Spring '14 Vol. 27 No. 2

HHMI BULLETIN

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Keeping Time

How do biological clocks control our daily schedule?

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Despite its small size, the brain of a developing fruit fly is remarkably complex, consisting of about 12,000 neurons and 3,000,000 synapses. Capturing this immense neural network in a map, says Janelia Group Leader Albert Cardona, would take him about 50 years. To speed up the project, Cardona has enlisted the aid of scientists from around the world. This snapshot shows the progress they've made thus far, with each lab's contribution marked in a different color. Though only 12 percent complete, the map has already revealed many new insights into the nervous system.

Albert Cardona

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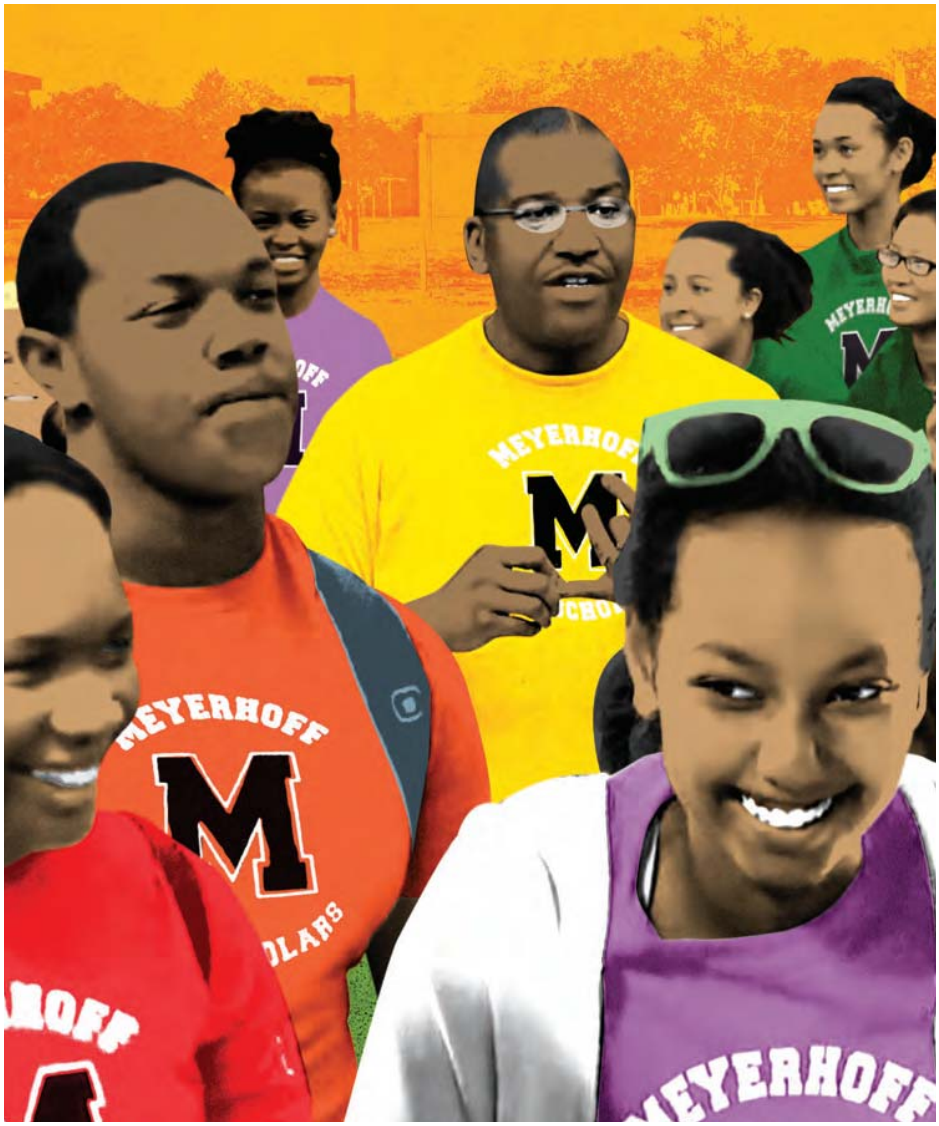

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- **See how fast cells earned their name in a video from a state-of-the-art microscope.**
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Cover image:
Michael Kirkham

Contributors

A self-described American Post-Pop artist, **John Ritter** ("Strength in Numbers," page 18) creates vibrant depictions of celebrities and popular culture that have appeared in *The New Yorker*, *Time*, *GQ*, *The New York Times,* and *Harper's*, among other publications. He lives and works in the wooded hills of Pennsylvania.

As HHMI's senior manager of marketing, **Sarah Goforth** ("Strength in Numbers," page 18) believes in the power of analytics to help science communicators reach and engage their audiences. She has 15 years of experience as a science writer, editor, and data junkie. Her last article for the *Bulletin* was a feature profile of Bert Vogelstein.

Brandon Keim ("In the Zone," page 4) is a freelance journalist specializing in science, environment, and technology. A regular contributor to *WIRED*, his work also has appeared in *Scientific American Mind*, *Aeon Magazine*, and *Nautilus*. Born and raised in Bangor, ME, and now based in Brooklyn, NY, he is an avid photographer, hockey player, and aspiring landscape gardener.

Clare Mallison ("Destination Science," page 24) is an illustrator living in London, England, whose work is created with a mix of marker pens, dip pen, ink, pencil, and Photoshop. She studied illustration at Kingston University and loves drawing on location to capture the whimsical moments of everyday life.

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John Ritter, Sarah Goforth, Brandon Keim, Clare Mallison

President's Letter

James Kegley

Opening Doors

DIVERSITY IN THE sciences has become a very important goal. In everything we do at the Howard Hughes Medical Institute, we are trying to cast the net wide. Quality is everywhere—and it's our job to go find it.

The Visitor Program at Janelia Farm Research Campus, highlighted in this issue of the *HHMI Bulletin*, exemplifies that philosophy. The idea of welcoming visiting scientists to Janelia to collaborate and make use of its resources was baked into the research center's earliest planning. In fact, that's how I got involved at Janelia in the first place, back in 2005, when I was still an HHMI investigator at the University of California, Berkeley. I saw Janelia as an opportunity to jump into an entirely new field—molecular imaging—which now represents some 85 percent of my research program. The imaging technologies that Janelia researchers shared were a complete game changer, both accelerating and deepening my studies of gene regulation.

And it's absolutely a two-way street. Janelia has reaped huge rewards from the influx of visiting scientists from across the United States and 23 other countries to date. Because Janelia's visiting scientists come in from such diverse institutions and locations, bringing technologies, expertise, and their unique viewpoints with them—as happened with my team—the effect on the whole enterprise becomes exponential.

"Diversity of all kinds gives our endeavors the deepest possible talent pool and the best chance for accelerating scientific progress."
—ROBERT TJIAN

Indeed, diversity of all kinds—not only of research field and expertise, but also of culture, ethnicity, and gender—gives our endeavors the deepest possible talent pool and the best chance for accelerating scientific progress. On the science education side, HHMI aims to increase the number of underrepresented minorities in the sciences through the expansion of a highly successful program from the University of Maryland Baltimore County (UMBC). The Meyerhoff Adaptation Project, also featured in this issue of the *Bulletin*, will attempt to identify the elements of UMBC's Meyerhoff Scholars Program that ensure the retention of minority students majoring in science, technology, engineering, and math. We're very pleased to be supporting the University of North Carolina at Chapel Hill and Penn State University in establishing scholar programs on their campuses, and for committing to rigorous documentation and evaluation. Our hope is that, by identifying a set of core principles and methods, this type of program can be put into place on university campuses across the country.

Finally, we're mindful of the need for more balanced gender representation in the sciences, including in leadership positions. We are pleased to report that half of HHMI's senior executive team is female, including both our executive vice president/chief operating officer and our chief scientific officer. But more needs to be done to encourage talented scientists and educators who are women. Indeed, we have plenty of work ahead to expand opportunities in the sciences for all who have talent and ability, and we're committed to progress. I'm optimistic.

Jorge Colombo

In the Zone

ON A MIDWINTER Saturday afternoon at the King Pong table tennis club in downtown Manhattan, two men are playing Ping-Pong. It's a sport best appreciated in person: when hit by a skilled player, balls can easily reach speeds of 30 miles per hour, traveling a table's length in an eye blink. Both these players are skilled.

One of them is Evgeny Nudler, an HHMI investigator and molecular biologist at New York University. Today he's just an intense guy with a paddle, moving with almost preternatural anticipation of what looks less like an orange ball than a laser beam.

"You don't focus on anything. You just empty your mind and use your reflexes," he says later. "That's why I really like Ping-Pong. You cannot think about anything else."

Sure, Ping-Pong is good exercise, a way of being more than hands and eyes attached to a computer. As the game goes on, Nudler works up a welcome sweat. But it's more than good exercise. After a 60-hour workweek, with many more ostensibly free hours spent thinking about research, Nudler seeks escape.

"I need some distraction from work," he says, "to completely switch off my brain."

He's not complaining. Nudler loves his work. But running a 25-person lab, with multiple lines of research—studying the molecular mechanisms underlying gene expression and cellular responses to heat shock, for example—is all-consuming.

Helping Nudler leave that effort behind, at least momentarily, is Slava Solganik, a former theoretical physicist and emigrant from Ukraine. (Nudler is from Moscow, arriving in New York in 1993, shortly after the collapse of state-funded research sent Russia's science into disarray.) They've met on afternoons like this for a decade, and now trade shots like fencers.

Slow rallies quickly turn into ballistic barrages with barely a pause in the staccato of ball on paddle. At times the game seems less like competition than choreography. Solganik's movements are short and compact, while Nudler puts his entire body into his shots. He's light on his feet, with flowing movements hinting at the Japanese martial arts he practiced for many years.

On this day, Solganik wins. Next Saturday they'll meet again, sending the ball blurring between them for another blissful hour. "It makes you empty your mind. I think it's good for you," Nudler says. "It's like a good sleep." *–Brandon Keim*

For more on the Prison University Project, visit www.prisonuniversityproject.org.

Science at San Quentin

TROY LIONBERGER NEEDED approval for every object used in the college-level physics lab course he designed in 2012. The tennis balls and tape measures, the pulleys and string—all had to be cleared. Sharp objects were forbidden. For one experiment, he and his co-instructors wrangled a hot plate, but they weren't allowed to bring in a glass beaker to heat water. "We ended up very carefully doing it with a plastic cup," he recalls.

This was no ordinary science class: It took place in a mobile trailer next to the old laundry building at San Quentin State Prison in California. The students, all wearing blue uniforms, were convicted felons.

Lionberger is a postdoctoral researcher in the biophysics lab of Carlos Bustamante, an HHMI investigator at the University of California, Berkeley. Evenings and weekends, he teaches in the nonprofit Prison University Project, which offers San Quentin inmates a chance to take free college classes and earn an associate of arts degree. The unique project runs on private donations and volunteer faculty from nearby universities.

Lionberger remembers his first visit inside San Quentin, as an undergraduate in 2001. "I was pretty terrified," he recalls. But soon he saw that prison students are just people eager to learn. "They come into the classroom to get away from their cells and their circumstances."

Inspired to pursue a career in academia, Lionberger earned a biology PhD and an engineering master's degree from the University of Michigan, then joined Bustamante's lab in 2011. But he also wanted to return to San Quentin and set up a math-intensive physics course that included a lab, a component that Prison U didn't offer but one that inmates needed to transfer credits to four-year colleges on the outside.

