune system blamed for allergy and asthma—"type 2 immunity"—also helps fend off parasitic worms known as helminths.

But Locksley saw holes in this line of thinking. For one, scores of vertebrate animals in the wild have worms. "They don't get asthma or allergy—but we do," he says.

Plus, he adds, "In developing countries, where people run around barefoot, 100 percent of the population has intestinal helminths. They come from the soil and water." Infected individuals have heightened type 2 immunity yet can't clear the worms; disease symptoms often don't show up for years, if at all.

Clues to the conundrum came when Locksley considered a different vantage point: the worm's. Could it be that worms tap into metabolic pathways that allow them to thrive in the human gut for decades?

The journey that led Locksley to explore this possibility began in the 1980s. As a postdoc, he became fascinated with the parasite *Leishmania*. In one mouse strain, *Leishmania* infection swelled the animals' footpads but seemed otherwise harmless. Yet in another strain, the parasite made the mice waste away and die. Through cell transfer experiments, researchers had learned that certain lymphocytes—CD4-expressing T cells, or "T helper cells"—determine if a mouse resists or succumbs to *Leishmania* infection.

"The same T cells can kill you or help you. Something's different about them, and I wanted to find out what," recalls Locksley.

It turns out that CD4 T cells are not homogeneous. These T helper (Th) cells assume different roles governed by their release of signaling proteins known as cytokines. Mice that produce mostly "Th2" cytokines—interleukin-4 (IL-4) and interleukin-13 (IL-13)—succumb to infection by *Leishmania*, Locksley and colleagues discovered. But mice that mainly make "Th1" cytokines, such as interferon gamma, resist infection.

At the time, scientists thought that subsets of CD4 T cells were the source of these cytokines. But when Locksley's team deleted the *IL-4* and *IL-13* genes in mouse CD4 cells, the animals could still fight off certain worms. However, blocking these cytokines with antibodies rendered the mice defenseless. This suggested that some other cell type also supplies the cytokines.

"The same T cells can kill you or help you. Something's different about them, and I wanted to find out what."
—RICHARD LOCKSLEY

To find the mystery cells, the researchers fluorescently tagged these cytokine genes in mice to see where the cytokines were expressed. They discovered that a rare cell—type 2 innate lymphoid, or ILC2—was a potent source of IL-13 and IL-5, a related Th2 cytokine.

Unlike most immune cells, ILC2s do not circulate in blood. Instead, they reside in skin, heart, lung, and, most prominently, small intestine and fat tissue. "That changed the game," Locksley says.

His lab's recent discovery of ILC2's role in metabolism challenges longstanding beliefs about other immune cells, including eosinophils. Implicated as initiators of allergic inflammation and in fighting worm infections, eosinophils can also accumulate in the small bowel—but no one knew why. "Some survival factor must be dialed up, because otherwise you wouldn't see these cells there," Locksley says. "They're too rare."

Analyzing reporter mice, his team identified that survival factor as IL-5, a cytokine produced by ILC2s in the small bowel. The results appeared in October 2013 in *Nature*. Earlier that year, Locksley and colleagues reported in the *Journal of Experimental Medicine* that ILC2s sustain eosinophils in fat tissue, too.

More recent work from Locksley indicates ILC2s also help keep lungs healthy. His team triggered asthma in mice by spraying purified chitin into their lungs. Chitin, the biopolymer that makes insect bodies brittle, is also found in molting helminths and their eggs, as well as in other common urban allergens such as fungi and molds. Locksley's team determined that chitin unleashes molecular signals that activate lung ILC2s in the mice. Turning on these Th2 pathways proved essential for restoring lung health, the team reported in March 2014 in *Immunity*.

The findings call into question the assumption that ILC2s, and the Th2 immune responses they activate, serve primarily to ward off parasitic worms. Instead, Locksley says, it might be that worms exploit "metabolic pathways that are good for tissues in order to expedite their own survival and replication over many years."

By exploring the signals that guide ILC2s in metabolism and other bodily processes, scientists may learn how to calm asthma and allergic reactions. Perhaps PB&J can even return as a school lunch standby for Locksley's grandchildren. *–Esther Landhuis*

Leslie Williamson

Richard Locksley wants to get to the root of allergy and asthma.

Bench Report

The Protein at the End of the Tunnel

Scientists reveal the structure of a protein that performs the final step in an iconic and long-studied signaling pathway in cells.

EMBRYONIC DEVELOPMENT AND cancer may seem wildly different, but it turns out they have a lot in common. A Rube Goldberg-like cell signaling pathway that scientists have been studying for 30 years holds the key to the unlikely connection. New research from HHMI Investigator Leemor Joshua-Tor at Cold Spring Harbor Laboratory has characterized the end point in that pathway.

One similarity between cancer cells and the undifferentiated stem cells of an embryo is a microRNA molecule called let-7, which is destroyed in both cases. MicroRNAs prevent certain genes from being expressed and translated into proteins; the let-7 microRNA turns off genes that promote cell division. Without let-7, cells keep dividing—allowing the expansion of embryonic cells or, later in life, of cancers.

A microRNA begins as a stretch of self-complementary RNA that folds up on itself like Velcro, forming a double-stranded molecule with a loop at the end called a microRNA precursor. This precursor is then processed to form a 22-nucleotide microRNA that targets protein-coding messenger RNAs, either preventing their translation into proteins or leading to their degradation. In cancers and in stem cells, let-7 doesn't make it to maturity because its precursor is marked for destruction by a "tail" of uridine bases—the U's in the genetic alphabet. Another protein, Dis3l2, recognizes this polyU tail and degrades it, along with the let-7 RNA precursor.

Joshua-Tor's lab group used x-ray crystallography to identify the structure of Dis3l2 bound to a string of U's, like those that tag let-7 for degradation. As they reported in *Nature* on October 9, 2014, her team found that the RNA fits into a funnel-shaped cavity in Dis3l2. Within the mouth of the funnel and along its narrow end, Dis3l2 makes uridine-specific contacts with let-7's tail—not just recognizing the tail as RNA but "reading" its individual letters.

"Seeing how many bases are read in a row is quite unusual," says Joshua-Tor. Once Dis3l2 makes its contacts along the string of U's, it begins to break down the RNA, letter by letter, until the entire let-7 precursor is destroyed. "Once it gets going, it just chews it all up," she says.

A Path of Twists and Turns

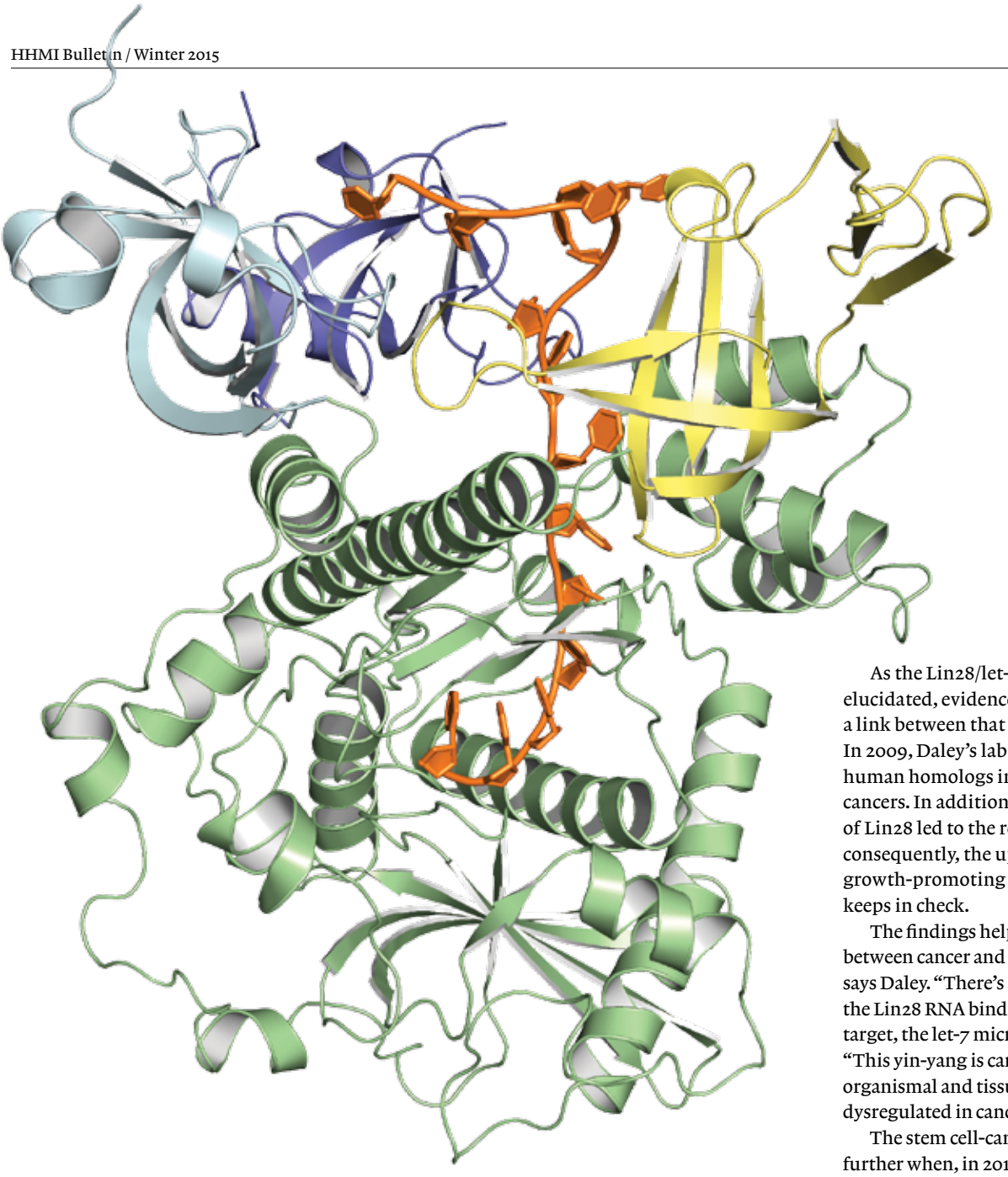
The demise of let-7 is the end point of a complex signaling pathway set in motion by a protein called Lin28.

The story of that pathway begins in the mid-1980s, in the Massachusetts Institute of Technology lab of H. Robert Horvitz, now an HHMI investigator. Horvitz's postdoc Victor Ambros was looking for genes that alter development in the roundworm *Caenorhabditis elegans*. In the mutants that Ambros (now at the University of Massachusetts Medical School) and Horvitz were studying, worms either fell behind or jumped ahead developmentally. One of the genes they identified was *lin-28*; worms in which the gene was defective jumped ahead in development.

Similarly, in both mice and humans, the Lin28 protein is abundant in embryonic cells but lessens throughout development, becoming undetectable in fully differentiated cells.

In 2000, let-7 came onto the scene when Gary Ruvkun, at Harvard Medical School, reported that in worms, a loss of let-7 had an effect opposite to a loss of *lin-28*: it caused differentiated cells to revert to earlier developmental stages. Ruvkun also identified let-7 as a microRNA—the second ever discovered (after *C. elegans*' lin-4, which Ambros discovered). Let-7 targets *lin-28* messenger RNA, repressing its gene activity. Ruvkun published a second paper in 2000 showing that the let-7 microRNA is conserved across most organisms, including humans.

Lin28 soon found a practical application. In 2007, scientists reported that turning on four genes, including *lin-28*, in human fibroblast cells returned the cells to an embryonic state—essentially, into stem cells.



Leemor Joshua-Tor's lab used x-ray crystallography to identify the structure of Dis312 bound to a string of uridine bases.

A Rube Goldberg-like cell signaling pathway that scientists have been studying for 30 years holds the key to an unlikely connection between development and cancer.

But though Lin28 and let-7 were known to have opposite effects on differentiation, their relationship was unclear. It was in 2008 that Richard Gregory and HHMI Investigator George Daley, at Boston Children's Hospital, collaborated to show at the molecular level that Lin28 represses let-7 maturation.

"To preserve the stemness, or pluripotency, of the cell," explains Joshua-Tor, referring to stem cells' ability to differentiate into many cell types, "you have to downregulate let-7, and the way the cell does that is by making Lin28."

Soon afterward, Narry Kim at Seoul National University reported exactly how Lin28 turns off let-7: by promoting the addition of the polyU tail. And by 2009, the Kim and Gregory labs had found the enzyme, called a TUTase, responsible for adding the tail.

As the Lin28/let-7 pathway was being elucidated, evidence was also mounting for a link between that pathway and cancer. In 2009, Daley's lab team found Lin28's two human homologs in 15 percent of human cancers. In addition, overexpression of Lin28 led to the repression of let-7 and, consequently, the upregulation of the growth-promoting genes that let-7 normally keeps in check.

The findings help to explain the connection between cancer and embryonic development, says Daley. "There's a yin and yang between the Lin28 RNA binding protein and its main target, the let-7 microRNA," he explains. "This yin-yang is carefully orchestrated during organismal and tissue development and [is] dysregulated in cancer formation."

The stem cell-cancer link was strengthened further when, in 2013, Gregory identified Dis3l2 as the protein that degrades uridine-tagged let-7 in mouse stem cells. It turns out that Dis3l2's human counterpart is mutated in Perlman syndrome, a genetic disease that leads to fetal overgrowth and a predisposition to a certain kidney tumor. It is also the protein whose crystal structure Joshua-Tor determined.

Researchers may now want to see if Dis3l2 degrades uridine-tagged RNA molecules other than let-7. "It's unlikely that this enzyme exists solely to degrade the precursor to let-7," Gregory says. "There must be some other relevant RNA substrates." As Daley notes, many messenger RNA molecules have polyU tails. Could Dis3l2 act on them, too? "There's at least the provocative possibility," he says. *–Ashley P. Taylor*

Fueled by a desire to help biologists, **Eric Betzig has spent the last decade** devising ways to break free from the constraints of light microscopy–

defying limits

work recognized with the 2014 Nobel Prize in Chemistry.

BY JENNIFER MICHALOWSKI

ILLUSTRATION BY MARKOS KAY

ERIC BETZIG IS a physicist and an engineer: he thinks in terms of light waves and energy, and when he tinkers in the lab, it is with lasers and mirrors and beam splitters. He's the first to admit that he is no biologist. But lately, his lab at the Janelia Research Campus has been bustling with experimental biologists who arrive toting vials of cells and leave with gloriously detailed videos of molecular life in motion. Those scientists return to labs all around the world with their minds racing, full of ideas about how Betzig's newest invention—a microscope that lets users collect high-resolution, three-dimensional images over prolonged periods—might transform their research.

It's not the first time biologists have clamored to get their hands on one of Betzig's groundbreaking imaging tools. New technologies have streamed steadily out of Betzig's lab as he has resolutely ticked off items on the ambitious to-do list that he laid out when he came to Janelia in 2005: faster microscopes, gentler microscopes, microscopes that look deeper, and microscopes that reveal more detail. Nine years later, he says, "I'm getting close to done."