Lionberger recruited others at UC Berkeley to help teach the new class, including his wife, Diane Wiener (a postdoc in the lab of Susan Marqusee), and grad student Sam Leachman (in the Bustamante and Marqusee lab groups), who later organized a new chemistry lab course.

San Quentin had hosted a few science courses with rudimentary lab instruction, but Lionberger "brought an added level of rigor and determination" to the task, says Prison U executive director Jody Lewen.

The experience has been deeply rewarding, Lionberger says. For some inmates, learning scientific knowledge they never imagined they could handle instills self-confidence and a desire to build a better future through education. Studies show that higher education can reduce the odds that ex-convicts wind up behind bars again.

"Education is transformative," says Bustamante, who at first questioned whether the volunteer teaching could really make a difference. Now a believer, he and Lionberger have even invited former San Quentin student Daniel Jackson to the Berkeley lab. The 52-year-old served 19 years in the state prison system for offenses related to drug dealing. He earned his degree from Prison U last summer and was released in October. In January, he began studying computer engineering part-time at San Francisco State University while holding a full-time job. He hopes to work with Lionberger on writing computer code for the lab.

Thanks to Prison U, Jackson says, "I got it together now. I can see the future." *–Ingfei Chen*

Daniel Stolle

The Method in Cancer's Madness

Computational approaches reveal that massive chromosome alterations give cancer an edge.

CANCER CELLS ARE known for the rampant disorder in their genomes: extra or absent chromosomes or parts of chromosomes, long stretches of DNA gone missing or present in too many copies. "It looks like someone threw a stick of dynamite into the nucleus," says HHMI Investigator Stephen Elledge of Harvard Medical School and Brigham and Women's Hospital. "It's a real mess."

This chaotic state is called aneuploidy. It stems from errors during cell division causing the daughter cells to have abnormal numbers of chromosomes or chromosome fragments. Aneuploidy affects hundreds or thousands of genes and can wreak all kinds of havoc, including miscarriages, lethal birth defects, and disorders like Down syndrome.

Whether aneuploidy foments cancer is a 100-year-old mystery that scientists have largely neglected, according to Elledge. They've focused instead on potent single-gene mutations called "cancer drivers." Some of these drivers disable tumor suppressor genes, allowing unruly cell division. Others send growth-promoting oncogenes into overdrive.

But Elledge suspected aneuploidy was an important part of the story. Based on his group's latest research, Elledge says these massive alterations have evolved because they give malignant cells an edge in the "brutal competition" to win out over normal cells. Their research is described in a paper in *Cell* in November 2013 that built on the group's July 2012 report in *Science*.

His team devised computational methods to analyze mutation patterns in 8,200 tumors containing more than one million mutations. From these results they identified a large number of tumor suppressors (also known as STOP genes) of varying potency. The more potent a tumor suppressor, the greater its potential for causing cancerous growth if disabled by a mutation. They also identified a list of oncogenes along a continuum of strength, based on their pro-growth effect if mutated. The researchers estimated the genes' potency by the frequency with which they were lost or gained in the cancer cells' evolution.

Elledge then predicted that chromosomes on which the cumulative impact of STOP genes outweighed that of GO genes (the oncogenes and "essential genes" needed for normal or cancerous growth) would be more frequently deleted in cancer cells—in effect, disabling the normal brakes on cell growth. He tested this hypothesis using statistical methods and found the predictions were accurate to a degree that surprised even him. He also found the converse to be true, that chromosomes on which the cumulative impact of GO genes outweighed that of STOP genes were often amplified.

"Knowing the identity and likely potency of these cancer drivers has allowed us to uncover a driving force behind the selection of losses and gains of chromosome arms or whole chromosomes," notes Elledge. "We have basically answered the question: Does aneuploidy drive cancer? We believe it does."

Wiping Out Entire Clusters

Because chromosomes exist in pairs, the loss of single chromosomes affects only one copy of a given gene. The second copy on the partner chromosome remains intact. As a result, these "hemizygous" losses have a weaker effect on cancer growth than the mutation of both copies of a tumor suppressor gene. But the additive combination of groups of hemizygous losses can have a large impact.

In addition to aneuploidy, there are also recurring deletions of specific segments of chromosomes. "It turns out there are more STOP genes in these focal deletions than you would expect to occur randomly," Elledge says. "So that brought up the idea that maybe a cancer cell is looking for these clusters of tumor suppressors, and wiping out an entire

"We have basically answered the question: Does aneuploidy drive cancer? We believe it does."
—STEPHEN ELLEDGE

Jon Han

cluster at once—getting a bigger punch, a bigger bang for their buck."

To those familiar with the "two-hit" model of cancer, it may come as a surprise that loss of a single gene copy can have an effect. According to this model, a mutation in a single copy of a tumor suppressor gene does nothing because the second copy compensates, and only if that second copy is subsequently "hit," or mutated, does the cell begin its malignant journey.

However, Elledge cites evidence that a large proportion of cancer-suppressing genes are "haploinsufficient"—loss of even one copy can contribute to cancer development. In fact, Elledge estimates that 30 percent of all genes in humans are haploinsufficient, which has important implications for human development and disease.

"Losing or gaining single copies of genes on their own may have small effects, but altering many at the same time gives the cancer cell an advantage," says Angelika Amon, a biologist and HHMI investigator at the Massachusetts Institute of Technology who studies aneuploidy. "Once you see [Elledge's findings], you realize these losses and gains are not random noise in tumors, and we can begin to understand them."
–Richard Saltus

Bench Report

Reduce, Reuse, Recycle

Supporting autophagy to fight disease.

TO SURVIVE IN an ever-changing environment, cells require an assortment of parts—but not all at the same time. As needs change, proteins and organelles become obsolete or fall into disrepair. This debris could muck up a cell pretty quickly. But a process called autophagy tidies up the mess.

Unwanted parts are engulfed in a double-membrane sac, which then fuses with an organelle called a lysosome, where enzymes break down its contents. The bits left behind can be recycled by the cell for new construction.

In her research lab at the University of Texas Southwestern Medical Center, HHMI Investigator Beth Levine is exploring the complex cellular pathways that control autophagy. Each discovery that links autophagy to a life-threatening disease spurs Levine—who began her career as a traditional MD and then gravitated to research—to dig deeper.

"We work on basic biochemical mechanisms, but disease is always in the backs of our minds," she says.

Since she discovered Beclin 1 in 1998 and demonstrated it to be the first known mammalian autophagy protein in 1999, Levine has directly linked the cellular housecleaning process to cancer signaling and shown that it helps animals fight off infectious disease. She's also demonstrated that autophagy is required for the longer lifespan associated with caloric restriction in worms and is a big part of the beneficial effects of exercise in mice.

Keeping Organisms Healthy

Levine first encountered Beclin 1 during research on viral pathogenesis. When the protein turned up in a screen for molecules that interacted with an inhibitor of programmed cell death, she noticed that its genetic code lay in a region of the genome often missing in breast and ovarian tumors of patients who have no family history of the diseases. This suggested that the protein encoded by the gene might play an important role in cancer prevention.

Levine's team soon found that Beclin 1 protects mice from lethal viral encephalitis. After her team reported those antiviral effects in the *Journal of Virology* in 1998, they learned that a yeast gene closely related to the mouse gene *beclin 1* is essential for autophagy. Levine's team was able to restore autophagy in cultured yeast cells lacking their own autophagy gene by giving them Beclin 1. Boosting levels of the protein also restored autophagy in cultured human breast cancer cells that were missing a copy of *beclin 1*.

The discovery that Beclin 1 enables autophagy suggested to Levine that it had a broad and vital role in keeping organisms healthy. "Autophagy is the only mechanism inside a cell for degrading pathogens and damaged organelles," she notes. Excessive accumulation of such debris had already been linked to aging, cancer, and neurodegeneration, and Levine suspected that understanding Beclin 1 might shine a light on those conditions.

Her team's 2003 finding that mice with only one functional copy of the *beclin 1* gene developed more spontaneous tumors than normal mice did provided convincing evidence that Beclin 1 interferes with mammalian cells' spiral into cancer.

Levine's lab group continues to study how autophagy helps suppress tumor formation. The team has discovered that the proteins produced by two potent oncogenes, *Akt* and *EGFR*, directly modify Beclin 1, inhibiting its ability to trigger autophagy. In work published in *Science* in 2012, they showed that Akt's interactions with Beclin 1 contribute to Akt's ability to transform healthy cultured cells into tumor cells. In 2013, they reported in *Cell* that EGFR must interact with Beclin 1 to drive tumor progression and resistance to chemotherapy in animals with non-small cell lung tumors grown from human cells.

Feeding Muscles

One of Levine's most recent discoveries concerns the impact of exercise on disease. Since debris recycling helps supply a cell's energy-producing mitochondria with the raw

Trevor Paulhus

"We're looking into whether exercise's effects on aging, certain types of cancer, and delaying the onset of neurodegenerative diseases are through autophagy."
—BETH LEVINE

materials for making the energy molecule ATP, Levine wondered whether autophagy might help muscle cells keep up with exercise-induced energy demands.

Levine's team showed that, in mice running on treadmills, autophagy increased in skeletal and heart muscle, as well as in the liver, pancreas, fat cells, and brain. Mice whose cells were unable to dial up autophagy during exercise had less endurance. They couldn't boost the uptake of glucose to fuel their muscles. In addition, exercise did not protect autophagy-deficient mice against diabetes induced by a high-fat diet, as it does in normal mice.

"I think it's going to help explain a significant part of the mechanism underlying the beneficial health effects of exercise," Levine says of the findings, which were published in *Nature* in 2012. "We're looking into whether exercise's effects on aging, certain types of cancer, and delaying the onset of neurodegenerative diseases are through autophagy."

Levine is optimistic that targeted drugs may help patients with diseases that may benefit from increasing autophagy. Through her studies of Beclin 1's interaction with a protein made by HIV, her lab has created a peptide that holds promise. A short, 18-amino-acid segment of Beclin 1 that binds to the HIV protein inhibited growth of the virus in cultured cells. The peptide also was remarkably effective in inducing autophagy in virtually all cultured cells examined and in multiple tissues in mice. "This small piece of Beclin was sufficient to do the job of the whole protein," Levine says.

In fact, the peptide induces autophagy that protects against West Nile encephalitis in mice and clears protein aggregates associated with Huntington's disease in lab-grown cells. Since describing the peptide in *Nature* in February 2013, Levine's team has been working to make it into a drug-like molecule.

–Jennifer Michalowski

Bench Report

A Killer's Weakness Exposed

New options for arresting the renegade protein known as K-Ras.

KEVAN SHOKAT HAS been trailing one killer after another since the early 1990s. The organic chemist and HHMI investigator at the University of California, San Francisco, has been investigating signaling pathways within a cell, domino chains of molecules that, when disrupted by a mutation, can trigger uncontrollable growth that turns an ordinary cell cancerous.

He began by trying to inhibit molecules at the front end of these pathways, but just over a decade ago, he moved down the chain and deeper into the cell, focusing on a protein called K-Ras. Normally, the protein is key to healthy cell growth. But when mutated, "it is the most frequently activated oncogene, the oncogene that is responsible for the cancers with the worst prognosis for patients," Shokat says. And, he adds, it had proven completely resistant to attempts to inhibit it.

That frustrating picture may be changing. Shokat has found a hidden pocket in a mutated, cancer-causing form of K-Ras that allows a drug molecule that his team built to bind to it. "K-Ras is like a switch when it comes to cancer, and this molecule keeps the switch in the off position," Shokat says. Even better, he adds, the molecule only sticks in the pocket in the mutated form of K-Ras, leaving the normal form of the protein alone. Shokat and his team reported their findings in the November 28, 2013, issue of the journal *Nature*.