Biologists are accustomed to designing their experiments around the limitations of available tools. For fluorescence microscopy—the method of choice for finding and following specific molecules inside cells—that has in the past meant choosing between imaging speed, detail, or the well-being of the sample. But Betzig has always been eager to hear about his colleagues' frustrations. "If you know the problem, that's half the battle to coming up with the solution," he says. "That's the source of creativity." Betzig has spent the past decade applying *his* creativity to finding ways around the usual imaging trade-offs, thus giving biologists the freedom to apply *their* creativity to investigating the questions they hope to answer.

In October, Betzig's creativity was recognized with the 2014 Nobel Prize in Chemistry; he shares the prize with Stefan Hell of the Max Planck Institute for Biophysical Chemistry in Germany and William Moerner of Stanford University. The prize committee lauded the three scientists for their development of super-resolved fluorescence microscopy—methods of visualizing objects so small that, until recently, distinguishing them with a light microscope was considered a feat that would defy the fundamental laws of physics.

Living Color

Betzig helped launch a revolution in super-resolution microscopy in 2006, when he developed a method that he and his collaborator Harald Hess called photoactivated localization microscopy, or PALM. The technique creates stunningly detailed images of cells by taking advantage of fluorescent labeling molecules that can be switched on and off with a pulse of light. In PALM, a sample labeled with these fluorescent tags is imaged many times, with a small subset of the fluorescent tags switched on each time. Because just a smattering of molecules is glowing in the image, each one can be pinpointed with precision. Compiling thousands of images yields a picture in which nearly all fluorescently labeled molecules show up as individual and distinct.

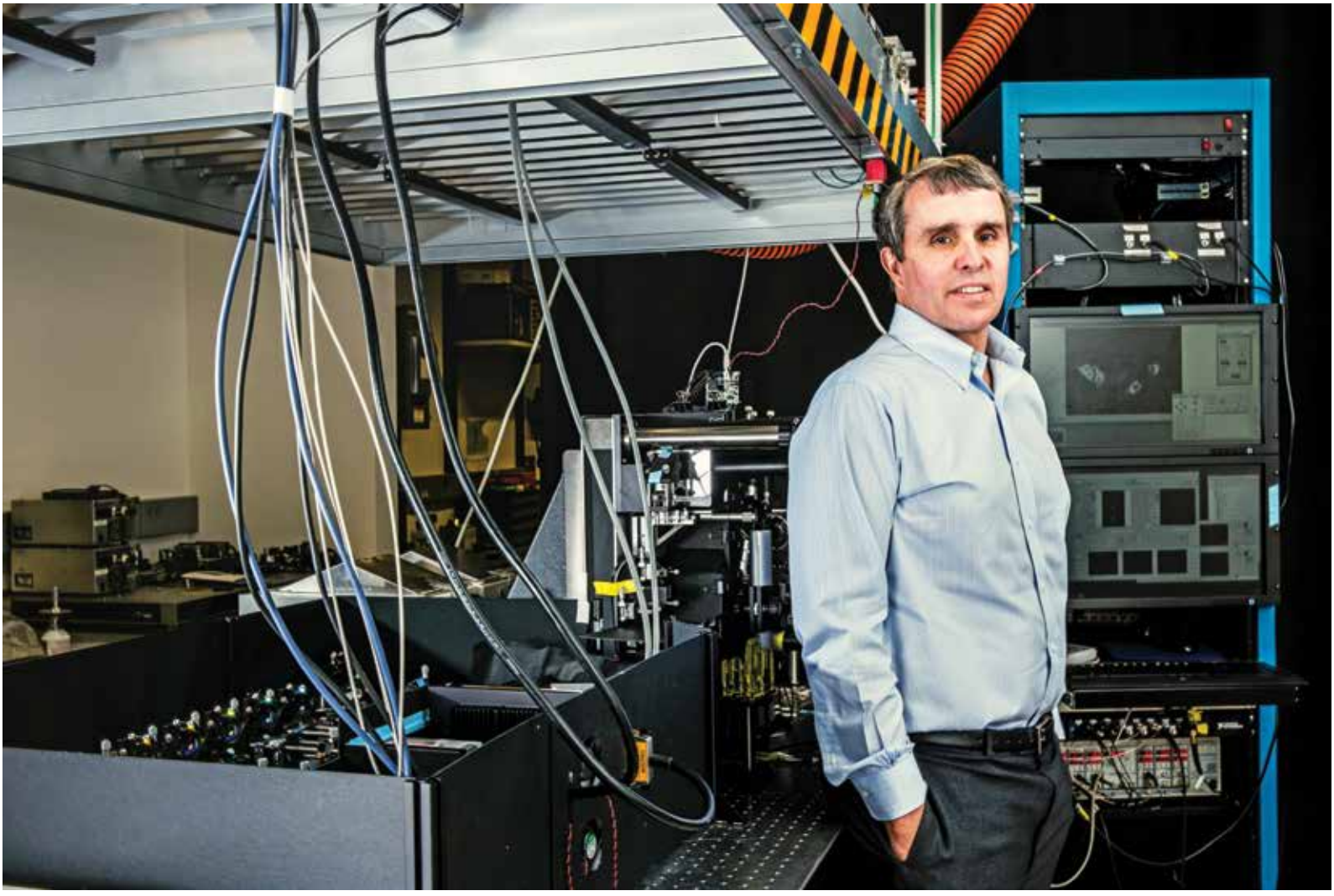
The Janelia campus—where both Betzig and Hess are now group leaders—was still under construction when Betzig first learned of the fluorescent probes that would make PALM possible. So he and Hess built a prototype of the microscope in Hess' living room in La Jolla, California. Later, they set up shop in a trailer on the campus of the National Institutes of Health, where they worked with cell biologist Jennifer Lippincott-Schwartz and others to generate the microscope's first spectacularly revealing images of cells at work. "The first time we put a dilute suspension of these [fluorescent] molecules on the surface, and we turned on the photoactivating light, we knew we had it," he remembers. "It felt like flipping a switch."

Betzig and postdoctoral researcher Hari Shroff "lived and breathed PALM" during his first few years at Janelia, adding features that made the technique more powerful, such as the ability to detect multiple colors—meaning more than one kind of protein can be tracked at once—and the possibility of imaging living cells. But Betzig knew the method had its limits. "It's too slow, and you throw too much light at the cells," he says, explaining that generating a single PALM image subjects samples to toxic light exposure time and time again. Because of that, Betzig realized, the principles of PALM would never translate into what biologists really craved: the same extraordinary resolution in three-dimensional images of living cells.

With a spate of other labs by then working to enhance super-resolution microscopy, Betzig decided it was time to move on. "Everybody and his kid sister was doing super-resolution then. You have to go to the areas where other people aren't," he says. "Those are the most fertile areas to find something useful."

Fertile Fields

He saw plenty of opportunities. Now that he was at Janelia, mingling daily with scientists working at the forefront of neuroscience research, he became intent on improving their capability to look inside the brain. To investigate how nerve cells mediate thoughts and actions, scientists need



Eric Betzig enjoys helping biologists answer challenging questions.

visual access to all parts of the brain—but most microscopes can't see very far beneath the surface. That's because the insides of cells bend light in unpredictable ways. The farther light travels through tissue to the microscope, the more distorted an image becomes.

"I knew that these guys probably didn't realize how crummy their imaging gets as they start peering into flies and mice," Betzig says. But he knew there was lots of room for improvement. He also knew that astronomers had already solved a similar problem. Turbulence in our planet's atmosphere disrupts light as it travels to telescopes on Earth, much the same way light bends and bounces as it passes through biological tissue. To correct these distortions, astronomers shine a laser into the sky toward the object they want to observe and measure how the atmosphere distorts the light from this "guide star" as it returns to Earth. Then they use those measurements to cancel out the atmospheric aberrations in the images they see through their telescope.

Betzig and Na Ji, a postdoctoral researcher in his lab who has since become a Janelia group leader, mimicked this strategy by inserting a fluorescent bead into brain tissue to act as a biological guide star. By imaging that guide star, they can calculate the corrections required to bring into sharp focus the blurry structures in images of the surrounding tissue.

At the same time, Betzig was thinking about another problem that limited biologists' ability to image living cells:

"If you know the problem, that's half the battle to coming up with the solution. That's the source of creativity."

—ERIC BETZIG

Stephen Voss

too much light. Light is essential to activate the fluorescence that makes chemically tagged molecules visible under an optical microscope, but it is also toxic to cells. What's more, it burns out fluorescent molecules, so labeled molecules' signals fade over time. Because of that, Betzig's biologist colleagues told him, they couldn't always monitor cellular processes for as long as they would like, and they often had to sacrifice detail or speed to prolong the lives of their cells.

Betzig says part of the problem is that, although a microscope's objective lens focuses on just one plane of a sample at a time, most microscopes shine a beam of light all the way through their samples, bombarding out-of-focus regions with no imaging payoff. At a Janelia conference, Betzig learned that Ernst Stelzer, now at the Buchmann Institute for Molecular Life Sciences in Frankfurt, Germany, was limiting light exposure by imaging with a sheet of light instead of a beam. "It was an elegantly simple solution to the problem of photo damage and out-of-focus background," says Betzig.

"The best ideas are the simple ones," he adds. This was one he thought he could adapt for high-resolution imaging. Stelzer's technique prolonged the period he could monitor the activities of whole cells, but the light sheets he used were too thick to reveal cells' inner workings.

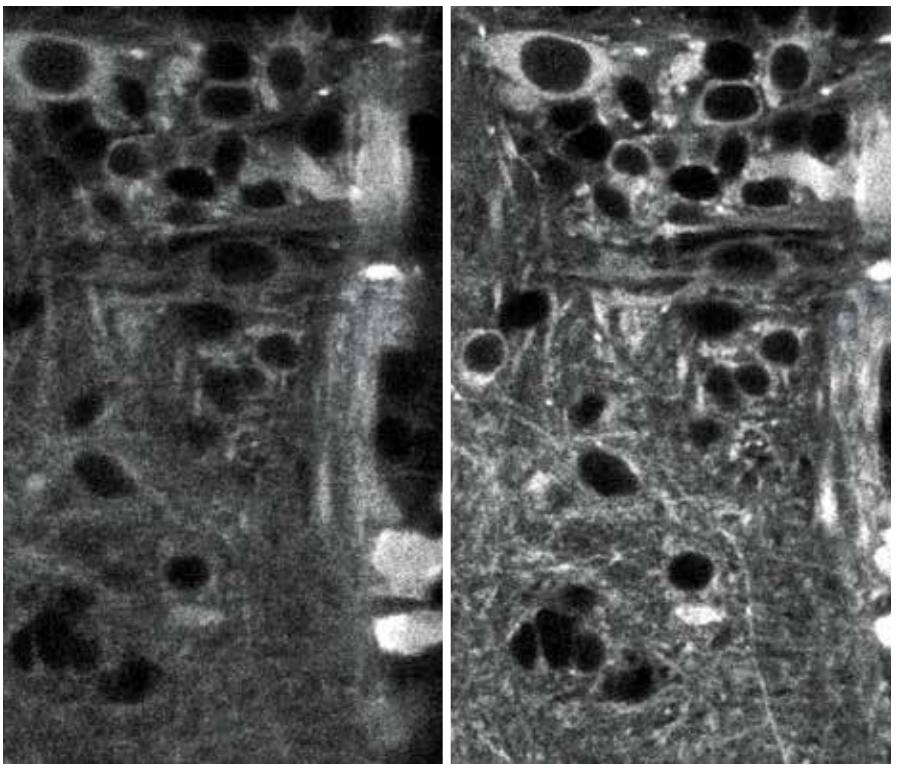
The thickness of the light sheet could not be reduced without significantly reducing the microscope's field of view, so Betzig came up with a different illumination strategy: a long but narrow beam of light called a Bessel beam that could be swept across the imaging field to create an extremely thin virtual sheet of light better suited for imaging inside single cells.

By 2011, Betzig and two members of his lab, Liang Gao and Thomas Planchon, had developed a microscope that collects unusually detailed images with minimal damage to living cells. Cell biologists were elated and immediately began harnessing the technique to monitor developmental processes in growing embryos, to witness subtle shape changes as cells moved in three dimensions, and to follow other fast-moving or prolonged processes. Still, Betzig's team suspected they could do more to amplify their microscope's impact.

During the Bessel beam microscope's development, Betzig had discovered that cells stay healthier when the light they are subjected to is spread out, reducing its peak intensity. He had first noticed the improvement when his team split its beam into seven parts to speed up imaging. That was a surprise benefit, and it reminded Betzig that he and Ji, working with Janelia Group Leader Jeff Magee, had had a similar success a few years earlier when they reduced the damage cells experienced during a different imaging technique, a process known as two-photon microscopy, by breaking up the microscope's pulses of light into sub-pulses. "I said, 'That's exactly what happened with the temporal division in the pulse splitter—so of course we have to spread the energy out.'"

So at this point, Betzig wondered whether splitting the beam still more might boost the benefits.

Each Bessel beam's central core is surrounded by concentric rings of weaker light, and he was concerned about how those might interact when the beams began to crowd one another. But when he modeled the interactions among a large field of Bessel beams, his model predicted certain arrangements where the undesirable lobes of light would destroy one another. If he could create those patterns, energy would be spread out, the already low levels of light exposure would drop further, and the pattern would lend itself to enhancing spatial resolution with a technique called structured illumination. "What you get is sort of a triple win," Betzig says.



Two-photon imagining of living zebrafish with adaptive optical correction (right).

Kai Wang, Davis Bennett, Misha Ahrens, Eric Betzig

Whole New Window

Ten years earlier, before he landed the job at Janelia, Betzig had been in need of a big idea. After several early successes at AT&T's Bell Labs, where he was the first to image single fluorescent molecules at room temperature instead of in extreme cold, he had become frustrated with academic science. He left Bell Labs and spent the next seven years leading research and development at his father's machine tool company in Michigan. But that didn't hold his interest forever. He was ready to return to the lab—but he needed to convince someone to hire him. "I figured it was my last chance to make a scientific career," he says.

His instinct was to delve into something totally new. But when he learned that biologists could now tag specific molecules inside living cells with fluorescent proteins—a power he'd longed for during his Bell Lab days—he knew it was time to renew his focus on microscopy. He retreated to a cabin in Michigan to immerse himself in theoretical

To see videos from Betzig's lattice light sheet microscope, go to www.hhmi.org/bulletin/winter-2015.

physics and devised a scheme to limit light damage to cells during imaging. The technique he proposed involved illuminating samples with a massive three-dimensional array of light foci. He'd never built that "optical lattice," as he called it, but now it was time to return to the idea.

"I realized that the 'magic periods' [of the theoretical Bessel-beam light field] were the periods that were predicted by my optical-lattice theory 10 years ago," Betzig says. So he used that theory to map out the light patterns he expected would be optimal for imaging. Once postdoctoral researchers Kai Wang and Bi-Chang Chen figured out how to best generate those patterns, their microscope began producing three-dimensional images of cells with the detail, speed, and low toxicity that they'd hoped for. The microscope is so gentle, Betzig says, that "there are many cells you could look at forever, in 3-D. In a way, it's pretty much the final solution for that."

Over the next year, Betzig invited 30 teams of biologists to visit Janelia to work with Chen and fellow postdoc Wesley Legant on the new microscope. Over thousands of hours, they worked out the kinks to better understand the technology's potential, generating terabytes of breathtaking movies for each group. Those movies were unlike any the scientists had ever seen. The long, thin microtubules that give a cell its form and structural support could be seen rapidly growing, shrinking, and regrowing as cells reshaped themselves and prepared to divide. Individual gene-activating proteins could be followed in three dimensions as they diffused through cells and bound to DNA. Development-regulating growth factors could be monitored for hours as small clumps of cells began to transform themselves into more complex organisms.

"We've worked with a new group of biologists every week, and each time it's a new adventure," Betzig says. "I'll never be a biologist, but I get a kick out of the art of it—out of the craziness of the cell. It feels like we have a whole new window on nature that we didn't have before."

Now that they have demonstrated a miscellany of applications for the lattice light sheet process, Betzig and his team are eager to continue developing the technology. Their highest priority is integrating a rapid adaptive optics technique that Betzig and Wang developed last year for use in transparent tissues. "We're going to try to extend the lattice light sheet so we can [image] through the entirety of transparent organisms like the zebrafish or [the roundworm] *C. elegans* and have all the benefits of the noninvasiveness," he explains.

Betzig doesn't want that work to impede researchers' access to the current lattice light sheet microscope, so his team built a duplicate instrument for Janelia's new Advanced Imaging Center (see "Microscopes for the Masses," page 36). The center's director, Teng-Leong Chew, says that months before the lattice light sheet microscope was officially announced, it was already the most in demand of the imaging center's many cutting-edge technologies. Betzig and his team also helped build two more of the instruments, for labs at Harvard and the University of California, San Francisco. He hopes the lattice light sheet process, which has been licensed to Zeiss, will eventually become standard technology in most labs. But until that time comes, interested researchers have the option of building their own instrument, by following detailed plans from Betzig's lab.

"I'll never be a biologist, but I get a kick out of the art of it—out of the craziness of the cell."

—ERIC BETZIG

His team reported on this new microscope in *Science* on October 24, 2014—just weeks after the Royal Swedish Academy of Sciences had named Betzig a Nobel laureate. In fact, Betzig worried a bit that the recognition for his work on PALM was misguided. "The lattice will be my lasting impact," he says. "My guess is this will be the high-water mark of my career."

Though work on the lattice is not yet done, Betzig expects the final optimizations to proceed smoothly. "I'm just dotting i's and crossing t's," he says. "I pretty much know that most of the stuff I'm doing right now is going to work."

That kind of stability makes Betzig restless. "It's time to throw the dice again," he says. "It's time to try something risky—the kind of stuff [Janelia] is designed to support." ■

Perfect Balance

Germline stem cells tread a fine line between replenishing themselves and producing sperm and eggs. They just may be the most important cells you've never heard of.

BY MEGAN SCUDELLARI

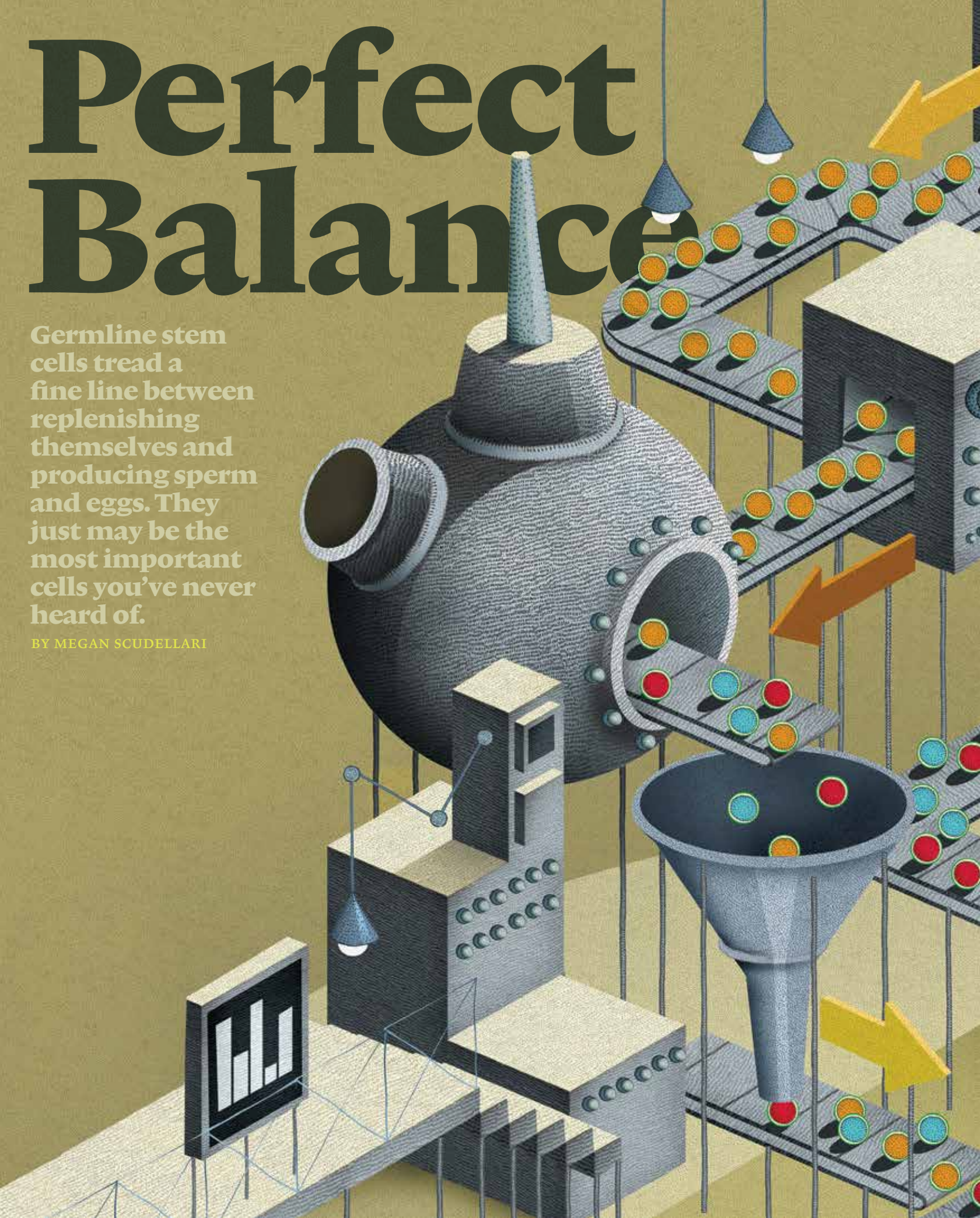


ILLUSTRATION BY ADAM SIMPSON

STEM CELLS ARE among the few cells in the body with the ability to generate new cell types, but because of this distinction, they are caught in a precarious situation. Stem cells walk a tightrope between the two most powerful cellular forces in the body: they are able to both self-renew, producing more stem cells, and differentiate, generating specialized cells with specific functions, such as blood or bone cells. If the balance tips in favor of self-renewal, stem cells can over-accumulate, in some cases leading to cancer. If the balance tips toward differentiation, the pool of stem cells can disappear, leading to the deterioration of organs.

Ruth Lehmann studies the earliest stages of germline stem cell formation in fruit flies.

Nowhere is that tightrope walk more vital or more perilous than in germline stem cells—the stem cells that ultimately produce sperm and eggs, or gametes. While all stem cells self-renew and produce specialized cells, germline stem cells (GSCs) are the only ones that make gametes, which are responsible for transmitting genetic information from one generation to the next. "They're the only cells that you don't actually need for your own survival, but you do need them for the survival of your species," says Ruth Lehmann, an HHMI investigator at the Skirball Institute of Biomolecular Medicine at New York University.

In this way, GSCs are the very foundation of a species and must walk that tightrope carefully. "In a germline stem cell, just being precise isn't enough," says Yukiko Yamashita, an HHMI investigator at the University of Michigan. "To make sure your daughter can make a daughter who can make a daughter, it has to be perfect."

To achieve that perfection, GSCs have evolved sophisticated strategies for their preservation and function. By studying those strategies, researchers have accomplished many firsts in stem cell biology, including identifying the first stem cell niches, discovering novel mechanisms of cell division, and pinpointing key signals that regulate stem cell actions. And many of the molecular pathways governing the precise balance between self-renewal and differentiation have been illustrated in vivid detail, making GSCs arguably the best understood stem cells in biology.

Walking the Line

Germline stem cells are a walking contradiction: they are ultimately able to generate every cell in the body but must produce only one type of cell—sperm or egg. "The germ cells are very primitive embryonic cells, and it's really important for them to avoid somatic differentiation. They shouldn't become muscle. They shouldn't become nerve," says Lehmann, who studies the very earliest stages of GSC formation in fruit flies.

From the very beginning, the precursors to GSCs, known as primordial germ cells (PGCs), are protected from becoming somatic cells by forming at the edge of an embryo, away from signals that might induce the wrong fate. This occurs by one of two mechanisms. In mice and other mammals, signals passed between extra-embryonic tissues and the embryo coax a specific set of cells at the periphery to become the germ cells. In flies, worms, frogs, and fish, on the other hand, a special protein-rich zone in the cytoplasm of the egg, called the germ plasm, determines which cells become PGCs. Germ plasm is a thick soup of proteins and RNA, with a much higher concentration of molecules than in the rest of the cytoplasm.

In 1992, at the Massachusetts Institute of Technology (MIT), Lehmann identified a key ingredient in that soup—a maternal effect gene she named *oskar*, which deposits a critical protein into the germ plasm. In *Drosophila*—those small, hairy fruit flies occupying laboratory vials since 1901 and a favorite for studying development—if *oskar* is inactive, no germ plasm forms, and no germ cells develop.

But when *oskar* is active, PGCs do form. From there, they adopt a clever defensive maneuver to avoid rapidly dividing and specializing like the rest of the cells in the embryo. The PGCs take a break; they pause.

This happens through extreme transcriptional repression, wherein DNA is prevented from being transcribed into RNA and is held temporarily silent, as if in a trance. Various molecular mechanisms control this repression, depending on the species, but the outcome is the same: little to no transcription and therefore no expression of the genome.

One of those mechanisms, recently discovered by HHMI Investigator Brad Cairns at the University of Utah, is the

Lehmann: John Abbott

"They're the only cells that you don't actually need for your own survival, but you do need them for the survival of your species."
—RUTH LEHMANN

modification of the genome by chemical tags that bind and silence developmental genes, thus preventing differentiation. In a project that took more than four and a half years, Cairns' team looked for and identified numerous modifications of DNA and histones—the proteins that package DNA—in multiple stages of male germ cell development in mice. They also validated their findings in human sperm.

Over time, the project became bigger and bigger. "We were astounded to find so many chemical marks and so many changes at different developmental stages," says Cairns. "We made a major effort for several years, as each new result helped explain the logic of the regulation process." As they reported in August 2014 in *Cell Stem Cell*, the group found a slew of tags—half "activating" tags and half "silencing"—stuck to key developmental genes. The activating tags help ensure that the genes can be turned on after fertilization. Until that time, the genes are held in check by the silencing tags.

With the DNA of genes promoting development in hibernation, some other system must regulate the activity of PGCs, which includes moving to the gonad and beginning to divide and produce daughter cells. It turns out that a hallmark of early germ cells is small, sticky balls of RNA and proteins that move around the cytoplasm and regulate RNA, leaving the DNA alone. Researchers believe that the DNA inside GSCs is so precious—the instructions for the survival of the species—that the DNA is kept in a pristine state in the nucleus, while the RNA is highly regulated by these sticky RNA-protein clumps in the cytoplasm. "Having less of an emphasis on transcription and more on RNA regulation may have something to do with preventing damage to the DNA," says Lehmann. "We don't really know, but it is an interesting phenomenon."

In *Drosophila*, Pumilio and Nanos—proteins Lehmann discovered as a graduate student—are two of the proteins that compose these RNA-protein clumps, and they can be found in germ cells from worms to humans. In the worm *Caenorhabditis elegans*, Judith Kimble, an HHMI investigator at the University of Wisconsin-Madison, identified a Pumilio-like protein called FBF, required for GSC maintenance, which represses more than a thousand RNAs in germline stem cells, many of which activate developmental pathways.

A Niche Role

Kimble's work on GSCs started much earlier. In 1981, as a postdoc at the MRC Laboratory of Molecular Biology in Cambridge, England, she used a laser to destroy cells in the tip of the developing gonad of *C. elegans*. By doing so, she identified a single cell—dubbed the distal tip cell—that was absolutely required for maintaining the worm's GSCs. This special regulatory cell was not a germ cell but a somatic cell, and when Kimble moved it around the gonad, the GSCs moved with it. She was the first to show that a nearby somatic cell is essential for GSC maintenance—suggestive of a stem cell niche in *C. elegans*.

Subsequently, Kimble demonstrated that the distal tip cell niche maintains stem cells by producing a molecule that binds to a Notch receptor protein on the surface of GSCs. It was the first time that Notch signaling was shown to control stem cells. Researchers would go on to discover that Notch is a powerful regulator of many types of stem cells and an important driver of cancer.

Kimble and her team have since focused on unraveling the elaborate network downstream of Notch signaling, a series of about 100 molecules that together control the balance between self-renewal and differentiation. By identifying and manipulating those molecules, her team has been able to expand and contract the pool of GSCs in *C. elegans*.

Kimble has no plans to stop until she's identified all the major links in the stem cell Notch signaling pathway.

Yukiko Yamashita is focused on why one stem cell stays in the niche while another moves away.

Brad Cairns' team identified a slew of chemical tags that activate or silence genes in germ cells.

Yamashita: Tony Ding; Cairns: Kevin Wolf

"I want the whole network that regulates this decision between self-renewal and differentiation," she says. And she's well on her way: just last spring, in the March 11, 2014, issue of *Proceedings of the National Academy of Sciences*, Kimble reported two new proteins essential for GSC maintenance that are direct targets of Notch signaling in GSCs—a long sought missing link between Notch regulation and the downstream regulatory network.

By the 1990s, most researchers believed in the existence of a stem cell niche—a specialized location inside a tissue where stem cells reside—but had never directly observed individual stem cells inside a niche. At the time, it was close to impossible to reliably isolate a single human—or mouse—stem cell, much less to identify where in the body the cell originated.

However, in *Drosophila*, the problem was tractable. "The small size and simplicity of their tissues make it easy to look at what individual cells are doing," says Allan Spradling, an HHMI investigator at the Carnegie Institution of Washington. "Nowhere in all of biology, that we know of, is this more true than with the stem cells of the *Drosophila* ovary."