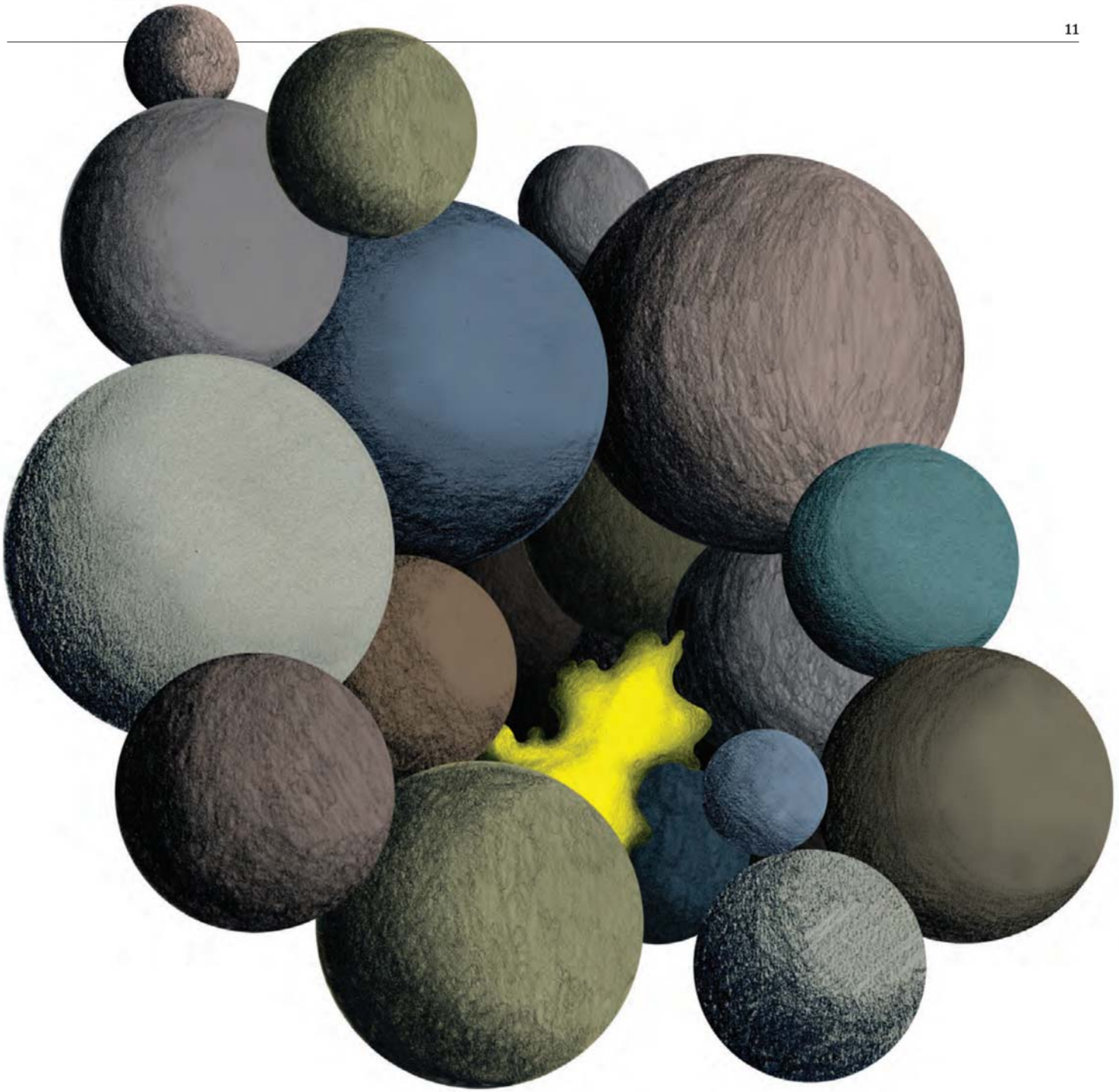
Multiple ways to inhibit the mutant may be emerging as other researchers are learning how to disrupt proteins that help mutant K-Ras encourage cancerous growth. The National Cancer Institute has seen enough recent progress to fund new work on the Ras family of oncogenes to the tune of $10 million. To move his team's compound into drug development, Shokat has cofounded a company, Araxes Pharma, which is partnering with Janssen Biotech to improve the potency and stability of the compound.

K-Ras was identified in the 1980s. The mutant form is active in at least 20 percent of human cancers. It is especially dangerous in pancreatic and colon cancers, where it is mutated in 90 percent and 40 percent of cases, respectively. It is also mutated in 20 percent of non-small-cell lung cancers. Shokat's team has been focusing on the most common form of these lung cancer mutations, a version of K-Ras called G12C.

Normal K-Ras signals cells to grow by binding to a nucleotide called GTP and helping transform it into another molecule called GDP, which stops the signal. Mutant K-Ras, however, doesn't switch the complex to GDP, and it binds very strongly to GTP, forcing malignant, ongoing cancerous growth. "The mutant has its foot on the gas all the time," says Shokat.

In G12C, an amino acid called cysteine is substituted for a glycine in one particular spot. Cysteine also has chemical properties that make it easy for another molecule—like a potential drug—to bind with it. The researchers screened 700 molecules over three years, and finally found one that fit tightly to the cysteine. When they examined the structure of the protein bound to the molecule, they learned that the docking occurred near a pocket no one had noticed. That pocket appears to be a crucial part of the K-Ras link to GTP, the "on" switch. Adding the new molecule kept the link from forming.

Stephen Fesik, who studies the structural biology of the protein at Vanderbilt University, says there are many positive things about Shokat's approach, but a few concerns, too. "Kevan has identified a way to bind a molecule to mutant K-Ras and not the wild type, or normal, protein. That was very cool." However, Fesik notes, researchers—including his own group—have had a very difficult time ensuring that drug candidates bind very strongly to the



Merijn Hos

"K-Ras is like a switch when it comes to cancer, and this molecule keeps the switch in the off position."
—KEVAN SHOKAT

K-Ras complex. He's also concerned that using cysteine as a target could create some toxic problems within the cell. Many cellular proteins have a free cysteine, he says, so a drug aimed at K-Ras could latch onto them as well and disrupt their normal function, harming the cell.

Shokat concedes that nothing is ready for the clinic yet. But he says his team has found ways of stabilizing the bond, and the cysteine approach he is using would not necessarily interfere with other proteins, only K-Ras. "We tested this," he says, "by asking if the abundant protein albumin, which has multiple free cysteines, reacts with our compound—and it does not." The compound he is working with also has a structure similar to a cysteine-reactive anticancer drug, called Ibrutinib (Imbruvica), which was recently approved by the U.S. Food and Drug Administration as a treatment for chronic lymphocytic leukemia.

Now, Shokat says, the search is on for singular features, like the G12C pocket, in forms of K-Ras that cause pancreatic cancer and colon cancer. He's found vulnerability in one killer, he says. Time to go after the others.
–Josh Fischman

Around the Clock. Scientists know how living cells keep what these circadian for human health.

BY SARAH C.P. WILLIAMS

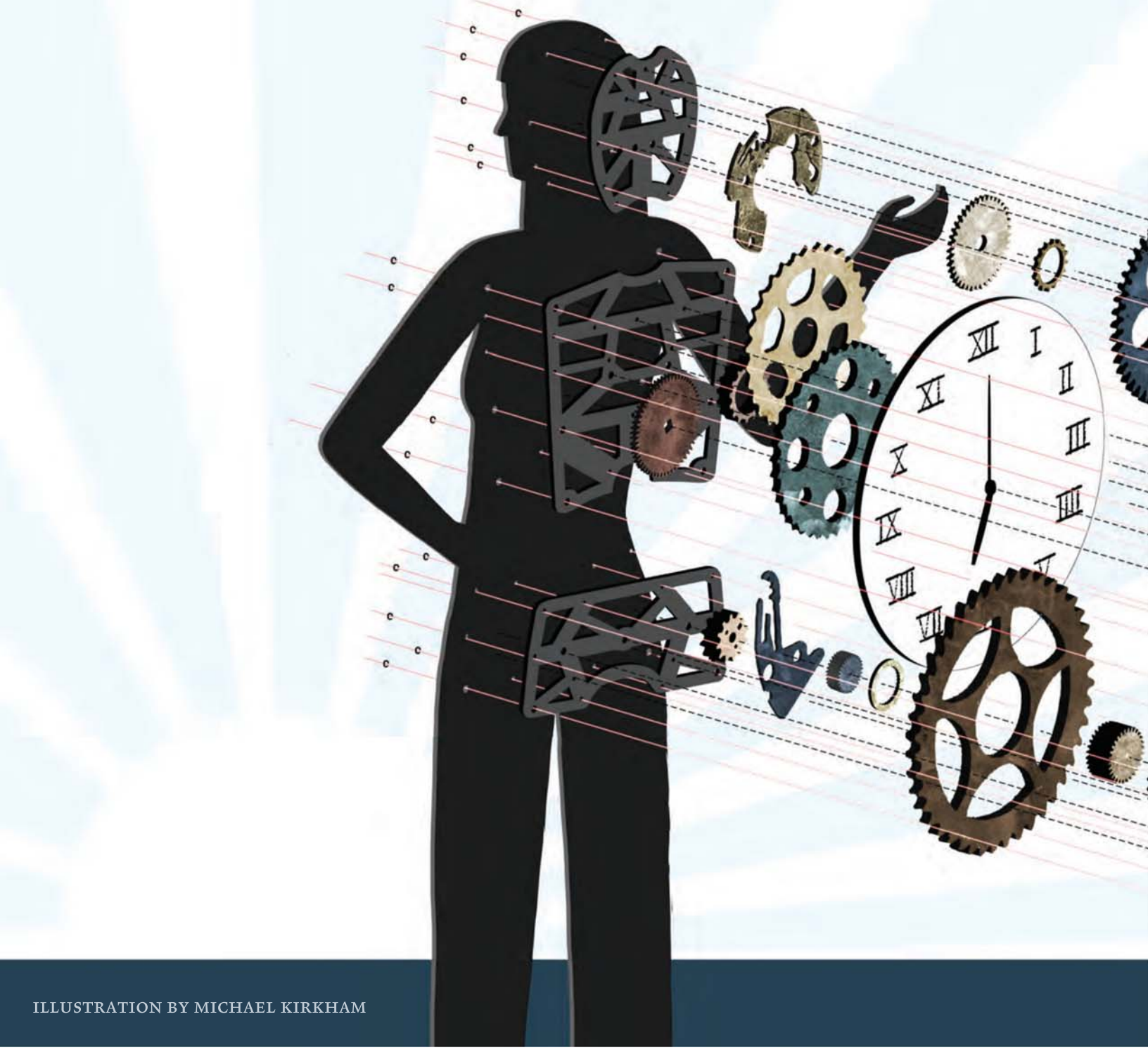
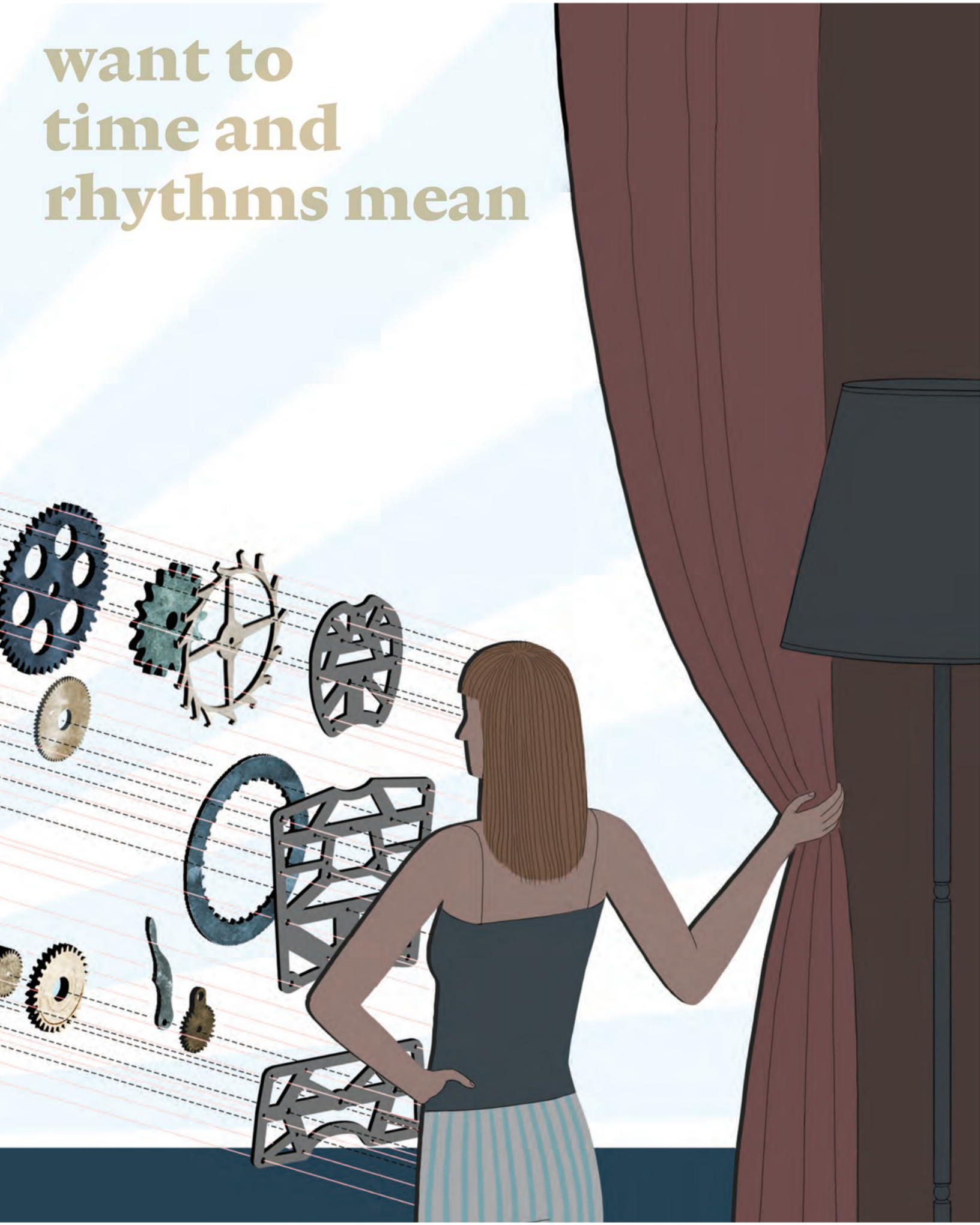