Judith Kimble was the first to show a somatic cell is essential for GSC maintenance.

Each fruit fly ovary contains 12 to 16 flexible tubes filled with chains of maturing egg cells. To locate the stem cells in the ovary, one simply follows a single tube back to its start, to the very tip where all the other cells originate. There reside two plump, round germline stem cells, the cells that produce eggs. Just how many animal species have female GSCs is still uncertain, though evidence suggests that female mammals, including humans, are born with all the eggs they are ever going to need.

Classical histologists identified *Drosophila* germline stem cells simply by looking under a microscope, but Spradling argued that the identity of such cells could be known with certainty only by tracing their cell lineages—that is, by identifying all the progeny of a single cell. In 1995, he verified the conclusions of the classical histologists about *Drosophila* ovarian GSCs by doing so, but in many other cases, such analysis showed that early guesses about other types of stem cells were not correct.

In 2000, he and postdoc Ting Xie also used lineage tracing to identify a germline stem cell niche in *Drosophila*. They marked ovarian GSCs with a gene encoding a fluorescent protein, making the cells glow under controlled conditions, and then manipulated them. When the researchers removed one of the two cells, the remaining cell replicated, and its offspring took the place of the absent stem cell. Spradling and Xie realized that the location, not some magical aspect of the cell itself, was key to the existence of a stem cell. "Our work showed a stem cell is really not a special cell; it is just an average, undifferentiated germ cell," says Spradling. "The location made all the difference."

"Our work showed a stem cell is really not a special cell; it is just an average, undifferentiated germ cell. The location made all the difference."

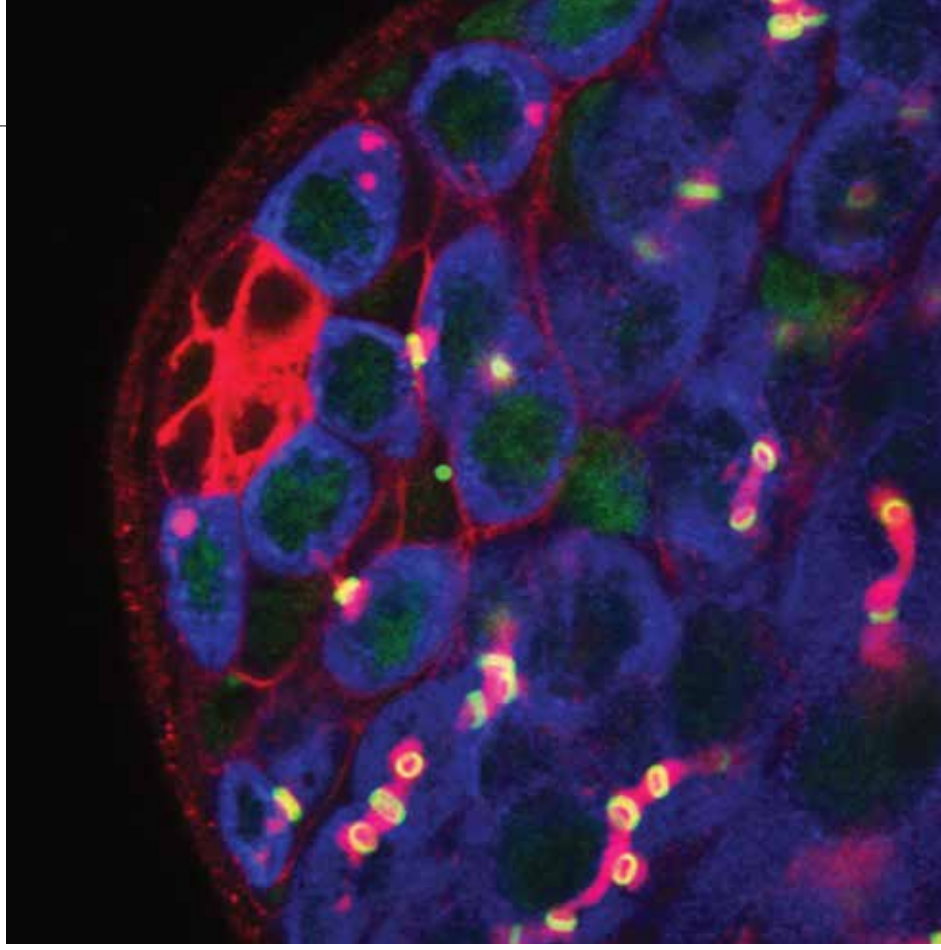
—ALLAN SPRADLING

Spradling then turned to the genes that might be involved in self-renewal and differentiation in the *Drosophila* ovary. He found one sterile mutation that caused the tip of each tube in the ovary to fill up with stem cells, like a bag of marbles. Naming the gene *bag of marbles*, or *bam*, Spradling's team concluded that the gene is required for differentiation. Next, Spradling's lab, in collaboration with the University of Texas Southwestern Medical Center lab of Dennis McKearin, now a senior scientific officer at HHMI, identified a signaling pathway in the niche that enables GSCs to silence *bam* and retain their stem cell identity. But when GSCs divide and daughter cells move outside the niche, they no longer receive the protein signal, so *bam* is active and the cells differentiate.

Orientation Matters

Around the time Spradling was identifying the signals regulating the female *Drosophila* niche, Margaret "Minx" Fuller, at Stanford University, was simultaneously studying GSCs in the *Drosophila* testis. Unlike the fruit fly ovary, the testis niche contains six to 12 germline stem cells clustered around a group of somatic support cells, called the hub, like petals on a flower. Yukiko Yamashita, then a postdoc in

Kimble: Kevin Miyazaki



In *Drosophila* testes, germline stem cells (violet) cluster around a group of support cells called the hub (bright red).

Fuller's lab, decided to take a quick look at mitosis–the process of cellular division–in GSCs. Yamashita and Fuller hoped that by studying how GSCs divide, they might gain insight into how one of the offspring remains a stem cell while the other differentiates.

"We both planned just to have a tiny paper out of that project," says Yamashita with a laugh. "That's not what happened." Yamashita found that the centrosomes, small, anchor-like organelles where the structural filaments needed for cell division originate, were not behaving as expected. Most biology textbooks have diagrams showing centrosomes separating *after* a cell commits to mitosis. Yet in the male fly GSCs, centrosomes were splitting apart from each other and moving around the cell *before* the initiation of mitosis. Because of this early movement, the centrosomes had marked where the mitotic spindle should be oriented–perpendicular to the stem cell niche, so that one cell stays in the niche and the other is pushed out–before mitosis even began.

"That [orientation] is probably responsible for the continued balance maintaining the stem cell population and continuing to produce [sperm]," says Fuller. "It is really critical for the stem cell program." In retrospect, says Yamashita, it makes sense that germline stem cells would take this extra, early precaution to prepare for reliable stem cell division. Their "tiny paper" was published in 2003 in *Science* and has since been cited over 415 times.

Yamashita has continued to explore how one daughter cell stays in the niche to be a GSC while the other moves away, to commit to becoming a specialized sperm cell. "What kind of precise mechanism can make sure that only one cell of two daughter cells, next to each other, cheek to cheek, gets a signal to make it a stem cell?" Yamashita asks. "It's like you have twin toddlers and try to give a snack to only one of them!"

Recently, one of Yamashita's postdocs came to her with a microscope slide and asked her to look closely. There, in *Drosophila* GSCs in the testis, she observed a slim whisker of microtubules, a nano-sized bridge poking out of the stem cell to touch the nearby stem cell niche. Yamashita was so surprised she went back to tissue images collected some 10 years earlier that were stored on her computer. Lo and behold, there was the same whisker. "It was there; I just hadn't noticed it," she says. "We missed that structure for such a long time." The team investigated and discovered that a critical signal is sent from the niche cells to the GSC on the surface of that whisker–a precise mechanism for signaling to the stem cell but not the daughter cell.

Model Behavior

Once a daughter cell does leave the niche, it begins the process of becoming a viable gamete, which starts with meiosis–the process of cell division that leads to gametes, each with only half the genetic information needed to form an individual.

At the Whitehead Institute and MIT, David Page and colleagues have deciphered the signals that initiate meiosis in both male and female vertebrates, including the powerful *Stra8* (*Stimulated by retinoic acid gene 8*), a gene expressed in the fetal ovary and juvenile testis (mammals continue producing sperm into adulthood, though not eggs), right at the time meiosis is initiated. "A defining feature of germ cells is they all know how to do meiosis," says Page, "so I wanted to know how this super-conserved program is initiated."

Allan Spradling's team was able to identify a stem cell niche in *Drosophila*.

Page and his team have shown that retinoic acid, a fleeting and hard-to-track chemical believed to be produced by nearby support cells in the gonad, activates *Stra8* in both ovaries and testes. In August 2014, Page's team reported in *PLoS Genetics* that retinoic acid activates a second gene, *Rec8*, which encodes a protein also critical for meiosis and is conserved all the way from yeast to humans.

"There is so much now known from the fly and worm male and female germlines about [numerous] things happening in stem cells," adds Fuller. "These have provided paradigms for people working in other adult stem cells–models for what kinds of things to look for." ■

Spradling: Paul Fetters; Science image: Yukiko Yamashita

Power to the Posse

Serving as both a lifeline and a launching pad, the Posse STEM program promotes persistence among multicultural college students.

BY ERIN PETERSON

ILLUSTRATION BY ALICE PATTULLO

"We know there are extraordinary numbers of talented students who fail to attend colleges that fit with their high school achievements."

—DAN PORTERFIELD

FOR BRYN MAWR COLLEGE sophomore Fransheska Clara, going to college wasn't just a personal goal: it was a family one. Neither her father, who hails from El Salvador, nor her mother, a native of Puerto Rico, had gone to college. And they had come to America—to Worcester, Massachusetts, an hour outside Boston—in the hope that their two kids could get a college education and fulfill a dream that they hadn't been able to achieve themselves.

Clara was driven to succeed in high school. She got good grades. She was president of her school's STEM club, a group focused on science, technology, engineering, and mathematics. And she spent a summer and a full semester doing internships through the biology and biotechnology departments of Worcester Polytechnic Institute.

So when she first heard about the Posse STEM program—which would send 10-person "Posses" of the very best students to top-notch colleges across the country to focus on science—Clara was eager to learn more. The program is designed to identify high-achieving student leaders in urban high schools and send them to college as a group so they can lean on one another for support. The Posse members are also paired with mentors at the colleges and receive significant access to science research opportunities. It is a program tailored to help these students, who often come from disadvantaged backgrounds, succeed.

A teacher nominated Clara for the program, and she spent months going through demanding leadership exercises, interviews, and problem-solving projects alongside some 100 other similarly talented students. "Everyone was so impressive," she recalls. "I always felt like I wouldn't get through to the next round."

But on December 3, 2012—three days before her 18th birthday—after a final, exhausting three-hour interview, she got the news: she had been selected for Bryn Mawr's first STEM Posse. "I really remember that call," she says. "My dad was crying. My mom was crying. It was a really big thing for us."

For students like Clara, the Posse STEM program represents both a lifeline and a launching pad. The four-year, full-tuition scholarship helps students whose families might not otherwise be able to afford a college education. And the wide-ranging support network of faculty, staff, and nine other high-achieving students, all aiming at similar goals, helps students focus on achieving their dreams, often faster than they ever thought possible.

With the help of Brandeis University chemistry professor Irv Epstein, who was supported with a grant as an HHMI professor, the first STEM Posse arrived at Brandeis in 2008. Since then, nine other schools have adopted the program, and each will support 50 new STEM Posse students over the next five years—a $70-million investment in 500 students who will help shape the future of science. The investment is significant. But the long-term payoff—for students' individual careers and for diversity in the sciences—could be even bigger.

Model of Success

The overarching Posse program, the brainchild of founder Deborah Bial, has been around since 1989. Bial was inspired to start the initiative when she heard a student who had dropped out of college say, "I never would have dropped out if I'd had my posse with me." She knew that kids from big—and often underfunded—urban public school systems often struggled and then dropped out of elite colleges, even though they were just as talented as their peers. The Posse program aimed to reduce these dropout rates by recruiting groups of students from specific cities around the country who would attend the same college, support one another along the journey, and help make the campus more welcoming to students from all backgrounds.

Each student received a four-year scholarship, on-campus mentors, and the camaraderie of fellow Posse members who had all graduated from high

As members of the Bryn Mawr STEM Posse, Fransheska Clara (left) and Carol Bowe help each other navigate social adjustments as well as academics.

Gene Smirnov

schools in the same city. "If you grew up in the Bronx, for example, but you ended up in Middlebury, Vermont, you'd be a little less likely to turn around and go home if you felt culture shock," explains Bial. The results have been nothing short of exceptional: there are more than 2,600 alumni of the program, and the graduation rate for Posse Scholars is 90 percent. The program does not explicitly seek students who have high need or who are minorities. However, because of the demographics of the schools the program draws from, 80 percent of Posse Scholars come from economically disadvantaged backgrounds and are African American or Latino. The program is extraordinarily competitive: this academic year alone, the Posse program received more than 15,000 nominations for 700 slots.

Brandeis' Epstein has seen the value of the program firsthand. He was involved in Brandeis' traditional Posse program when he was the school's provost, and he knew students thrived in it.

When he stepped down as provost and returned to teaching, he became frustrated by the lack of diversity in his science classrooms–and concerned that some of the dynamics of the Posse program might be pushing smart kids away from the sciences. "When a [Posse student] got a 40 on her first chemistry test, she might say, 'I can't do it,' and the other Posse students might say 'You're smarter than we are, and we're doing just fine in our classes; maybe you should switch out of that class,' " Epstein says.

The solution, he believed, wasn't to put more pressure on those individual students pursuing the sciences–it was to create Posses in which science was the focus. "I thought if they were all doing science and all committed to it, then the pressure would be inward, toward science, rather than outward, away from it," he says. Working together with Bial and Brandeis administrators, and with the support of his HHMI professorship, Epstein helped bring the first STEM Posse to Brandeis' campus in 2008.

Many components of the Posse STEM program at Brandeis were nearly identical to those of the traditional Posse models: a rigorous, months-long recruiting process to identify great students who were potential leaders; pre-college meetings for students to get to know other Posse members; and weekly meetings with staff and faculty mentors and their Posse during the school year. But the Posse STEM model layered on two additional pieces. One was a two-week immersion program before students' freshman year that introduced them to their campus, to science at an elite college, and to important support services such as tutoring centers. The second was a mentor for Posse STEM students who had a background in the sciences.

Initial results from the Brandeis program have been promising: 29 of the 30 students in the first three Posses have graduated. Of those, 22 earned degrees in the "hard sciences" of biology, chemistry, physics, math, or neuroscience; the other seven earned degrees in psychology or science policy.

Encouraged by Brandeis' pioneering work, four other schools–the University of Wisconsin–Madison, Bryn Mawr College, Franklin & Marshall College (F&M), and Texas A&M University–adopted the Posse STEM program between 2008 and 2013. According to F&M President Dan Porterfield, his school's investment in the program makes perfect sense. "We know there are extraordinary numbers of talented students who fail to attend colleges that fit with their high school achievements," he says. "We invest in programs, including Posse, to provide students access to higher education that's commensurate with their talent. And we believe they will go on to make a disproportionate difference in the world."

In 2015, with encouragement from President Obama at a White House Summit on higher education, five more schools–Davidson College, Georgetown University, Smith College, Middlebury College, and Pomona College–will begin making that investment by adding STEM Posses to their student bodies. Bial believes the impact could be transformative. "We hope that we'll see similar success rates at all of these schools," she says. "It will prove to the nation that there's a pool of young people out there who are not only capable of being, but who will be, superstars. And they will help these institutions bring diversity into their STEM majors in a way that hasn't happened before."

Practical, and Moral, Support

STEM Posses are designed to help students with whatever problems they're facing.

Fransheska Clara, for example, says she leaned on her Posse for support when she missed her family and friends from home. "The others

When University of Wisconsin sophomore Caroline Rozado (left) needs support, she can turn to her mentor, Emilie Hofacker.

were also struggling with homesickness and transitioning into a new environment," she says. "It really helped to have them around and to talk about things. I related to them. I knew they felt the exact same way."

For University of Wisconsin-Madison sophomore Caroline Rozado, her Posse was there to help prevent small problems from spiraling into big ones. Rozado had been an accomplished student at her New York City high school, and her extracurricular activities included three years of internships focused on ecogenetic and environmental research. But even so, she was worried about her introductory chemistry class at Wisconsin. "My high school had low funding, so they couldn't find a chemistry teacher," she says. "Instead, they had a biology teacher teach chemistry, but even our biology teacher couldn't get us the information we needed."

It wasn't just the course material at Wisconsin that seemed daunting: she'd never been in a classroom with 300 students before. "It was nerve wracking," she says. "Was I supposed to sit in the front? The middle? The back?"

The first couple of weeks of her chemistry class were tough. But her Posse–and her mentor, Emilie Hofacker–had her back. Hofacker, who is assistant director for STEM initiatives at Wisconsin, gave her a pep talk, and an older Posse student gave her tips on how to read the textbook and what problems to focus on. Soon, she and a Posse member from her cohort worked up their courage to go together to office hours with the course's professor. By the end of the first month, Rozado was on track.

The help she needed was simple, but the impact has been profound. "I'm not afraid to ask professors questions anymore, even if they seem really basic," she says. "Professors actually want to know what we don't understand, and I just needed to have those first experiences to get to the point where I felt it was okay to talk to them."

For Hofacker, helping students have epiphanies like these are central to her work. "Sometimes, students just want to know that it's going to be okay," she says. "There will be days when they want to give up, but we'll help them work through it."

STEM Posses are helpful to navigate some of the subtler aspects of the social adjustment as well. Bryn Mawr sophomore Carol Bowe says that despite an overwhelmingly positive experience in college, she has had to deal with students who make condescending remarks to her because she's in a STEM Posse. "Sometimes there's a stigma," she says. "There's an idea of, 'You're on a scholarship, and had you not been on a scholarship, you wouldn't be here.' People say so many things about money."

She says her Posse discussed how to handle such issues during their first meetings in Boston, before they arrived on campus. "It's easy to say, 'Oh, I'll just educate them,' " Bowe says. "But when you're standing in front of a person who said something ignorant and you feel really hurt, you might not actually know how to do that." She says she was relieved to be able to talk to other Posse members to figure out how to navigate the nuances of such conversations.

As Posse STEM students focus on their science studies, they also benefit from two other Posse perks: research opportunities and lab time. Posse STEM students often hear about research opportunities before other students on campus, and some on-campus researchers specifically request to work with Posse STEM students. Even if students don't pursue research from the outset, they still often rub shoulders with researchers in campus jobs scrubbing beakers in a lab instead of pots and pans in the cafeteria.

For students coming from disadvantaged backgrounds, that tiny distinction can be critical. "So many students have a notion that science is something where a lone scientist works in the dead of night with no human contact," says Brandeis' Epstein. "But these kinds of work experiences help them see that research groups are tight-knit, multi-generational, multi-ethnic communities that support each other. If you wash dishes in a lab, eventually you'll probably end up doing research in a lab."

Such was the case for Rozado, who was able to parlay her experience cleaning glassware in the school's research demonstration lab into work with genetics professor Jerry Yin. She's helping Yin with a research project studying protein signaling and protein levels in people who

Kat Schleicher

have neurodegenerative diseases. "It's so great to know that researchers are open to students working in their lab with them. They're patient enough to teach us these protocols, and even if we make mistakes, they'll help us fix them," she says. "It's amazing. It feels like an honor."

Metrics of Success

The four most recent Posse STEM programs are too new to have significant data come out of them, but the schools' administrators are already thrilled. F&M's Porterfield, for example, says that from the three Posse STEM groups currently on campus, not one of the students has dropped out. The oldest cohort, now juniors, has an average GPA that is higher than their non-Posse STEM classmates in a science-heavy curriculum. Meanwhile, Bryn Mawr reports that all but one member of its first STEM Posse have continued on to their second year, with the 10th likely to return for the spring semester. Wisconsin's Hofacker reports that not only are her school's Posse STEM students persevering in the sciences, but half are on track to graduate with STEM majors in four years, even though the typical trajectory at the school for such majors can demand an extra semester or two.

Epstein adds that, in some ways, these students are defying expectations. The average math and verbal SAT score for an incoming Brandeis student is 1353; for a Posse STEM student it's in the mid-1100s. Yet despite that 200-point gap, STEM Posse students, so far, have a four-year graduation rate of 97 percent, compared to the school's overall average of 86 percent. To Epstein, it's an indicator that the Posse STEM program is focusing on the right things. "I think we've picked people whose abilities are higher than their test scores alone might suggest," he explains. With the right support, they're not just persisting in school—they're succeeding at the highest levels.

STEM Posse students aren't the only ones benefiting from the program: faculty discussions about the Posse STEM program and teaching science more effectively have spilled over in ways that benefit all students. At Bryn Mawr, for example, the Posse STEM program opened up a larger conversation at the school about supporting students in the STEM fields; the college is currently piloting a course called "Fundamentals of Mathematics for Science and Social Science Students," which gives students the quantitative skills they'll need to succeed in introductory science courses. At F&M, administrators have been so pleased with the cohort-mentoring model that they've expanded it to six groups of first-generation college students who meet regularly with faculty mentors to bolster their chances of success.

And F&M chemistry professor Ken Hess, who served as a mentor to the first group of Posse STEM students when they arrived on campus in 2012, says his experience with the Posse students has helped him be a better mentor to all of his students. "I'm much more holistic in the way I think about things now," he says. "I'm far more patient. I'm a better listener. I'm more sensitive to, and appreciative of, the challenges that students bring to the classroom and how that might affect their performance."

> **"Sometimes, students just want to know that it's going to be okay. There will be days when they want to give up, but we'll help them work through it."**
>
> —EMILIE HOFACKER

A Path Forward

Because the Posse STEM model so closely follows the wildly successful traditional Posse model, and because early Posse STEM outcomes data seem so promising, it's not difficult to imagine that its long-term success will be similar. Alumni from traditional Posse cohorts have earned 43 Fulbright scholarships since 2007. And 41 percent of Posse alumni who are two or more years out from graduating have earned a graduate degree or are pursuing a graduate degree.

Posse STEM students are just as ambitious: Caroline Rozado, who plans to pursue majors in neurobiology and communications, says her dream is to be the next Dr. Oz, someone who uses her deep knowledge of science to teach the world how our bodies work. She credits the Posse program for giving her the chance to talk with other bright science students who helped her unlock her passion—and who fuel her relentless drive to succeed. "Those conversations made me realize how happy I am when I can help others learn, but I didn't know that until I was in Posse. Now I understand why I'm doing this work in science."

For Fransheska Clara, Posse has given her the one piece of the puzzle she was previously missing: confidence. "I used to be very doubtful and uncertain, but now, I know I can match up with anyone," she says. "That's helped me with academics. It's helped me with everything." ■

Perspectives & Opinions

Rethinking GMO Regulation

Are we overregulating transgenic crops?

In the United States, as in most countries, genetically modified crops are heavily regulated at every stage of their development–from research through marketing. While oversight is valuable, too much can be detrimental, believes HHMI-GBMF Investigator Jorge Dubcovsky. He explains why our current approach to transgenic regulation may not be in society's best interest.

Why are people afraid of GMOs? People don't know what GMOs, genetically modified organisms, are. They just know somebody is messing with their food and worry whether that person has good or bad intentions. Though fear is sometimes irrational, it's valid to fear what you don't know–we need to respect that feeling.

Unfortunately, governments often respond to such fear by increasing regulation. In the case of wheat, for example, the testing and regulatory process is so burdensome it's almost impossible for public breeding programs–at land-grant universities or the Department of Agriculture–to release transgenics. It's okay to regulate GMOs, but currently we regulate the technology, not the products. We should look at what we're putting in the plant, not how it's made.

What's wrong with regulating the technology? It's irrational. If I add a new wheat gene to a wheat seed to increase its resistance to disease or its nutritional value, the resulting plant is considered transgenic. Even if it has no foreign DNA, the new variety can be released only after lengthy and expensive tests. But if I achieve the same result by random mutagenesis or by recombination of a wheat chromosome with a related wild species, it's not considered a GMO, and I don't pay a cent.

Using the same process, you can make harmless products, like more nutritious varieties of wheat, or products with higher risk, like plants that express part of a virus to confer resistance against pathogens. I don't see the logic, scientifically speaking, of regulating the process rather than the product.

What are the consequences of regulating the process? It's now so difficult to develop transgenic crops and so expensive to release them that public breeding programs can't afford to do so. This poses a risk that large corporations could control the seed market–and they may make decisions based on the bottom line rather than public benefit.

Fortunately, we're not in that situation yet, at least for wheat. In 2012, roughly 68 percent of the wheat planted in the U.S. represented varieties produced by the public sector. But in the future, if current regulations remain in place, the public wheat-breeding sector won't be able to compete. Overregulation could lead to big companies controlling the seed supply. Keeping public-sector involvement offers several advantages. Public breeding programs help train new plant breeders; they serve as a backup for industry efforts; and they can focus on longer-term goals, like increasing crop diversity, and on traits not economically attractive to corporations.

Jorge Dubcovsky

—

Jorge Dubcovsky heads the Wheat Breeding Program and Wheat Molecular Genetics Laboratory at the University of California, Davis.

How can we improve things? I believe a hybrid model, with both public- and private-sector breeding programs, offers the right balance. The public sector has freedom to explore traits beneficial to consumers, while companies are generally better at distributing and selling seeds and can bring additional resources to breeding efforts. We have a system like that in California, and it works well. Companies get new technology and germplasm from public breeding programs, growers get the best seeds, and society gets improved products and reduced use of fungicides and pesticides.

I also believe public breeders can help ease fear of transgenic crops. For example, we can create transgenics by introducing DNA only from the same or related species (processes known as "cisgenics" and "intragenics"). Or we can use approaches known as RNA interference and CRISPR, which just knock out certain genes. Such applications eliminate introduction of foreign DNA, so may reduce the fear, but they're still very powerful. For example, RNA interference can be used to reduce gluten intolerance and increase the fiber in a grain. If regulations are reduced for these less controversial technologies, public researchers can use them to generate useful products.

Are things likely to change? Yes, people's fear will likely diminish. There were similar concerns many years ago when growers introduced hybrid crops. Today, nobody cares about hybrids. Genetic engineering is a powerful technology. It lets us do lots of positive things in a faster, more efficient, very controlled way. And it's happening. If you look at maize and soybeans, most acreage in the U.S. and elsewhere is now planted with transgenic varieties–but no big cataclysm has occurred, reducing the traction of the "we haven't proved it's safe" argument. We can't prove planes are safe, either, yet we still fly.

Everything has risk. What we need to do is evaluate the balance between risk and benefit. Seven billion people need to eat every day.

–Interview by Nicole Kresge

Marc Olivier LeBlanc

Jorge Dubcovsky believes transgenic regulation needs to change.

Perspectives & Opinions

Q&A

How can scientists help ease society's fear of GMOs?

GMOs make many people nervous. They're afraid of health risks, effects on the environment, and what could come of tampering with nature. But some of those fears may be unfounded. Here, four scientists suggest ways to ease GMO anxiety.

–Edited by Nicole Kresge

Dominique Bergmann
HHMI-GBMF Investigator
Stanford University

It's important to think about separating scientific from social and economic concerns. For example, there are people who worry about corporate ownership of biological materials; I think this is a legitimate concern, but we have to talk about such things in a separate breath from the actual genetic modification.

When I talk to people who are anti-GMO, I try to figure out their reasoning. If they're concerned about safety or putting foreign genes in food, I ask them when they first tried quinoa or acai berries. Usually, they say not until adulthood. I then ask why they were willing to take the incredible risk of consuming something with upwards of 20,000 genes they had never before been exposed to. I follow it up with a little light history of how we've manipulated plants for thousands of years. I doubt that I've actually convinced my Palo Alto neighbors they shouldn't fear GMOs, but I've at least taught them a little science.

Steven Henikoff
HHMI Investigator
Fred Hutchinson Cancer Research Center

Quelling irrational fears of low-probability events, such as catastrophic meteor impacts and adverse health effects from GMO foods, puts science on the defensive. The debate over labeling GMO products pits science against anti-science disguised as consumer protection, when the unstated implication is that GMOs require a health warning like cigarettes. But the two major GMO technologies—herbicide tolerance and insect resistance—have had an undeniably positive impact on the environment. Herbicide-tolerant crops promote no-till farming that reduces erosion, water use, and pollution. Both technologies cut down on the application of toxic chemicals, and the accompanying improvements in yield reduce the acreage devoted to agriculture. Debating the ecological impacts of GMO crops might make it more difficult to promote deceptive economic and political agendas.

Luis R. Herrera-Estrella
Senior International Research Scholar
Center for Research and Advanced Studies of the National Polytechnic Institute, Irapuato, Mexico

In the 1980s, papaya production in Hawaii was almost completely destroyed by the papaya ring spot virus. No natural resistance to this disease existed in papayas, and the immediate solution was to apply large amounts of insecticides. Using genetic engineering, researchers at Cornell University went on to develop genetically modified papaya plants that were resistant to the disease. These GM papayas saved papaya production in Hawaii; they also generated a cleaner product that was not contaminated by toxic insecticides. Consumers in the U.S., Canada, Mexico, and Europe have been eating GM papayas for the past 18 years and, to date, not a single health or environmental problem has been scientifically linked to this product, supporting the safety of GM crops.

Many other examples can be used to show that GMOs are not only safe, but they also help us develop sustainable agriculture with a less negative environmental impact.