ILLUSTRATION BY MICHAEL KIRKHAM

want to time and rhythms mean



Circadian cycles in mammalian cells are the focus of Joseph Takahashi's work.

IN NEARLY EVERY place on Earth, life pulses with a daily rhythm. As the sun rises over the savannah, plants spread their leaves and animals blink open their eyes. As light hits the oceans, night-loving crabs bury themselves in sand and fish emerge from deep water in search of food. In towns and cities, people wake to alarm clocks and read the morning news. These routines of life are more than a matter of habit and convenience. They are driven by deeply rooted biological programs that keep the planet's inhabitants on a 24-hour schedule called a circadian rhythm.

The circadian clock influences far more than daily habits, according to a few decades of research. Obesity, diabetes, cardiovascular disease, liver disease, cancer, and depression have all been linked to malfunctions of the internal timekeeping scheme. Almost every cell in a living organism, it turns out, sees daily fluctuations in levels of genes and proteins, and when these fluctuations are dampened or stopped, things can go awry.

"The circadian system has its tentacles around everything," says HHMI Investigator Michael Rosbash. "It's ticking away in almost every tissue in the human body." And in plants, too—including major food crops—circadian rhythms are tied to disease susceptibility, growth rate, and fruit size.

Studies by Rosbash and other HHMI scientists are revealing links between the internal clock and health, and they are detailing the complex cellular machinery that drives the circadian clock in organisms from plants to flies to humans. They hope that discovering these components—like understanding the gears of a wristwatch or the swinging pendulum of a grandfather clock—will allow them to fix the system when it's off-kilter, or at least prevent it from losing track of time when a person travels across time zones or pulls an all-nighter.

Noon

The sun hangs high in the sky and shadows have all but disappeared. At Sonny Bryan's Smokehouse in Dallas, the line of lunchtime patrons snakes out the door and the smell of Texas barbecue wafts across the parking lot. But it's not just the smell that has everyone's stomachs growling. Inside each waiting customer's body, the digestive system is preparing for the midday meal. Anticipating food, hormones in the liver and stomach spike, and neurons in the brain send hunger signals throughout the body.

Across the street, on the University of Texas Southwestern Medical Center campus, scientists in Joseph Takahashi's lab can't smell the barbecue, but they can tell that it's the middle of the day just by glancing at their cages of mice or by measuring protein levels in cells—both cycle in predictable ways each day.

Twenty years ago, Takahashi, an HHMI investigator, watched daily behavior patterns of mice to discover the first gene—dubbed *Clock*—that controls the circadian rhythm in mammals. He found that healthy mice follow a 24-hour schedule of sleeping, eating, and exercising, even in steady darkness. But without normal CLOCK proteins, mice kept in a dark environment alternate these behaviors at all times of the day and night. Since that 1994 discovery, researchers have identified a host of other circadian control genes.

"This field has gone through many different phases," says Takahashi. "At the turn of the century, it was incredibly exciting because all these genes were being discovered. Then, we found that clocks were in almost every cell of the body. Now, we're beginning to understand the role of clocks in cell and organismal physiology and metabolism."

Before Takahashi's work on mammalian clock genes, former HHMI Investigator Michael Young at Rockefeller University, along with Rosbash and his Brandeis University colleague Jeffrey Hall, had already cloned circadian genes from the fruit fly *Drosophila melanogaster*. They'd established that the genes' expression cycled over 24 hours using a feedback system: in the flies, genes called *timeless* and *period* produced proteins that paired up, traveled to cell nuclei, and turned off the very genes that encoded them. Interestingly, the mammalian CLOCK and BMAL1 proteins could regulate the fly *period* and *timeless* genes, and this led to the concurrent discovery of the fly versions of the *Clock* and *Bmal1* genes by Takahashi and Steve Kay and by Rosbash and Hall. Young then went on to discover mutants that made this cycle last longer or shorter than the typical day length, and Rosbash and Hall pinpointed and cloned a host of other genes that influenced the clock.

Takahashi and others found that in mammals, similar cycles occur: In mammalian brain cells, the CLOCK protein and its partner BMAL1 pair up and turn on *Period* and *Cryptochrome* genes. Once the Period and Cryptochrome proteins reach a certain level, however, they shut down CLOCK and BMAL1.

Michael Rosbash studies the daily changes that occur in the circadian neurons in the fruit fly brain.

"The circadian system has its tentacles around everything. It's ticking away in almost every tissue in the human body."

—MICHAEL ROSBASH

The competition between the two sets of proteins leads to an endlessly fluctuating cycle—one that takes 24 hours to complete. Levels of CLOCK and BMAL1 peak during each day, and Period and Cryptochrome rise each night. As they rise and fall, the quartet of proteins turns on and off many other genes throughout the body. That's how the circadian rhythm has such wide-ranging effects.

Recently, Takahashi focused on how the circadian cycle influences the liver. "If you look in the liver at the targets of CLOCK and BMAL1, and plot those genes on a chart of metabolic pathways, essentially the entire chart would be covered," he says. "Every fundamental metabolic pathway is under circadian control."

The link between the clock and metabolism explains why humans and animals tend to eat at the same time every day, and why digestive molecules increase even before a person sits down for a meal. But that isn't all—Takahashi's group discovered that CLOCK and BMAL1 not only bind many of the metabolism genes, they also recruit RNA polymerase II—a protein needed to read every gene in the body—throughout the genome on a circadian basis.

The implications of this observation are huge, Takahashi says, and suggest that the majority of genes in the body may be under the control of the clock through the cycling of RNA polymerase II.

"What this means is that, even genes that we can't directly measure as having circadian cycling are, in fact, cycling in some ways," he says.

In *Drosophila*, Young has used high-throughput genetic technology to screen all 14,000 fly genes for daily cyclical activity. In the fly's head alone, he found, the levels of more than 500 genes fluctuate in circadian patterns. But over the past few years, scientists have also realized that the circadian rhythm doesn't just control protein levels by altering when genes are transcribed from DNA to RNA, the first step in gene expression.

Rosbash, also still working in *Drosophila* as a model for circadian rhythms, is revealing the many ways that protein levels are controlled by the clock. He uses a technique called nascent RNA sequencing to get a snapshot—at any given time—of which genes in a cell are being actively transcribed into strands of so-called nascent RNA. This is different from looking at what strands of processed messenger RNA are present.

"What we see is a disconnect between the nascent RNA profiles and some messenger RNA profiles," explains Rosbash. That observation suggests that circadian regulators control the production of genes at two levels: both when genes are transcribed into RNA, and also when that RNA is processed and translated into proteins. His findings were published January 22, 2013, in the *Proceedings of the National Academy of Sciences*.

Rosbash has also focused his attention on the dramatic changes that happen every day in the approximately 150 circadian neurons in the fruit fly brain. These neurons are the equivalent of the cells in an area of the mammalian brain called the suprachiasmatic nucleus (SCN), which is responsible for receiving light signals from the eyes and then adjusting the timing of the circadian clock in the rest of the body. When the 20,000 or so neurons in the SCN are obliterated, the clock goes awry. The fly brain, Rosbash says, is a simple model for the more complicated mammalian SCN.

"During the day, the branched ends of certain fly circadian neurons spread out," he notes. "And then at nighttime, these ends all come together, curling back in. The neuron may go from touching 20 other neurons to just a few, then back to 20."

In a paper published in *Neuron* in July 2013, Rosbash reported that the gene *Mef2* links neuron morphology to the circadian clock. Proteins similar to CLOCK and BMAL1, he found, bind to *Mef2* regulatory signals, which—when turned on—reshape brain cells. Rosbash doesn't yet understand the implications of these daily neuron changes, but he suspects that they're one more way the internal clock controls physiology. "The clock governs so many processes," he says. Understanding those processes could lead to the development of ways to entrain the circadian clock for optimal health.

Takahashi: Brandon Wade, Rosbash: Paul Ferters

Amita Sehgal is trying to sort out how circadian rhythms affect sleep.

Dusk

The streets of Philadelphia have stilled after a whir of rush-hour commuters. Street lights flicker on, casting a warm glow on the pavement. Inside Amita Sehgal's lab at the University of Pennsylvania, graduate students hunch silently over their computers as janitors sweep up and down nearby hallways. Inside the lab's cages of *Drosophila*, things are quieting, too—flies have ceased flying and instead pose like statues, unmoving.