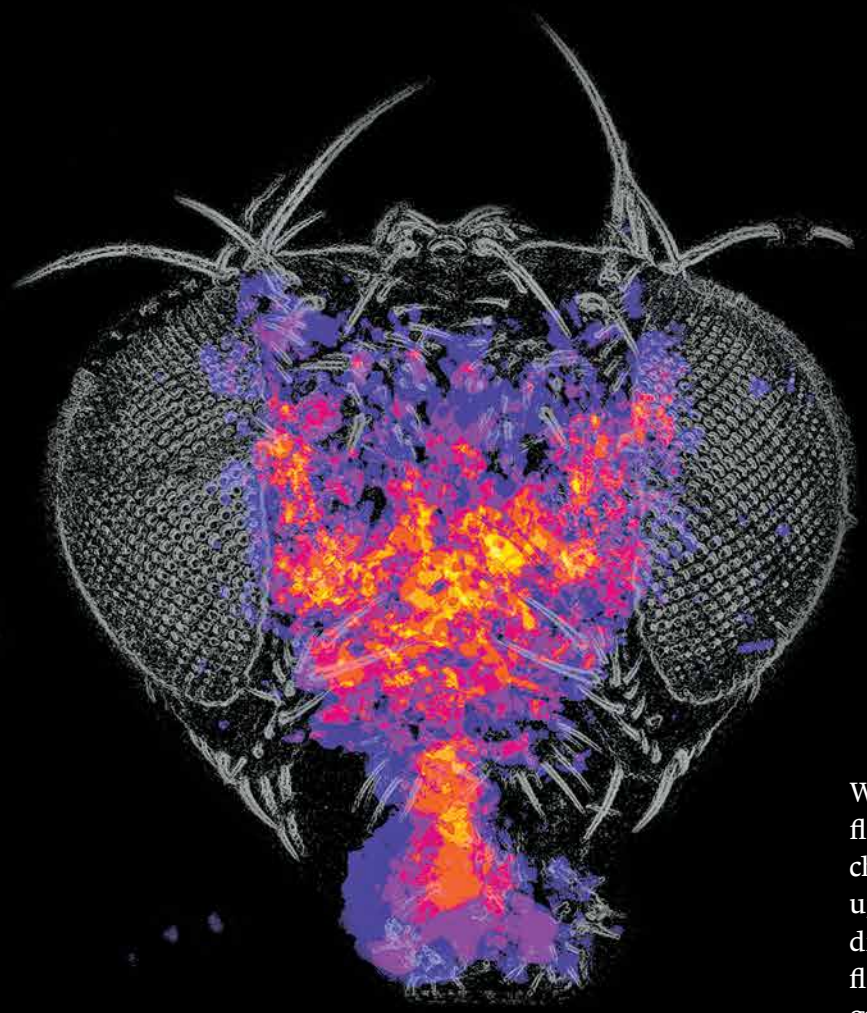
Michael Eisen
HHMI Investigator
University of California, Berkeley

The simple answer is education. Opposition to GMOs is born largely of ignorance, and scientists should take every opportunity they can to explain the technology and motivation behind genetic engineering to the public.

But even if we do that, I think strong opposition to GMOs will remain, because people don't see a good reason to "mess with Mother Nature." So I think the most important thing we can do to promote acceptance of GMOs is to develop GMOs that offer something to consumers, not just to farmers. If citrus greening wipes out the orange, for example, I think consumers will embrace engineered, disease-resistant varieties, and resistance to the technology will begin to disappear.

Bergmann: Tony Avelar; Herrera-Estrella: David Rolls; Eisen: Noah Berger; Henikoff: Kevin Wolf

Chronicle

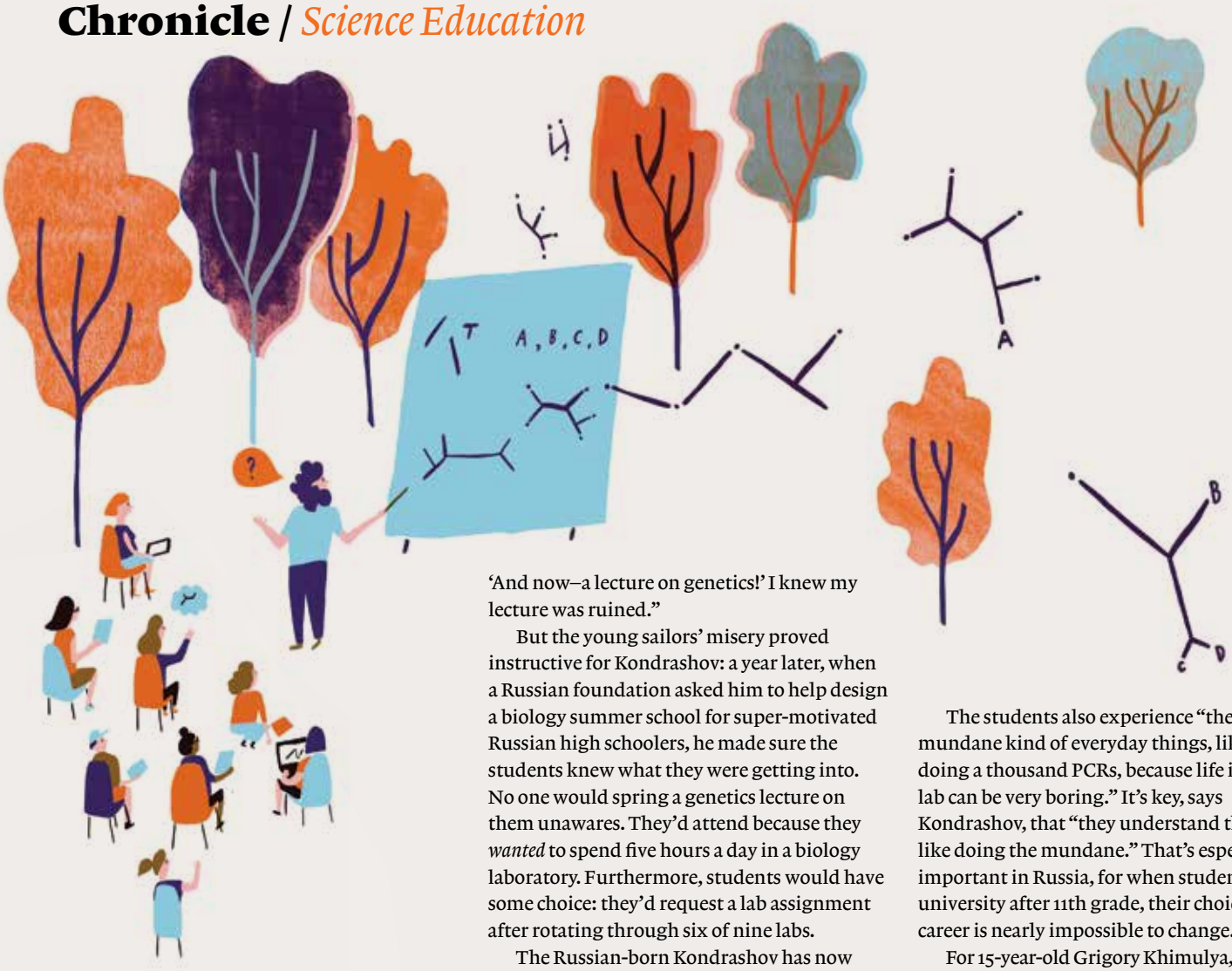


Andrew Seeds and Julie Simpson

When it comes to primping, fruit flies are hard to match. Given the chance, the winged insects will spend upwards of 20 minutes removing dirt from their bodies. By covering flies in dust and watching them groom, Julie Simpson's team at the Janelia Research Campus discovered that the insects perform a meticulous sequence of moves that starts at their eyes and ends at their thorax. This heat map of a fly's head reveals the areas that remained dusty after a minute of grooming, with the dustiest glowing yellow.

Read more about Simpson's research in "Habitual Grooming" on page 39.

Chronicle / *Science Education*



Immersion in the Lab

An international summer program gives talented Russian high schoolers a taste of life at the bench.

BIOLOGIST FYODOR KONDRASHOV'S lecture on DNA at a Russian summer program went badly. He'd been invited to talk at a Soviet-style encampment where thousands of teenagers were divided into interest groups: a space squad, a sports squad, and so on. Kondrashov would be talking to future mariners.

The sailors had spent the afternoon rowing on the Black Sea. "They docked at the pier," recalls Kondrashov, "totally tired after three hours of rowing. Their team leaders were like, 'And now—a lecture on genetics!' I knew my lecture was ruined."

But the young sailors' misery proved instructive for Kondrashov: a year later, when a Russian foundation asked him to help design a biology summer school for super-motivated Russian high schoolers, he made sure the students knew what they were getting into. No one would spring a genetics lecture on them unawares. They'd attend because they *wanted* to spend five hours a day in a biology laboratory. Furthermore, students would have some choice: they'd request a lab assignment after rotating through six of nine labs.

The Russian-born Kondrashov has now served for three summers as scientific director at the 17-day Molecular and Theoretical Biology School, or "bioschool." The students, who pay nothing and number from 60 to 80, come from all over Russia—some from elite high schools in Moscow, others from "somewhere in the middle of Siberia." They gather in August in Pushchino, a research community four hours from Moscow. The scientists, postdocs, and graduate students who run the labs come from Russia, the United States, or, like Kondrashov, Europe; he is an HHMI international early career scientist at the Centre for Genomic Regulation in Barcelona, Spain.

The students work on real experiments that advance the work of the lab heads. "There's tons of education literature where they talk about how authentic education experience enhances kids' motivation," says Kondrashov. Part of authenticity is seeing how scientists respond when something goes wrong, he says. "If you have a well-rehearsed project, nothing ever is supposed to go wrong."

The students also experience "the mundane kind of everyday things, like doing a thousand PCRs, because life in the lab can be very boring." It's key, says Kondrashov, that "they understand that they like doing the mundane." That's especially important in Russia, for when students enter university after 11th grade, their choice of career is nearly impossible to change.

For 15-year-old Grigory Khimulya, bioschool was "one of the most important events that happened in my life so far, and I feel like this is the case for most of the kids." Now 17 and a freshman at Harvard, Grigory says he loved working on "real research questions with a real principal investigator ... to research something that no one had explored before."

Grigory worked with José Luis García-Pérez, an HHMI international early career scientist from Spain who studies human transposable elements, or "jumping DNA." The 10 students on his team proposed testing whether ibuprofen would cause DNA sequences in human cultured cells to jump to new positions within the genome. They discovered that the common painkiller did seem to increase the rate of retrotransposition, and García-Pérez is continuing to study the effect.

All too often, says García-Pérez, students "don't know what science is until late in college or in graduate school." But at bioschool, they get a feel for "living in the lab."

They also "come to see [science] as a collaborative process," says bioschool

For a taste of the 2014 summer bioschool, see the slideshow at www.hhmi.org/bulletin/winter-2015.

Hannah Warren

"For many of them, it was the first time they'd ever talked to adults about science outside a classroom."
—MAXIM IMAKEV

lab head Mikhail Gelfand, a prominent quantitative biologist and former HHMI international research scholar. "It's important psychologically." Gelfand's lab listed bioschool students as co-authors on a recent paper.

Doctoral student Maxim Imakaev has noticed a stark difference between how Russian and American students view the chance to do research. For the Boston-area high schoolers he supervises at the Massachusetts Institute of Technology, he says lab work is one among many extracurricular activities–"just another program." Bioschool students, on the other hand, were ecstatic about doing real research. "For many of them, it was the first time they'd ever talked to adults about science outside a classroom," says Imakev. "It showed students that there can be other adults in their lives who respect them, who believe in them."

That was memorable for 17-year-old Aygul Minnegalieva, who'd never before done a lab experiment but said the bioschool scientists treat students like "you are equal to them. They don't look at you like you're just a high school student."

Kondrashov says bioschool validates students who "feel they are outcasts because they're smart, because they're interested in different things than their peers."

This was a revelation for Anastasia Goshina, 17: "I was surrounded by people who understood me. I could ask them what they think on biological topics, and they'd give their opinions. We'd maybe argue on things, or agree. I don't have opportunities like that at my school." Anastasia–whose graduating class in Togliatti, 1,000 kilometers from Moscow, has just 20 students–keeps in touch with her bioschool lab head and friends via email and social networking.

She plans to apply to university in Moscow. Kondrashov says one measurable outcome of the summer school is how many students are willing to relocate to enroll in the best programs, since it's unusual for Russian students to do so. Half the bioschool alumni now in university left their hometowns to further their education.

The bioschool will reconvene this summer, funded primarily by the Dynasty Foundation, Russia's first family foundation, and in part by HHMI.

But "bioschool doesn't end when the summer ends," says Grigory. "I'm still part of the network of researchers and students.... I'm in Boston, but I'm still finishing projects for labs in Moscow and Barcelona."

– Cathy Shufro

Chronicle / *Toolbox*

Microscopes for the Masses

Janelia's Advanced Imaging Center gives visiting scientists access to cutting-edge microscopes.

AT JANELIA, SCIENTISTS build tools. Some are small and easy to share: sensors that can be purchased, software that can be downloaded, websites that can be visited. Others, like innovative microscopes, are not. These hulking collections of lenses, mirrors, lasers, and electronics need to be precisely aligned and bolted to 800-pound optical tables. Furthermore, many are prototypes, meaning that outside scientists like Hernán López-Schier of Helmholtz Zentrum München in Munich, Germany, have little access to them.

López-Schier uses zebrafish to study nerve cells that transmit sensory information to the organism's brain. By cutting an axon—a threadlike extension of a nerve cell—with a laser and watching its recovery under a microscope, López-Schier learns how Schwann cells—which insulate and maintain axons—respond to the damage. With commercially available microscopes, he can get a general idea of the process. But to see it in detail, he needs a machine that can capture the action at high speed, in a live fish, without damaging its tissue.

Fortunately for López-Schier, the Janelia Advanced Imaging Center (AIC) recently opened its doors, offering access to the microscopes built at the Ashburn, Virginia, research campus. "Janelia is a tool-building place, and we want our tools to be used," explains Janelia Executive Director Gerry Rubin. "Our goal is to enable cutting-edge work by providing people with instruments that just didn't exist before."

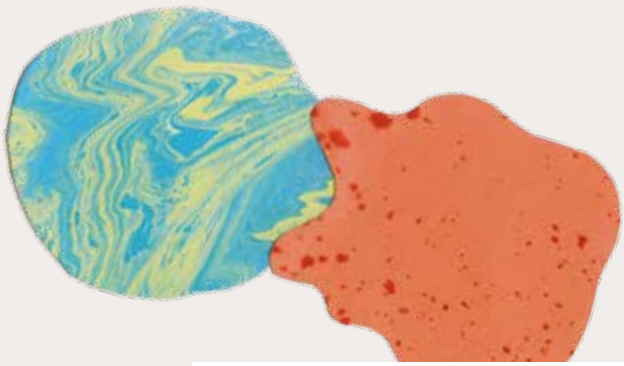
The AIC is a joint venture between HHMI and the Gordon and Betty Moore Foundation (GBMF). It houses five microscopes (see sidebar, "A Spectrum of Tools") and has a support staff of four scientists to help carry out experiments. "GBMF was already interested in imaging and is funding several groups developing new microscopes. Together, we concluded that providing widespread access to prototypes of new instruments would be very complementary to our ongoing activities," says Rubin.

Whenever a call for proposals goes out from the AIC, experimental biologists anywhere in the world can submit an application to use its microscopes. Successful applicants are given free lodging at Janelia and access to all the scientific facilities on campus. The first round of proposals included studying microfluidic devices, squid hatchlings, transgenic moss, and bacterial membranes.

"We want to encourage scientists from fields that historically don't leverage advanced microscopy in their work," explains the center's director, Teng-Leong Chew. The AIC is targeting users who lack access either to cutting-edge imaging instruments or to a support team versed in the latest microscopy techniques.

And by pushing the microscopes' experimental limits, users can also provide valuable feedback to the builders. "In a way, AIC becomes the convergent point where biology meets engineering," explains Chew. "It's not just that Janelia puts forward this really wonderful equipment for science to use; it's also that the visitors will inform the continued development of the instruments." Their feedback will help further refine the

"Our goal is to enable cutting-edge work by providing people with instruments that just didn't exist before."
—GERRY RUBIN



microscopes and enable the instruments to be used in a variety of ways in the life sciences. The hope is that, eventually, some AIC prototypes will be fine-tuned to a point where they might be commercialized for broader distribution.

López-Schier was one of the first scientists to use the AIC's lattice light sheet microscope to look at a fish. But adapting his relatively large sample to the microscope proved a challenge. "Initially, we couldn't image anything because we couldn't make the fish fit within the holder that would place it under the objective lenses of the microscope," recalls López-Schier. But he and the AIC support team spent a couple of days modifying the mounting procedure and were able to make it work.

He was rewarded with live video of Schwann cells repairing damaged axons. The footage revealed that the cells eat up dead axons, a process known as phagocytosis. "With the lattice light sheet microscope, we could actually see these intracellular vacuoles forming from the phagocytic activity of the Schwann cells as they consumed the dead axons," he explains.

Back in Germany, López-Schier has submitted a paper with his results. He also has his sights set on building a similar microscope in Munich. *–Nicole Kresge*

Hye Jin Chung

A Spectrum of Tools

Lattice light sheet microscope: Invented by Janelia Group Leader Eric Betzig, this microscope allows users to peer inside living cells, giving them the ability to create detailed, three-dimensional movies of biological processes like cell division. (See page 12 for more on Betzig's work.)

Interferometric photoactivated localization microscope (iPALM): Developed by Janelia Group Leader Harald Hess, the iPALM is especially suited for high-resolution studies of how molecules interact with each other, such as in a signaling complex.

Single molecule total internal reflection fluorescence microscope (smTIRF): Used for in vitro imaging of complex biochemical reactions like DNA transcription, the smTIRF was designed and constructed by the research teams led by HHMI President Robert Tjian and physicist Steven Chu of Stanford University.

Aberration-corrected multifocus microscope (acMFM): Designed and built by the research team led by the late Janelia Group Leader Mats Gustafsson, the acMFM can image nine focal planes simultaneously, making it perfect for tracking the movement of molecules inside living cells.

Live cell multicolor structured illumination microscope (SIM): Also developed by Mats Gustafsson, the AIC's SIM is one of the fastest instruments of its kind and is capable of doing three-dimensional, multicolor, high-resolution imaging, making it ideal for observing macromolecular interactions and subcellular structures in a live cell.

Chronicle / *Lab Book*

Random behavior often helps animals escape predators.

Decisions, Decisions

A stress hormone can flip the switch between strategic and random behavior in rats.

WE'RE HARDWIRED TO learn from our mistakes. But sometimes the brain abandons lessons learned and acts less strategically. In the wild, for example, animals often move unpredictably to escape predators. How the brain switches between paying attention to past experiences and proceeding randomly is clearer now, thanks to new findings from Janelia Group Leader Alla Karpova.

"Normally, we determine the best course of action strategically, by making a model of the world based on all the information available to us—in other words, our experience," explains Karpova. "But sometimes it can be better not to use all—or, for that matter, any—of that information."

Karpova and postdoc Gowan Tervo created a game in which rats were presented with two holes, only one of which contained food. The food's location was determined by one of three increasingly tough computer programs that learned from the rat and attempted to thwart its strategy. The first was predictable and fairly easy to beat, the second a bit trickier, and the third essentially unbeatable because the program used a very sophisticated prediction algorithm that was hard for the animal to counter-model.

The animals used a strategic approach when playing the easy opponent. But when they went up against the sophisticated competitor, they stopped basing choices on previous experience, and their decisions became haphazard. In fact, many of the rats got stuck in random mode for hundreds of trials, even when the food became easy to find—an effect resembling a psychological condition called learned helplessness.

As Karpova and Tervo reported September 25, 2014, in *Cell*, they discovered that altering levels of a stress hormone called norepinephrine in an area of the brain called the anterior cingulate cortex changed the rats' operating mode. Increasing norepinephrine suppressed the rats' strategic mode and caused them to behave randomly; inhibiting release of the hormone had the opposite effect.

"Just by manipulating a single neuromodulatory input into one brain area, you can dramatically enhance the strategic mode," says Karpova. "The effect is strong enough to rescue animals out of the random mode and successfully transform them into strategic decision makers."

Karpova suspects that the same neural mechanisms govern the way humans act, which could lead to a future therapy for learned helplessness. *–Nicole Kresge*

IN BRIEF

A GROWING FAMILY TREE

About 8,000 years ago, a band of brown-eyed, pale-skinned farmers made their way to Europe from the Near East. They mingled with the indigenous blue-eyed, swarthy hunter-gatherers, and their offspring eventually became modern Europeans. But that might not be the entire story. HHMI Investigator David Reich of Harvard Medical School has found evidence that modern Europeans have a third branch in their family tree.

Reich and a team of more than 100 collaborators worldwide compared the DNA of 2,345 modern Europeans to DNA in the remains of a 7,000-year-old farmer from Germany and eight 8,000-year-old hunter-gatherers from Luxembourg and Sweden. "What we find is unambiguous evidence that people in Europe today have all three of these ancestries: early Near Eastern farmers who brought agriculture to Europe, the indigenous hunter-gatherers who were in Europe prior to 8,000 years ago, and ancient north Eurasians," says Reich.

Although nearly all modern Europeans tested showed evidence of DNA from the third group—north Eurasians—the team did not find that same DNA in ancient hunter-gatherers or ancient farmers. This means the north Eurasians came to Europe after agriculture had been established—a scenario most archaeologists had believed unlikely. The scientists' data, published September 18, 2014, in *Nature*, also reveals that the first Near Eastern farmers to reach Europe had ancestors from a previously unidentified lineage, which Reich's group named the Basal Eurasians.

CRUNCHING BIG DATA

Nowadays, microscopes capture images of the brain in unprecedented detail. But with that detail come mountains of complex data that can slow even the fastest computer to a crawl. On a single machine, "you can load the data, start it running, and then come back the next day," explains Janelia Group Leader Jeremy Freeman. "But if you need to tweak the analysis and run it again, then you have to wait another night." For larger data sets, the lag time might be weeks or months.

Freeman joined with Janelia Group Leader Misha Ahrens to find another way. The scientists realized that a new distributed computing platform called Spark, which divvies up tasks across a cluster of computers, was particularly well suited to the challenges of neural data. Building on the technology, Freeman and Ahrens developed an open-source library, dubbed "Thunder," for analyzing large-scale neuroscience data. With their library, tasks that before would take days can be completed in hours or minutes—ideal for supporting high-throughput, exploratory analysis of large data sets.

In a report published July 27, 2014, in *Nature Methods*, the Janelia team illustrated Thunder's capabilities by using it to rapidly identify patterns of biological interest in high-resolution images of the brains of mice and zebrafish.

Thunder is designed to run on a private computer cluster or on Amazon's cloud computing services. It is totally open source; information and tutorials can be found via the GitHub project page at freeman-lab.github.io/thunder.

HIPPOCAMPAL STORAGE SPACE

The neurons in the brain's hippocampus store recent information about people, places, and events. But how does the hippocampus avoid running out of storage space

Photo: Stephen Dalton/Science Source; Illustration: Leif Parsons

Cancer's Niche Problem

In some bone marrow cancers, a stem cell's surroundings play a surprising role.

UNTIL NOW, SCIENTISTS believed that cancer was driven by changes inside cells: genes become mutated, cells grow unchecked, and a tumor forms. But there may be more to the story. New research from HHMI International Early Career Scientist Simón Méndez-Ferrer indicates that, in some cases, what goes on just outside a cell also plays a role in cancer formation.

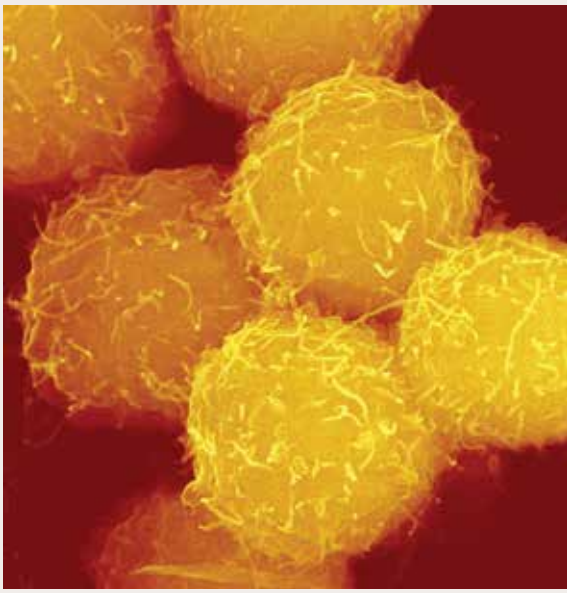
In a group of bone marrow disorders known as myeloproliferative neoplasms, a defective gene causes hematopoietic stem cells (HSCs) to make too many blood cells. As the blood cells build up, the disease worsens, sometimes evolving into cancer. The only real cure is a bone marrow transplant. However, some transplant patients develop a new bone marrow cancer originating from the donor transplant cells, hinting that something other than the patient's mutant HSCs are to blame.

Studying the process in mice, Méndez-Ferrer's team discovered that the mutant HSCs produced an abundance of small inflammatory proteins called cytokines that were damaging nearby neurons. This damage, in turn, prevented the nerves from activating other cells that help regulate HSCs. The end result: unchecked growth of HSCs and the potential of myeloproliferative neoplasms.

"Even though the disease is initiated by a mutated blood stem cell, this stem cell cannot drive the disease until it destroys particular elements of the microenvironment," explains Méndez-Ferrer at the Spanish National Center for Cardiovascular Research. "First, it has to damage the nerve cells in the marrow."

When the researchers added an analog of the neurotransmitter adrenaline to compensate for the damaged neurons' inability to fire, the mouse cells were once again able to control HSC proliferation. The team reported its findings August 7, 2014, in *Nature*.

Most efforts at treating myeloproliferative neoplasms are focused on blocking the mutant HSCs, according to Méndez-Ferrer. "We showed that an alternative approach might be to protect the niche—the environment—and control the expansion of the mutated cell," he says. With that in mind, Méndez-Ferrer, Radek Skoda, and other colleagues will soon start a clinical trial to see if adrenaline analogues can be used to effectively treat patients with myeloproliferative disease. *–Nicole Kresge*



The environment around these hematopoetic stem cells plays a role in cancer formation.

if, say, an environment is larger than predicted or an experience goes on longer than expected? According to Janelia Group Leader Albert Lee, it's all about division of labor.

Lee and his team created a 48-meter-long maze and recorded which neurons in the hippocampus fired when a rat explored a small area of the maze for the first time. Then the researchers incrementally increased the portion of the maze the animals could access and monitored how the brain added the new information to its spatial map.

As they reported August 15, 2014, in *Science*, the team discovered that each neuron contributes to the map at its own rate. As the rat explored the maze, some cells fired early and often and associated themselves with the new space immediately, while others held back. These slower cells rarely contributed unless the space expanded beyond the original size captured by the early-firing neurons.

"Instead of the hippocampus having to adjust in time as the animal notices that the maze gets larger, it anticipates all different sizes of mazes from the beginning," Lee says. "It does this by dividing up its population of neurons so that certain cells are ready to represent smaller mazes, others are ready to represent medium-size mazes, and still others, large mazes." Lee believes that similar mechanisms are at work when the human brain records a new experience.

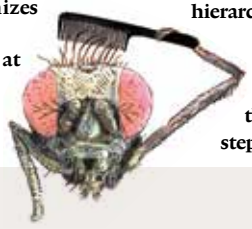
HABITUAL GROOMING

Fruit flies are very fastidious when it comes to cleanliness. A dust-covered fly may spend upwards of 20 minutes removing grit and grime in a habitual sequence that starts at its eyes and ends at its thorax. Studying this structured grooming has helped Janelia Group Leader Julie Simpson explain how the brain organizes sequential behavior.

"You can't do everything at once," says Simpson. "How do you manage competing demands on your brain and limbs so that you execute things in order of importance?" As she reveals in the August 19, 2014, issue of *eLife*, it's all about hierarchy.

Simpson, postdoctoral researcher Andrew Seeds, and their colleagues devised a way to trigger particular grooming tasks by turning on different sets of neurons. They discovered that dust-covered flies always started their grooming program at the beginning—cleaning their eyes—but weren't able to progress beyond the point that the scientists switched on. For example, when the researchers activated neurons for abdominal cleaning, the fly would clean its head and then its abdomen but then would continue cleaning its abdomen, ignoring the dust on the rest of its body.

"It progresses through the hierarchy to that particular point, and then it gets stuck," Simpson explains. This means that earlier grooming steps have the ability to halt those that happen later. These findings may help explain other behaviors that involve sequences of tasks, like nest building in birds.



SENSITIVE SEQUENCING

Techniques known as Sanger sequencing and whole exome sequencing are great tools for identifying defective genes that are present in all of an individual's cells. But they aren't as reliable when mutations occur in just a few cells. Although not as common, these "mosaic" mutations are increasingly recognized as a cause of disease, especially in the brain. "If a mutation is in only 5 or 10 percent of the cells, then that's going to be in a very small fraction of the data, which will be hard to separate from the noise," explains HHMI Investigator Christopher Walsh of Boston Children's Hospital.

To overcome that challenge, Walsh and his colleagues developed a new strategy that flushes out these hard-to-find mutations. The team focused on a set of genes associated with brain

Photo: SPL/Science Source; Illustration: Leif Parsons

Chronicle / *Lab Book*

Jamming Jumping Genes

Cells have a rapidly evolving arsenal to counter wayward DNA.