Like humans, fruit flies sleep in response to the daily rhythms of their bodies, making them a valuable tool for studying how circadian rhythms control sleep.

"Sleep isn't necessarily the number one best readout of the circadian rhythm when you compare it to molecular methods," says Sehgal, an HHMI investigator. "But it's by far the most obvious and the easiest to observe."

Almost a century ago, scientists discovered that sleep—in humans—syncs with the circadian rhythm. By the 1960s, experiments had shown that even in a constant light or dark environment, people will follow close-to-usual sleep and wake cycles.

To understand the sleep-circadian link, Sehgal is working her way through *Drosophila* chromosomes, one by one, searching for sleep-related genes. In 2008, she identified the *sleepless* gene on chromosome 2. More recently, her lab examined chromosome 3, introducing random mutations into genes and then observing which of them changed flies' sleep patterns. This turned up a gene—which Sehgal dubbed *redeye*—related to how much, and when, flies sleep.

Sehgal's team has observed that *sleepless* and *redeye* mutations do more than change the sleep patterns of flies; they impact their long-term health. *Sleepless* mutants live half as long as normal flies, and the gene mutation dials up the activity of stem cells in the testes of flies.

Sorting out how *sleepless* and *redeye* are linked to the circadian rhythm, however, is tough. When and how much an organism sleeps is affected not only by time of day, but also by how long it's been since its last slumber. The new *redeye* gene, Sehgal says, may relate to the second influence: the longer a fly has been awake, the higher the levels of *redeye*. The *redeye* levels continue to vary over the course of the day, even when fly clock genes are mutated, suggesting that *redeye* is not controlled entirely by the circadian rhythm.

"Sleep is subject to all sorts of influences," Sehgal says. "What we really want to know is where all these influences converge." The answer may help scientists treat sleep disorders and jetlag, or make shift work easier.

Midnight

At the medical intensive care unit at Yale-New Haven Hospital, it's hard to tell when it's the middle of the night. The fluorescent lights still burn brightly, pagers beep, and patients are shaken awake hourly. Inside each person's body, cells struggle to stay on schedule. The metabolic processes that normally wane at night are sputtering, confused by the lights and the noise.

HHMI Investigator Erol Fikrig is all too familiar with this 24-hour chaos—he's an infectious disease clinician at Yale School of Medicine. He's also a researcher who's recently discovered that daily circadian rhythms play a key role in how the human body fights infections.

Fikrig's research group, which studies mosquito- and tick-borne diseases like Lyme disease, dengue fever, and West Nile virus, began exploring whether the timing of insect bites affected how well people fight off disease. "People kept anecdotally telling us, 'I always get bitten by mosquitos at night,' or saying, 'I only get bites right around dusk,'" says Fikrig. "We wondered whether the human immune response is most vulnerable at a certain point in the day."

His lab group measured levels of immune molecules in the bloodstream of mice at different times. One—a receptor called TLR9 that recognizes invading pathogens—fluctuated over 24 hours. During the rodents' sleeping hours, levels of the *TLR9* gene plummeted; during their active hours, levels rose again, according to the group's 2012 report in the journal *Immunity*.

To see whether the changing levels of TLR9 had consequences, the scientists gave mice infections at different time points. The survival rates of the mice, they found, were linked to TLR9 levels at the time of infection. In a subsequent experiment, Fikrig's group found that a vaccine was most effective if given to the mice when TLR9 levels were highest.

"When do you get your flu vaccine? Whenever the clinic is available or you have time," says Fikrig. "None of us put any thought into what time of day we get the vaccine, but maybe we should."

Why would levels of an immune molecule rise and fall throughout the day? It may be taxing to keep the immune system on high alert all

"None of us put any thought into what time of day we get the flu vaccine, but maybe we should."

—EROL FIKRIG

Kevin Wolf

Erol Fikrig wants to understand how immune defenses change from day to night.

the time, Fikrig speculates, and organisms are less susceptible to many pathogens while they're sleeping.

"If you think about people who lived twenty thousand years ago, they were out in their environment all day. At night, when they were resting in their shelter, their susceptibility to diseases changed," he says. They were less likely to get a scrape or cut, but more likely to be bitten by a night-loving bug, so the immune system had different challenges to face.

Today, those daily cycles of pathogen risks may be dampened—at least in developed countries—but the circadian cycle of the immune system remains. Fikrig wants to know how to take advantage of this knowledge to help patients.

"Does this make certain patient populations more susceptible to infections at certain times in the day? If so, what can we do about that?" Fikrig asks. "And what happens when a person's circadian rhythm is off, like when she is jetlagged or in the intensive care unit with the lights on all night?" He's launched studies to explore those questions.

Dawn

In the moments before the sun's first rays peek above the horizon, plants already anticipate the light. Sunflowers tilt expectantly east and bean vines unfurl their leaves. Time-lapse movies of plant growth reveal some of the most visually enticing examples of the circadian rhythm. But—as in animals—many of the influences of the internal clock in plants are invisible to the eye because they happen inside cells.

In 2011, HHMI Investigator Xinnian Dong, at Duke University, was studying how *Arabidopsis*—a small, flowering plant common in labs—fights off downy mildew, a fungus-like microbe. She and her colleagues screened thousands of *Arabidopsis* genes and identified 22 gene mutations that weakened the plants' defenses. Fourteen of the genes, they discovered, had a DNA-binding site that allowed control by circadian proteins.

"We started sampling the expression of these genes every few hours," Dong says, "and were incredibly surprised to find out that these defense genes have a daily rhythm." The fungus-fighting genes, she found, were turned on each evening. This pattern of gene expression suggests that the resulting proteins likely accumulate throughout the night.

In retrospect, this makes sense, Dong says. Fungi tend to form spores at night, when it's cool and damp. In the morning, as moisture on a plant evaporates, the spores dry and are disseminated into a plant's cells. By rallying the maximum number of defense proteins in the morning hours, plants might effectively fight off downy mildew and related pathogens. Indeed, when Dong's team exposed *Arabidopsis* to mildew at dusk, the plants developed a more severe infection; the researchers reported their results in *Nature* in 2011.

Since then, Dong has shifted the focus of her lab to study how plant defenses are linked to daily rhythms. The group has discovered that it's a two-way street: the circadian clock controls the fluctuating levels of defensive proteins, but an infection with a pathogen can also affect the clock. In fact, they've learned that a plant's circadian control genes begin fluctuating much more strongly when a plant is fighting an infection.

"The clock runs at the same speed, but the changes in gene expression throughout the day are even more extreme," Dong says. She thinks that this helps the plant maintain its daily rhythms even while excess energy is being used to rid the plant of an infection. "You have this reinforcement of the clock to make sure it ends up back on track," she says.

As in mammals and flies, sorting out which daily changes in a plant are truly circadian can be tricky. Many plant behaviors—growth speed and direction, for example—are mediated by light, but aren't truly circadian.

"Ninety percent of the genes in a plant are expressed only at certain times of day," says Joanne Chory, an HHMI investigator at the Salk Institute for Biological Studies. "But many of those patterns go away if the plant is in constant light or darkness." That means that the genes are being controlled by the presence or absence of light, not by an internal clock. So outward appearances of the plant behavior can be deceiving.

"Since so many biological processes are regulated by the circadian clock, the big challenge for the future is to study the dynamic interplay among these processes," says Dong.

Synchrony

Human, mouse, fly, and plant circadian clocks are full of intricacies that make them seem more complex with every discovery. But even in the most basic of circadian clocks, questions still remain.

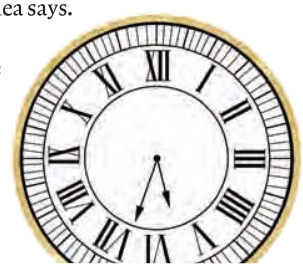
Since the mid-2000s, HHMI Investigator Erin O'Shea's lab group at Harvard has focused on studying the circadian clock of cyanobacteria, a type of aquatic bacteria that obtain their energy through photosynthesis. Consisting of just three proteins, the clock, as in plants, lets the organisms anticipate daylight to ramp up their metabolic processes. However, O'Shea found that, rather than being regulated entirely by gene expression, the cyanobacteria clock was controlled over time by the addition and removal of phosphates to or from the three involved proteins.

"The biggest surprise with this clock was how long it can continue keeping time even in constant light," says O'Shea, who is also HHMI's vice president and chief scientific officer.

A group of cyanobacteria, without communication between the cells, can remain in synchrony—with each other and the time of day—for weeks. And the simple clocks have a lot in common with more complex clocks: a pulse of darkness, for example, can reset the clock—a phenomenon that also occurs in mammalian cells.

In 2013, O'Shea published a paper in *Science* that added complexities—and questions—to the cyanobacteria clock. She found that, although the three-part protein clock can maintain a 24-hour cycle in the absence of certain gene fluctuations, circadian regulation of gene expression of clock proteins is required to maintain synchrony.

"There are still a lot of huge, unanswered questions, not just about this clock but about all circadian clocks," O'Shea says. The answers to those questions, she says, will most likely be found in studies focused on the basic molecular underpinnings that keep the circadian clock ticking in organisms from cyanobacteria to people. ■



Destination

Science

An innovative collaboration program opens Janelia's doors to visiting scientists from around the world.

BY NICOLE KRESGE

ILLUSTRATION BY CLARE MALLISON

IN MARCH 2013, Lillian Fritz-Laylin packed a cooler full of human immune cells and hopped on a plane from San Francisco to Virginia. Once she arrived, she spent almost every day in a small, dark room in Eric Betzig's lab at HHMI's Janelia Farm Research Campus, watching the cells crawl across a microscope's stage. A month later, she returned to the West Coast with more than 10 terabytes of data and information that would change the way a whole field looks at cell movement.

Fritz-Laylin, a postdoctoral researcher in HHMI Investigator Dyche Mullins' lab at the University of California, San Francisco, studies "fast" immune cells, which zip around about 100 times quicker than most other cells. They need this speed to respond to infections and other problems before things get out of hand. She'd like to know how they get around so swiftly. Unfortunately, microscopes that can capture the details of cells moving at this speed are few and far between. Except in Betzig's lab. He's built several microscopes that can visualize live cells, up close, in three dimensions. An avid collaborator, Betzig invited Fritz-Laylin to bring her cells to his lab.

What the microscope revealed was striking. A typical cell moves by oozing: extending a thin surface-attached edge, while simultaneously retracting its backside. The fast cells, as seen moving in 3-D, were different. They were covered in large, dynamic projections that extended and retracted in all directions [see Web Extra movie]. Fritz-Laylin suspects the fast cells are using forces produced by the rear retractions—like a tube of toothpaste being squeezed from the bottom.

The Janelia Visitor Program welcomes scientists such as Fritz-Laylin from around the world to the Ashburn, VA, campus to do research. To date, more than 180 visiting scientists from the United States and 23 other countries have participated in the program since it launched when Janelia opened in 2006.

Some collaborations last a few weeks; others go on for years. Some involve huge endeavors like mapping all the nerve cells in the fly brain. Others tackle smaller problems like sleuthing out the neurons that affect hunger in mice. By bringing together people with different expertise, the collaborations accomplish more than a single lab could have done on its own.

"Scientists come here and get access to equipment, budget, scientific reagents, and the ability to do things they couldn't do at their home institutions," says Janelia Executive Director Gerry Rubin. "It's a very special opportunity that doesn't really exist elsewhere."

"We really want to make sure that our state-of-the-art instrumentation or any new technique that we've developed can reach the community at large," explains Science Program Manager Zarixia Zavala-Ruiz.