FOR MILLIONS OF YEARS, bits of DNA called retrotransposons have been duplicating and inserting themselves randomly throughout the genomes of different organisms, including humans. Sometimes the jumping genes alight in places that help species evolve new traits. Other times they do nothing. But all too often they touch down in the middle of a gene, altering the way it's regulated or knocking it out of commission. Fortunately, as HHMI Investigator David Haussler recently showed, cells have evolved a way to keep these wayward sequences in check.

"Over generations, our genome becomes bloated with copies of retrotransposons," explains Haussler. "They do damage by disrupting normal genetic mechanisms. Something has to be the security patrol to try and shut these things down."

In humans, one form of security patrol comes from more than 400 rapidly evolving genes that produce watchdog proteins called KRAB zinc-fingers. These watchdogs scan the genome, clamp onto retrotransposons, and then call on other proteins to silence them.

To see KRAB zinc-fingers in action, Haussler's team at the University of California, Santa Cruz, used mouse cell lines containing a copy of human chromosome 11, which includes hundreds of retrotransposons commonly found in primates like humans, but not present in rodents. Since mice have rodent-specific KRAB zinc-fingers to control their retrotransposons, the rodent cells couldn't stop the rogue human retrotransposons from expressing themselves. That is, until the researchers added two human KRAB zinc-fingers—ZNF91 and ZNF93—that halted the primate-specific retrotransposons in their tracks. The team published its findings September 28, 2014, online in *Nature*.

Haussler explains that mutations in retrotransposons allow them to escape detection by the KRAB zinc-fingers, which in turn drives the evolution of new KRAB zinc-finger genes. "By reconstructing molecular evolutionary histories, we can see that these KRAB zinc-finger genes have been major players in this battle with the retrotransposons and will probably continue to be so," he says.

Primates have evolved specialized weaponry to home in on bits of jumping DNA.

There is a silver lining in this seemingly endless arms race within our own DNA. Once KRAB zinc-fingers are no longer needed to suppress retrotransposons, many of these watchdog proteins adopt new roles as regulatory proteins, controlling the activity zof genes near retrotransposon landing sites.

–Nicole Kresge

IN BRIEF

malformations. They isolated DNA from patients with unexplained brain malformations and then sequenced each of these genes hundreds to thousands of times.

"We said we'd shoot to sequence them a thousand times over," Walsh says, "thinking that if a mutation is only present in 5 percent of the cells, it will be obvious that it's a mutation, because we'll see that mutation 50 times." The team reported August 21, 2014, in the *New England Journal of Medicine* that they had found disease-causing mutations in more than a quarter of the patients in their study, demonstrating the effectiveness of their method and further supporting the idea that mosaic mutations are an important cause of diseases that affect just a part of the body.

APPETITE CONTROL

Sometimes science is serendipitous. Researchers—like HHMI Investigator David Anderson—occasionally find something they weren't looking for. In Anderson's case, the accidental discovery was a few thousand cells that turn appetite on and off.

Harnessing a technique called optogenetics, Anderson and his colleagues at the California Institute of Technology used a beam of light to activate a small group of neurons in a part of the mouse brain called the amygdala. In earlier research, Anderson had pegged these nerve cells as part of the fear response. But, as the team reported September 2014 in *Nature Neuroscience*, rather than eliciting fearful or anxious behavior, the light caused the mice to abruptly stop eating. The scientists were able to rule out the possibility that the animals were too scared to eat, and they also established that the neurons were also activated by bad tastes, nausea, and feelings of satiety.

"We think the neurons make up a central node that integrates the influences of multiple [feeding-inhibitory] signals and relays this information to inhibit other brain centers that normally promote feeding," explains Anderson. Many patients with eating disorders also have emotional disorders like depression and anxiety, which may be explained by the neurons' dual appetite and fear functions. Next, Anderson wants to examine how the nerve cells manage to regulate both feeding and emotional behaviors.

QUALITY PATROL

Like intricate pieces of origami, proteins need to be folded in just the right way. An incorrect bend here or an extra loop there can render a molecule useless—or even toxic—to the body. To dispose of these defective proteins, cells detect misfolded proteins and tag them with a protein called ubiquitin, marking them for degradation.

In the yeast *Saccharomyces cerevisiae*, there are two well-established roving protein complexes that add ubiquitin to proteins in the endoplasmic reticulum (ER). One complex is for proteins whose misfolded domains occur in the cytosol, and one is for proteins with misfolded domains in the lumen or membrane of the endoplasmic reticulum.

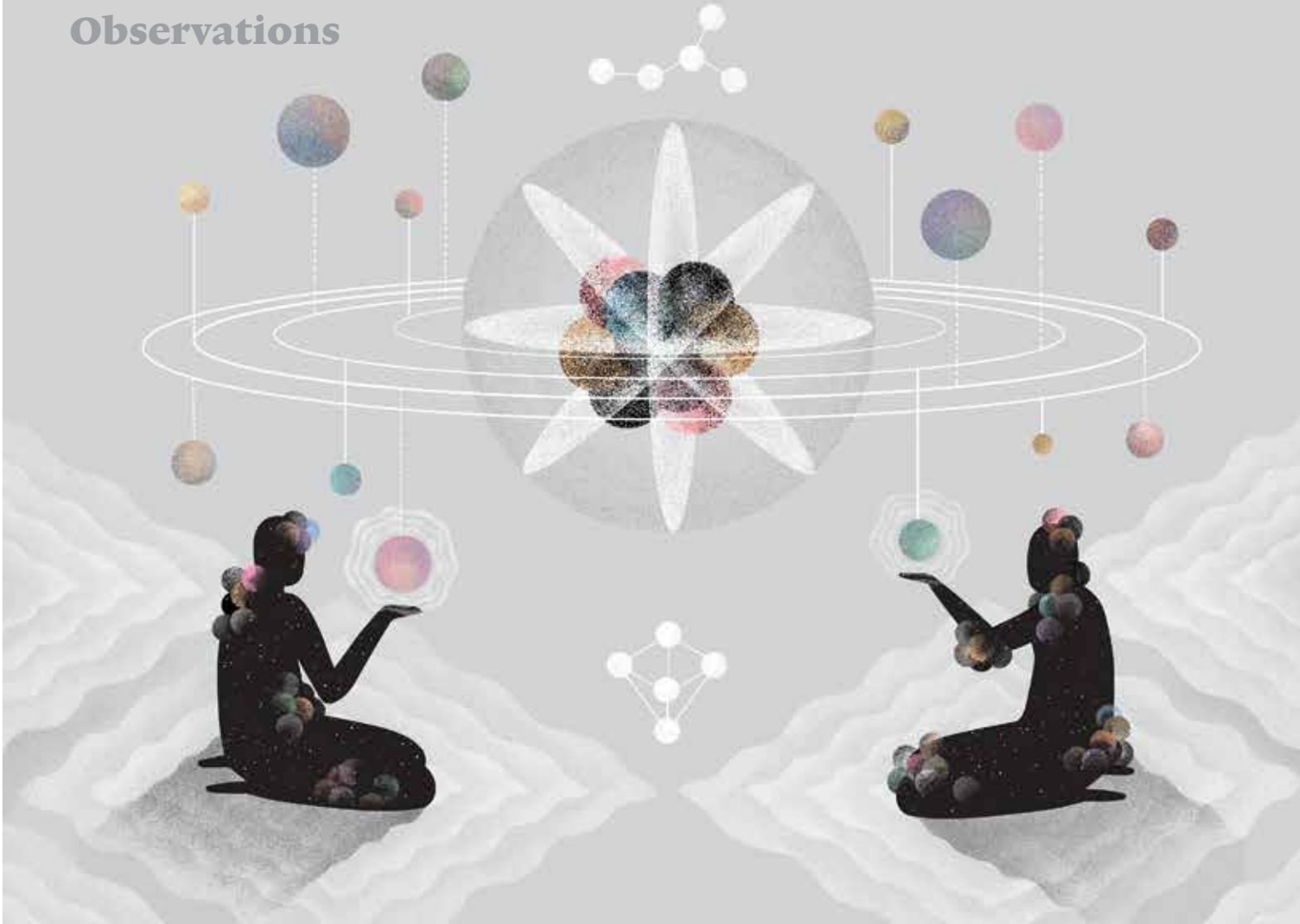
International Early Career Scientist Pedro Carvalho recently uncovered a third complex that targets proteins associated with a specialized ER domain in the inner nuclear membrane (INM).

When Carvalho and his team at the Center for Genomic Regulation in Barcelona, Spain, removed the two known protein patrol complexes from yeast, they noticed that some molecules were still being disposed of, suggesting that another scout was at work. As they reported September 18, 2014, in *Science*, this mystery warden turned out to be the Asi complex—a gang of three molecules in the INM that not only gets rid of misfolded proteins, but also removes certain correctly folded proteins that are incorrectly directed to the INM.

Carvalho and his team are now looking for other proteins that bind to this patrol complex.

Primate Illustration: John C.W. Carroll / Gladstone Institutes; Quality Patrol Illustration: Leif Parsons

Observations



Marina Muun

The Essence of our Being

We humans may think of ourselves as solid objects, all flesh and bone. But take a close look, and it's clear our bodies are composed largely of oxygen and hydrogen. We are essentially ephemeral–akin as much to wind, water, and fire as to earth. No matter how heavy you feel, says biologist and author Curt Stager, you are more like a "porous froth of atomic Styrofoam" than a dense mass. Understanding the world in atomic terms can give us a richer appreciation, he says, for how we interact with each other and the environment around us.

What do atoms have to do with you? Everything. They were present and intimately involved when you and everyone you have ever loved–or hated–did everything that you and they have ever done. Every scent you've ever savored, every sight you've ever seen, every song you've ever enjoyed, every cry or sigh that ever passed your lips sprang from atoms at work within the atmosphere and the darkest recesses of your body. When you eat, the bodies of other living things become part of you. If you cut yourself, the wreckage of dying stars runs out in a stream of ancient atoms that triggered some of the most violent explosions in the cosmos. When you flush your wastes, you scatter the atomic echoes of lightning bolts and volcanoes into a global cycle that may some day return them to you, as unpleasant as that may sound. And whenever you grin, the sparkling of your teeth conceals the dim afterglow of nuclear fallout from Cold War bomb tests over the Pacific.

You are not only made of atoms; you *are* atoms, and this book, in essence, is an atomic field guide to yourself. All you need in order to interpret your life in primal elemental terms is access to some of the latest scientific information, some new ways to reconsider your world in light of it, and an active imagination. In doing so you will begin to experience a revolution in self-awareness that is playing out on a larger scale around the world.

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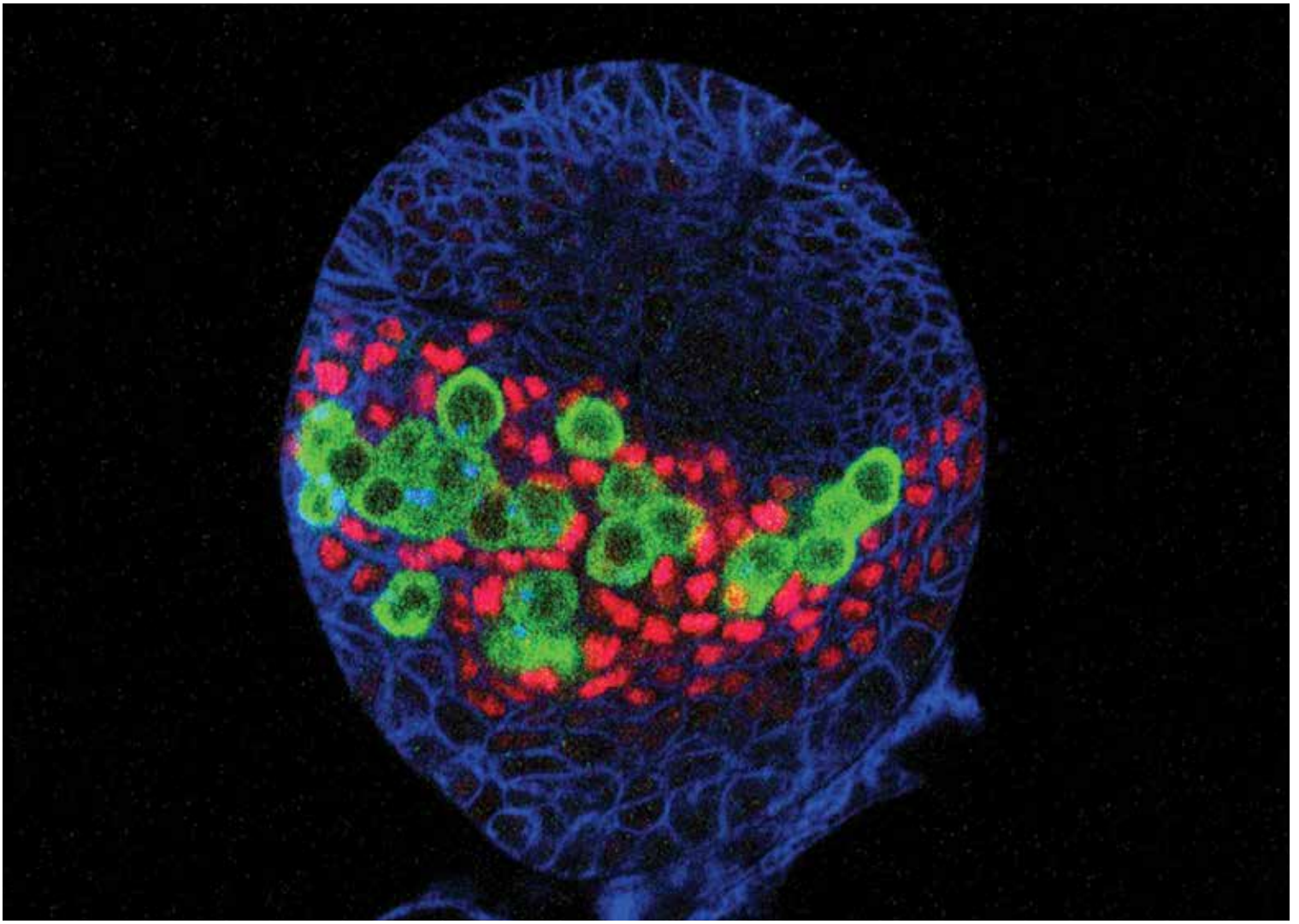
Cradle of Life

Mystery and awe surround the genesis of life, whether observed from afar or through the lens of a microscope. Even in the ovary of a fruit fly, as seen here, the beauty and wonder are undeniable. The orb-like organ is where primordial germ cells, or PGCs (green), rub shoulders with special "intermingled cells" (red), contact that is required for the germ cells to proliferate. Later in development, some of the PGCs will become germline stem cells–destined to start the process all over again–while the others will differentiate into eggs. Learn more about how germline stem cells hold the key to survival of a species, including humans, in "Perfect Balance," page 18.



Lilach Gilboa