Fly Fighters

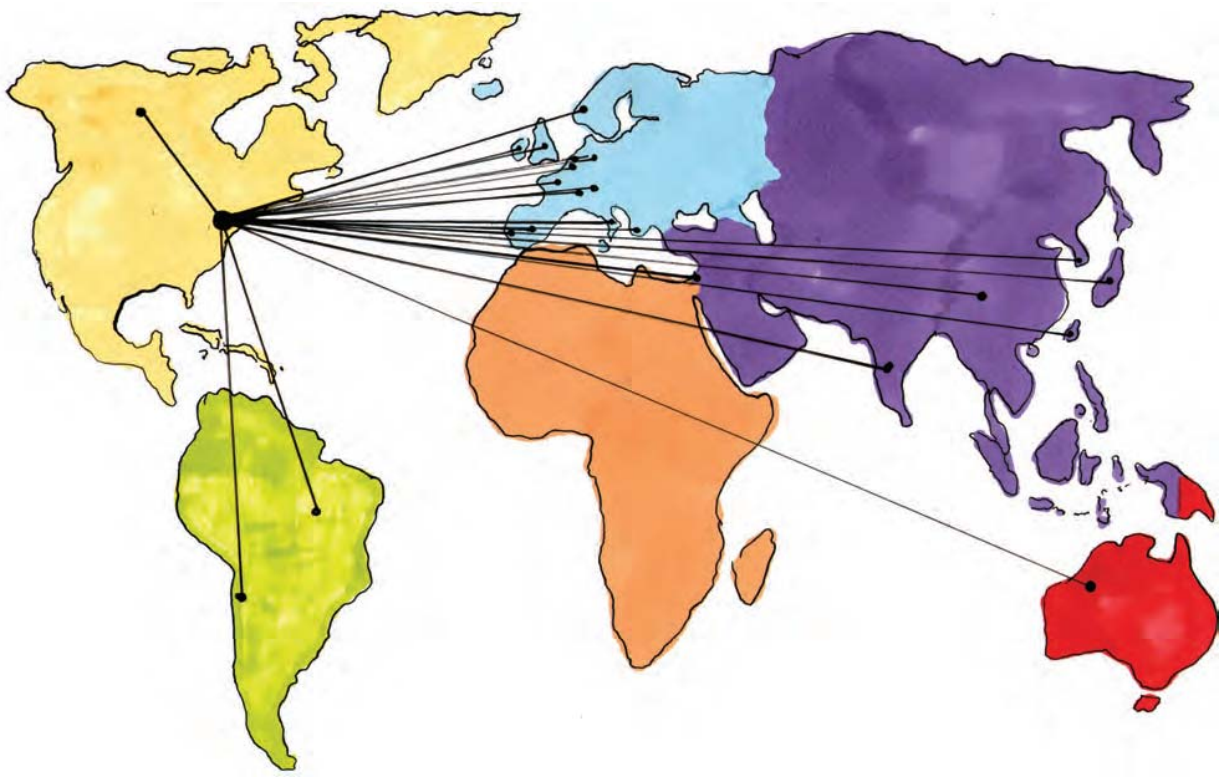
One of the very first visitors to Janelia was longtime HHMI Investigator David Anderson. His California Institute of Technology lab group is trying to understand how the brain processes emotional behavior such as fear, anxiety, and aggression. Over three years, Anderson traveled to Rubin's lab every couple of months to develop an assay to learn whether the fruit fly *Drosophila melanogaster* experiences fear.

"I would spend a week here and there, buried in the laboratory, running my experiments," Anderson recalls. "I really enjoyed the opportunity to get away from my administrative responsibilities at Caltech and immerse myself in doing bench science with my own two hands."

Anderson's initial project grew into a second, larger one focused on aggression. His postdoc, Eric Hoopfer, moved to Janelia to carry out the five-year project, which centered on a collection of 8,000 transgenic *Drosophila* lines. Created by Rubin and his team, each line has a different set of nerve cells that can be turned on and off. The entire collection covers the estimated 150,000 neurons that make up the central nervous system in the adult fruit fly. At the time, the lines were an extremely hot commodity for scientists interested in nerve cell function.

Hoopfer systematically turned on sets of neurons in the fly brain hoping to transform normally docile flies into aggressive fighters. "In the vast majority of days, they'd do

"The ability to do this project has been a great help to my career."
—ERIC HOOPFER



Visiting scientists have come to Janelia from 23 countries to collaborate on projects.

nothing," he admits. But once in a while, the flies would start going after each other.

"There are very specific populations of neurons that are involved in aggression, and looking for them is like looking for a needle in a haystack," Hoopfer explains. He ended up screening about 3,000 fly lines. Twenty of those lines contained neurons that increased aggression.

Back at Caltech now, Hoopfer is writing up his results and starting to figure out how the labeled neurons contribute to aggressive behavior. "The ability to do this project has been a great help to my career," he says. "The lines that I found are basically tools that I'll be able to use to start my own independent research group. There are years of work to be done figuring out how just these 20 lines work."

Hunger Circuitry

Janelia Group Leader Scott Sternson also likes to collaborate. Even before he set up his lab at Janelia, he had a list of scientists he wanted to work with. So it was perfectly natural for him to propose a project with neuroscientist Anirvan Ghosh within a few hours of meeting him.

Sternson studies hunger. Back in 2010 when he first met Ghosh, he was walking neuron by neuron through the paths that sense when a mouse's brain needs fuel, hoping to create a map of the circuitry that controls hunger. Unfortunately, the circuits weren't linear. Instead, the nerve cells' axons—the finger-like regions that transmit signals to neighboring neurons—branch into different parts of the brain, making it hard to figure out which fork in the road to follow. Sternson knew he could determine the correct path by blocking the axons' output points, called synapses, and seeing if that made the mouse hungry. But he needed help targeting the synapses.

That's where Ghosh came in. He was studying the molecular biology of synapses at the University of California, San Diego. Sternson realized that by collaborating, they might be able to build a "circuit breaking tool" that could home in on selected synapses and turn them off. The pair decided to base their tool on a molecule called hM4D, which turns off nerve cells in the presence of a drug called clozapine-N-oxide (CNO).

For the project, Ghosh recruited Tev Stachniak, a postdoctoral fellow from McGill University in Montreal who was interested in neural circuits. With guidance from both Ghosh and Sternson, Stachniak tinkered with hM4D and eventually got it to specifically target the synapses of interest. The effects of CNO last about an hour, just enough time to test the circuit breaker's impact on mouse feeding behavior.

The project, which is winding down, was a great success. Stachniak discovered that part of the hunger pathway involves neurons that mediate reward-motivated behavior in general, hinting at the existence of a core circuit shared by these behaviors. The tool, described in a publication in press at *Neuron*, can be used to target synapses involved in other circuits as well, making it widely useful for neuroscientists. Ghosh, who moved to Roche as the Global Head of Neuroscience Discovery in 2011, is starting to think about using similar tools in more complex animals such as primates. And Sternson, of course, is already on to his next collaboration.

Brain Maps

The only way Group Leader Albert Cardona will accomplish his ambitious project is if he engages lots of visiting scientists. He wants to create a neural wiring map of the fruit fly larva brain. That's about 12,000 neurons, 3,000,000 synapses, and 50 person-years of work. So far, he's brought in 39 scientists

Clare Mallison

Learn more about collaborative projects with international visiting scientists at www.hhmi.org/bulletin/spring-2014.

from 19 labs around the world. He teaches them how to trace the neurons in electron micrographs created by Rick Fetter, a principal scientist at Janelia, from a *Drosophila* larva that was sliced into about 5,000 sections, each 50 nanometers thick.

One of his visitors is Katharina Eichler, a PhD student from Andreas Thum's lab at the University of Konstanz in Germany. She's been working with Cardona for about six months, spending her days hunched over a computer and clicking a cursor on the black-and-white micrographs. She expects to be doing this for at least another six months.

Eichler and the other visiting scientists do their mapping using a Web application called CATMAID (Collaborative Annotation Toolkit for Massive Amounts of Image Data) that is like a Google Maps for the brain. The roads—neurons, in this case—are already on the map. "It's just like the OpenStreetMap.org project that asks contributors worldwide to annotate the place they live in with their knowledge of the local geography," says Cardona. Once the scientists learn the program, they can log in from their own computers and trace neurons from anywhere in the world.

Most of the scientists stay with Cardona for one to six months, learning how to map the fly brain. Eichler is an exception because she's decided to complete her PhD at Janelia in collaboration with the University of Konstanz. Eichler could have done the project in Germany, but, she explains, "I think it's important to be here because it's really helpful to be able to talk to people who have worked with a certain neuron or area of the brain before."

Eichler's studying the mushroom bodies—a pair of structures involved in olfactory learning and memory.

Janelia's Albert Cardona welcomes visiting researchers like Germany's Katharina Eichler to map the fly brain.

Paul Fetters

By the time she leaves Janelia, she hopes to have traced all of the approximately 320 nerve cells that make up the structures.

So far, Eichler and the rest of the team have mapped about 12 percent of the nervous system. But this is not the best measure of their progress. "Biologically speaking, there are so many questions you can answer with a fraction of a reconstruction," says Cardona. "You don't have to finish the whole thing to extract enormous value. You extract value as you go." Eichler has already proven this: even though she's only about halfway through her mapping, she's already showing how the connections between the mushroom body neurons contribute to the various roles the structure plays in the fly brain.

Massive Sequencing Experiments

Sacha Nelson and his postdoc Ken Sugino had a big idea that needed some big resources. Resources like immense computational power and instrumentation that could only be found at a place like Janelia.

The pair had figured out how to isolate genetically similar cells from a mouse's brain, pool the RNA from the cells, and determine the RNA's sequence. The result was a transcriptional profile—a unique fingerprint—that could be used to distinguish different cell types. If they could create a database of RNA produced by each of the cell types in the mouse brain, it would be an incredible resource for neuroscientists.

"Ultimately, if this database were big enough, any scientist could just look up a favorite neuron and read out that neuron's profile," explains Janelia Group Leader Adam Hantman. That information could help the scientist figure out what the neuron does and how it does it.

The problem was that, by Sugino and Nelson's estimate, the brain has between 5,000 and 10,000 different cell types. Sequencing each of those cell types would require more resources than were available in a small academic lab such as Nelson's at Brandeis University. So Nelson started casting around for collaborators. "I looked for people at Janelia," he says, "who were interested in using the scale available there to do the massive sequencing experiments that were not practical to continue in my own lab."

That's how they teamed up with Hantman and Janelia Group Leader Sean Eddy. Hantman needed help characterizing some mouse brain cells he was studying. Eddy, on the other hand, was interested in the genomic implications of the project. "It would be really cool if you could get each cell type and say, 'This is the genetic program this guy is running, and this is the program that guy is running,'" he explains. "Then you could start to ask how transcription regulation works, how it evolved, and how is it different between species."

Eddy also happened to have two postdocs, Lee Henry and Fred Davis, who were working on a similar technique to purify fly brain cells and sequence their RNA. Thus, the big project got even bigger. The new goal was to sequence all the cell types in both the mouse and fly brains.

As word got out about the project, many Janelia scientists approached the team, asking them to create

Ken Sugino, Adam Hantman, and Sean Eddy collaborated to produce a massive library of RNA profiles of mouse and fly brain cells.

Paul Ferters

"We need to show that the tools are useful. And I need outside collaborators to do that."
—ERIC BETZIG

RNA profiles for the mouse and fly brain cells they were studying. As a result, in September 2012—one and a half years after it started—the visitor project morphed into a larger-scale, permanent project called NeuroSeq.

Sugino, Henry, and Davis are now full-time employees at Janelia on the NeuroSeq team. Nelson is back in his lab at Brandeis, overseeing the project from afar. The team is also spending one-third of its time helping Janelia scientists ask specific questions about brain cells. Sternson has already approached them to learn how changes in feeding affect the RNA produced by certain cells in the mouse brain.

A New Adventure Weekly

Janelia Group Leader Eric Betzig calls the Visitor Program his "secret weapon." Without it, he wouldn't be able to share the cutting-edge, but very large, immobile microscopes he builds. People have to come to him. "We need to show that the tools are useful," he explains. "And I need outside collaborators to do that."

One of his creations is the Bessel beam microscope that Fritz-Laylin used. It's a hulking assembly of lasers and lenses that uses a thin sheet of light—similar to a scanner at a checkout counter—to acquire tens of thousands of images from a living specimen. By piecing together the frames, Betzig can assemble dazzling three-dimensional movies that show the inner workings of cells.

In 2012 and 2013, Betzig averaged about 20 visitors per year. A quick glance at his calendar for January 2014 shows four collaborators visiting back to back: a researcher from Duke University, a scientist from the National Institutes of Health, a collaborator from Johns Hopkins University, and finally, a group from Harvard University.

Typically, visitors—who range from individual scientists to teams of specialists—arrive on Sunday and jump into data collection first thing Monday morning. The microscopes are complicated enough that they have to be operated by Betzig's postdocs, who are willing to work 18-hour days to make sure the collaborators leave with as much data as possible.

Betzig will say yes to any collaboration that could benefit from his microscopes. "The variety of things we've looked at is all over the map," he says. "We've gone from bacteria up to huge fish, and everything in between. Every week is a new adventure." Two visitors—one from Harvard and one from the University of California, San Francisco—are even using the Visitor Program to build their own light sheet microscopes in collaboration with Betzig and Janelia's Instrument Design & Fabrication team.

Adding On

"I think everybody benefits from the Visitor Program," says Janelia Scientific Program Director Ulrike Heberlein. "Janelia benefits from having really wonderful and smart people contribute to the intellectual discourse. The visitors benefit from being in this really fantastic environment where they can try things they otherwise might never be able to do."

When Heberlein first came to Janelia two years ago, she thought the Visitor Program might benefit from a little more structure. "I wanted to have more rules and criteria for what gets funded and what doesn't," she explains. She soon shelved that idea. "It turns out that the real beauty of this program is the flexibility," she says.

Rather than changing the program, Heberlein has decided to add to it. This spring, Janelia launched its Visitor Graduate Fellowship. Aimed at students from around the world, the program will allow the young scientists to create collaborative projects that will become part of their thesis research.

And, like the Visitor Program, the sky is the limit. Even longshot projects find a home at Janelia.

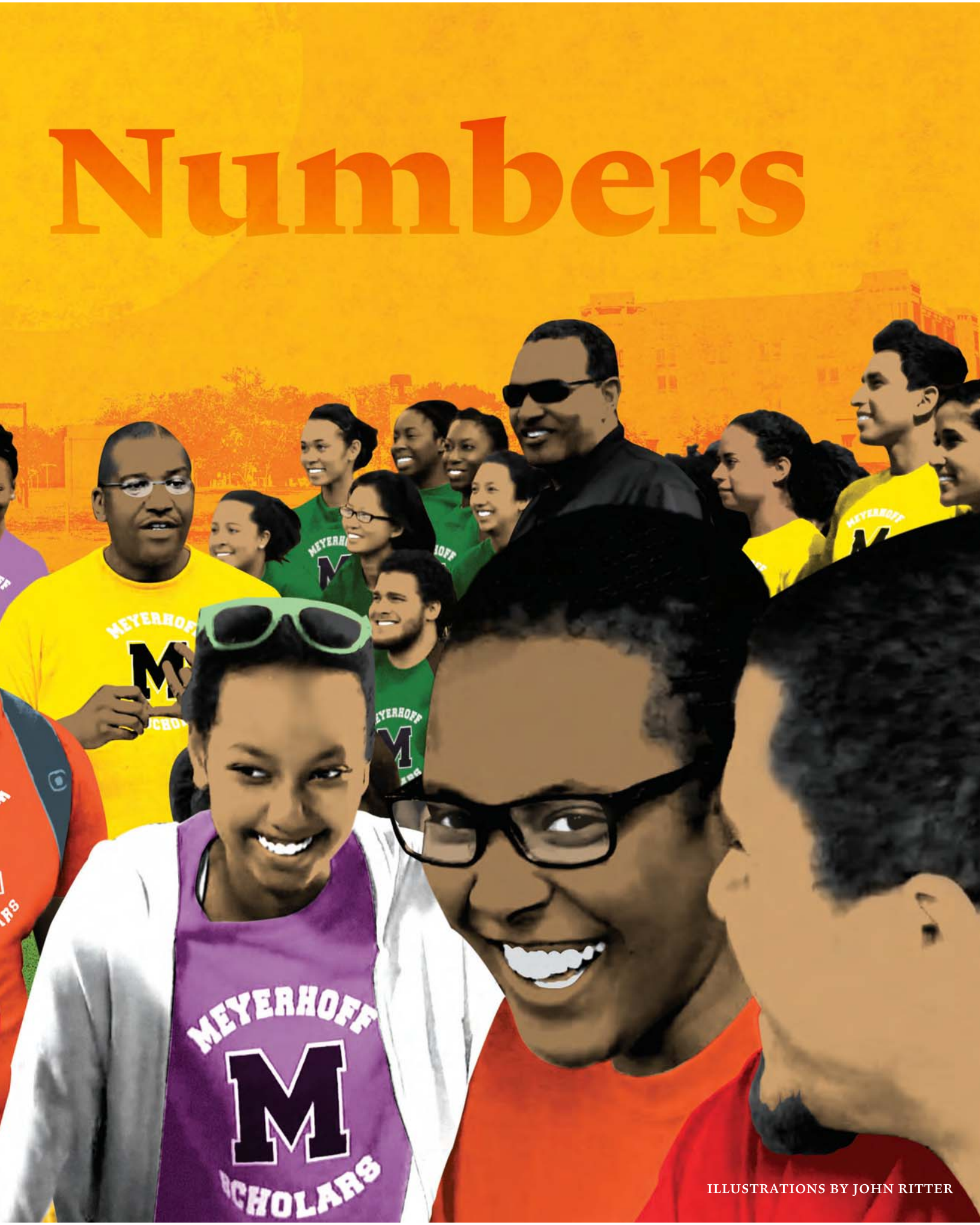
"We're willing to say, 'Well, this [visitor project] may only have a 20 percent chance of working, but if it works it's going to be really transformational, so we'll do it,'" says Rubin. "We have a high-risk, high-reward attitude toward these projects." ■

Can a successful STEM program for minority students succeed outside of one school in Baltimore?

Strength in

BY SARAH GOFORTH

Numbers



ILLUSTRATIONS BY JOHN RITTER

IF YOU HAPPENED upon Centennial Park in Ellicott City, MD, one breezy Saturday last September, you probably saw what looked like a large and lively family reunion: Teams battled across a hand-made obstacle course. An animated crowd cheered on competitors in a pie-eating contest. People hugged.

But this was a gathering of scientists, not relatives. Meet the Meyerhoffs. They are united by a University of Maryland Baltimore County (UMBC) scholarship program that is steadily boosting the number of African Americans and other underrepresented groups in the upper ranks of math and science. Started 25 years ago with funding from Robert and Jane Meyerhoff, the Meyerhoff Scholars Program has graduated more than 880 students—the majority of them African American—with science and math degrees. More than three-quarters of them have gone on to graduate school.

Other universities around the country are clamoring to do the same. Meyerhoff administrators get two to three calls per week from faculty at other institutions who want to know more. What is it about this model that works so well? Can those elements be duplicated elsewhere in a nationwide push to make science more inclusive?

As she fields MD-PhD program offers, Blossom Tewelde knows that she will always be a guide to younger Meyerhoff Scholars.

Attracting Talent

"People sometimes ask, 'Why is it important to have diversity in science?'" says 20-year-old Meyerhoff scholar Blossom Tewelde, an African American biochemistry major who will graduate this spring. "It's not because I think I'll learn better from a black professor. It's not about quotas. It's about diversity of mindsets." It's also, she adds, about offering young people role models who look like them. "I can count on one hand all of the black female physician-scientists I've met in my life," she says. "It can be hard to imagine yourself somewhere without that picture in your mind."

Good science requires constant fresh ideas and a self-replenishing talent pool, and diversity is a foundation for both, echoes David Asai, senior program director for science education at HHMI. A recent study published by the National Bureau of Economic Research, for example, showed that scientific papers authored by ethnically and geographically diverse teams are cited far more often (and therefore generally have greater impact) than those from homogeneous teams.

"Many of the easy problems in science have been solved," Asai adds. "If we want to tackle the really difficult ones, we have to bring as many creative approaches as possible, and that requires every kind of diversity—ethnic, geographic, gender."

Yet diverse teams are not the norm. College-bound African American, Hispanic, and Native American students express similar levels of interest in science, technology, engineering, and math (STEM) majors as do white and Asian students. Yet many fewer of the minorities complete STEM degrees. A 2000 study found underrepresented minority students switch out of STEM disciplines at more than twice the rate of white and Asian American students, and a 2005 study found much lower completion rates of STEM degrees among minorities. Although African Americans represent nearly 13 percent of the U.S. population, they earn only 2 to 3 percent of science and engineering doctorate degrees.

"If we want to maintain a strong scientific workforce in the United States, we have to attract the best and brightest from all groups and all parts of the country," says Asai.

A Model for Many

The statistics reveal a challenge for those who wish to take the Meyerhoff model nationwide: What makes this community so successful? Is it its leader, UMBC President Freeman Hrabowski? The close-knit UMBC campus, which focuses on the natural sciences and engineering? The early access to research experiences its participants are granted? The social cohesion of the group? All of the above?

To answer those questions, leaders at UMBC and two other universities—Pennsylvania State University and the University of North Carolina at Chapel Hill—have partnered with HHMI to study these questions at UMBC and at the other two campuses, both of which have started pilot programs with similar goals. With $8 million in support from HHMI over five years, they are expanding and studying the Meyerhoff model to learn how to apply it to new, often dramatically different, environments. Social scientists at all three universities will gather data about how the students live and study, whether the students face similar challenges and pressures, and the positive "halo effect" these programs can have on faculty and students who are not direct participants. At the end of the

Watch a video of the Meyerhoff family retreat at www.hhmi.org/bulletin/spring-2014.

trial period, dubbed the Meyerhoff Adaptation Project, the participants hope to know enough to offer a set of guidelines for others.

It won't be easy. The Meyerhoff program has advantages that aren't shared by the other institutions: UMBC is located in an urban area with a diverse population; the program was built by UMBC President Freeman Hrabowski, a charismatic and passionate leader with a peerless ability to rally students; the program's components have been honed over 25 years of trial and error in a single setting.

Some priorities are obvious, even before the studies begin. Having a group of faculty and staff who are committed to the program, and the students' success, is key, says Meyerhoff Scholars Director Keith Harmon. Originally from a small town in southern Georgia, Harmon knew well the value of an attentive community when he was recruited in 2005 to help lead the Meyerhoff Scholars Program (originally as assistant director).

"I was born in a small Southern town at the tail end of the civil rights era," Harmon says. "There was just a way that the community had oversight of you as a youngster. My friends and I could ride our bikes all day long on a Saturday, and as long as we checked in with someone's mom at some time during the day, it was a normal thing. You would be walking down the street and someone would literally stop you and say, 'I expect great things from you, Keith Harmon.' It wasn't until many years later that I realized how rare this is for young people today." But it's the norm for Meyerhoff scholars.

Keith Harmon says Meyerhoff Scholars are told often that they are expected to achieve.

From day one of the six-week "summer bridge" that welcomes Meyerhoff scholars to the program before their freshman year, the expectations placed upon them are high and unyielding. Students are expected to make A's consistently and to coach each other if they see another scholar falling behind. They are told, again and again, that they are ambassadors of a name synonymous with integrity and achievement, a name they are responsible for upholding. If they make a mistake, it never goes unnoticed. Each new scholar is paired with an older mentor, who is accountable for the younger student's success. When one scholar breaks a rule—and there are many rules—the entire cohort is held accountable.

"I remember sitting with the other members of my cohort in a courtyard at 4:45 a.m.," says Tewelde, recalling a time during her summer bridge when her cohort's wakeup call had inched back every time someone forgot to check in with his or her peer mentor, or relay a piece of information from program staff to the rest of the cohort. "Fifty-four zombies sitting there in the dark in ill-fitting suits because we'd never had to dress professionally before. I thought it was crazy." In hindsight, Tewelde takes those early morning lessons to heart: "If I need to stay up late or wake up early to meet a deadline, I know I can do it. If I have committed to do something, I know it's worth it."

In general, the program operates less like a top-down academic program than a fraternity or sorority. Scholars, who are selected on merit and receive full academic scholarships and opportunities in some of the best research labs in the country, are held to strict codes of conduct. They must study together. They must sit in the front row of class (all classes). They can't use social media while engaged in Meyerhoff-related activities. They never travel alone. Tight-knit groups of alumni, parents, faculty, and administrators

"If we want to maintain a strong scientific workforce in the United States, we have to attract the best and brightest from all groups and all parts of the country."
—DAVID ASAI

UMBC President Freeman Hrabowski launched the Meyerhoff Program 25 years ago.

are connectors in a network that now spans generations, disciplines, ethnicities, and socioeconomic backgrounds.

Connecting it all is Hrabowski, who was recruited to UMBC in 1987 after a series of protests over racial discrimination on campus. He started the Meyerhoff Scholars Program less than two years later and is responsible for its signature togetherness. Hrabowski can often be seen leading his apprentices in a recitation of the Langston Hughes poem "Dreams," which they all know by heart:

> Hold fast to dreams
> For if dreams die
> Life is a broken-winged bird
> That cannot fly.
> Hold fast to dreams
> For when dreams go
> Life is a barren field
> Frozen with snow.

The program's impact is impossible to deny. Meyerhoff scholars are more than five times more likely to have graduated from or be currently enrolled in a STEM PhD or MD program than students who were invited to join the program but declined and attended another university, according to UMBC psychology professor Ken Maton in a 2012 study published in the *Mount Sinai Journal of Medicine*.

Keys to Success

"The past 25 years have taught us a lot," says Mike Summers, an HHMI investigator and biochemist at UMBC who has played a crucial role in sustaining the Meyerhoff program and building alliances for it with faculty and administrators across the campus. "Freeman has shown what's possible at a medium-sized institution in an urban area with a brilliant leader. The question is: Could something like Meyerhoff be done elsewhere?"

Summers has hosted dozens of Meyerhoff scholars in his lab over the years, including Tewelde. "I had spent my whole life with people telling me, 'Why are you asking so many questions?'" says Tewelde. "I was used to getting in trouble for it. But I went to Dr. Summers' lab, and at the end of a presentation you had two choices: you could ask questions or do pushups. Something I had been chastised for my whole life was suddenly worthy of praise. I thought, if research is where you get to ask questions, then that's where I'm supposed to be."

Building on what he learned as a mentor and program advocate, Summers visited the UNC campus in 2011 to meet with administrators who were thinking of adopting the Meyerhoff model. Together they created a roadmap for what is now the UNC Chancellor's Science Scholars Program, which welcomed its first cohort of 24 students last fall.

"The problem was staring us in the face," says Lauren Thomas, coordinator of the UNC program. In a good year, she says, two African American students who earn a bachelor's degree at UNC go on to get a PhD. This from a campus with 18,400 undergraduates, about 3,700 of whom are underrepresented minorities. Faculty who teach upper-level science classes say they don't ever see underrepresented students. Adds Thomas: "They can't recall when they had an African American student in their lab. Mike Summers' visit was the impetus to make a change. The university was reminded that the potential existed in the student body. It just needed to be tapped."

UNC faculty who attended Summers' 2011 talk were inspired to do something about it. They raised money from internal sources and HHMI, and looked to the Meyerhoff model for the essential program elements: summer bridge, early research experience, shared housing, and rules designed to get scholars to stick together.

"That visible cohort is so important," agrees Summers, adding that it influences not only the students but the faculty as well. "Think about it: You're a typical white male lecturer in a huge class with 300 students. If this program supports 15 minority students in a typical class, and those 15 students are scattered about, they won't have the same impact on the faculty as they will sitting as a group right in front of the class. When they see eight or 10 minority students in their face, asking questions, it changes perceptions. It's subtle, but it works."

Changing Minds

Summers recalls a faculty colleague at UMBC who was convinced, in the early years of the Meyerhoff program, that it wasn't productive to qualify students for programs based on their race or gender. "He pounded his fist on the table when he argued and told me he didn't have a racist bone in his body," Summers remembers. Five years later, this same faculty member was describing the Meyerhoff program to a visitor, and he said something Summers will never forget.

"He said that at one time, if he had a black student who sat in the back of the class and made a C, he would write a strong letter of recommendation for that student," Summers recalls. "Now, there are large numbers of black students in his class, and they sit in front. If one of them makes a C, he calls that person into his office because he wants to know what the problem is."

When Penn State leadership invited Summers to present the Meyerhoff model to the school's faculty and administrators, he brought stories like this with him.

For a Q&A with HHMI Science Education Fellow Clifton Poodry on the Meyerhoff Adaptation Project, go to www.hhmi.org/bulletin/spring-2014.

"Last fall was hard, but the kids came out better for it. Now they're telling parents of potential scholars that they should be a part of this."
—STARLETTE SHARP

Starlette Sharp, program director of Penn State's Millennium Scholars Program, says the program would not have been possible at Penn State without a small group of committed advocates who were inspired by what they heard. Their enthusiasm "spread like a rash," she says. "It started with the deans, who knew what this could do for Penn State. They walked around and rallied people. They used all their political capital to start this program." With tight budgets and competing demands, administrators are hard pressed to start new programs without a clearly defined map to their return on investment. And while no one doubts the credibility of the Meyerhoff model, plenty of people doubted it could be applied to a mega-university with 40,000 students in predominantly white State College, PA.

"Parents were worried that their kids wouldn't be able to find anywhere to get their hair cut," says Summers, noting the cultural challenges. "You couldn't blame them for asking why they should entrust this somewhat foreign community with their children."

Last fall, when the first Millennium Scholars arrived on campus, Sharp admits she was nervous. After all, if a key element of the Meyerhoff program is mentoring from older students and alumni, Penn State scholars would not have the peer-mentor network established. "Our program is modeled after Meyerhoff. I read their papers, and whatever I can find about Freeman. Our parents' association is like theirs. We have a summer bridge. Until we try something that doesn't work here, why change something that has such a high success rate?" She knew her students would face the same pressures and demands of the Meyerhoff Scholars Program without the full support network. That job would fall to her, in one sense, and the students would have to support each other.

Taylor Soucy, a 19-year-old freshman Millennium Scholar from a small town in Delaware, says the intensity was surprising but worthwhile. "I didn't understand the cohort mentality at first," she says. "We couldn't have cell phones during the day, and I couldn't get away and just be by myself because the whole process was meant to bring us together. It felt like prison, in a way, but we formed a bond. I can't imagine going through college without my cohort."

"Last fall was hard, but the kids came out better for it," says Sharp. "Now they're out there telling parents of potential scholars that they should be a part of this. It's transformational." The first cohort had just 19 students. "It was important to do it right and well before we do it big," Sharp says. Next year the program will scale up to 25, and some of the rules will be different. For example, Meyerhoff Scholars are required to sit in the front row of their classes, but at Penn State, where introductory science classes can include more than 400 people, the front row fills up quickly. "I was getting text messages on the first day of school from our scholars, saying, 'Star, all the front-row seats are full; what do we do? Do we sit on the floor?'"

Mike Summer is helping other schools adopt the Meyerhoff mantle.

Sharp responded that they could sit anywhere as long as they sat together. "Students call me all night long. I answer every phone call. I don't have mentors yet, but they will become mentors."

Mentors like Meyerhoff senior Blossom Tewelde, who is fielding offers from four prestigious MD-PhD programs and knows that her involvement in the program does not end when she tosses her cap in the air this spring.

"My mentor is in a PhD program at the University of Miami, and I know she's just a text or a call away if I need anything. She still checks on me if she hasn't heard from me in a while," she says. "Now, I'm a peer advisor, too. I will support younger scholars the same way I've been supported. It's just something about Meyerhoff—there's a sense of ownership. Any Meyerhoff anywhere will take you in with open arms."

"It is life changing to see someone as a high school senior and, years later, see them as a postdoc or college professor or leader in their field," says Harmon. "You stop and say, 'What a blessing it is to be a part of their story!'" ■

Perspectives & Opinions

The Art of Mentoring

Winston Anderson says mentors need to prepare science students to be adaptable to meet real-world demands. The cell biologist and HHMI professor at Howard University says it's short sighted to mold students in your own image.

Winston Anderson

—

Received the 2011 Presidential Award for Excellence in Science, Mathematics, and Engineering Mentoring

REMEMBER THAT BILL WITHERS song? "Lean on me, when you're not strong, and I'll be your friend, I'll help you carry on." That song sums up my philosophy of mentoring. Students—undergraduates, graduate students, and postdocs—need someone they can depend on who can provide opportunities and encouragement to do better.

We don't need to create clones of ourselves. As mentors, we can provide foundational support, promote creativity, and enhance a student's abilities to analytically reason. We want to shape young people who will be independent. Who will listen and benefit from mentoring, and then go out into the world and be mentors themselves—to pass on the tradition of helping people grow, test boundaries, and contribute.

Several mentors have supported me throughout my career. When I left Washington, DC's, historically black Howard University to attend graduate school at Brown University, in Rhode Island, I was scared. I was one of two African Americans. I saw no Latinos or Asians. But I found mentors, none of whom looked like me. They communicated with me about ideas in science and academics. They were compassionate. They became colleagues. I was in a place surrounded by peers who were moving fast, competitive, and accomplished. I knew I had to produce.

If you look around at all the accomplished black scientists today, each one had mentors like mine who provided the environment, the means, the encouragement, and the guidance. My mentors offered opportunities to develop skills as a scientist as well as a teacher. Harvard's Morris Karnovsky was what I'd call an "ideas" person. And Hewson Swift at the University of Chicago was a compassionate fellow who championed his students. The first African American scientist I met in my career was Harold Amos, department chair at Harvard and an excellent example of what a good scientist is.

Students have to master basic skills so they can explore and branch into new areas that stimulate them. We must train our students to be adaptable. Some of the most adaptable students I've encountered have come to the United States from other countries. Because their access is limited, they are very motivated and learn to adjust. This is what we should do with many of our U.S. minority students: put them in environments where they must learn to adapt. And then support them. Keep the students on their toes by creating an environment where they are continuously forced to produce.

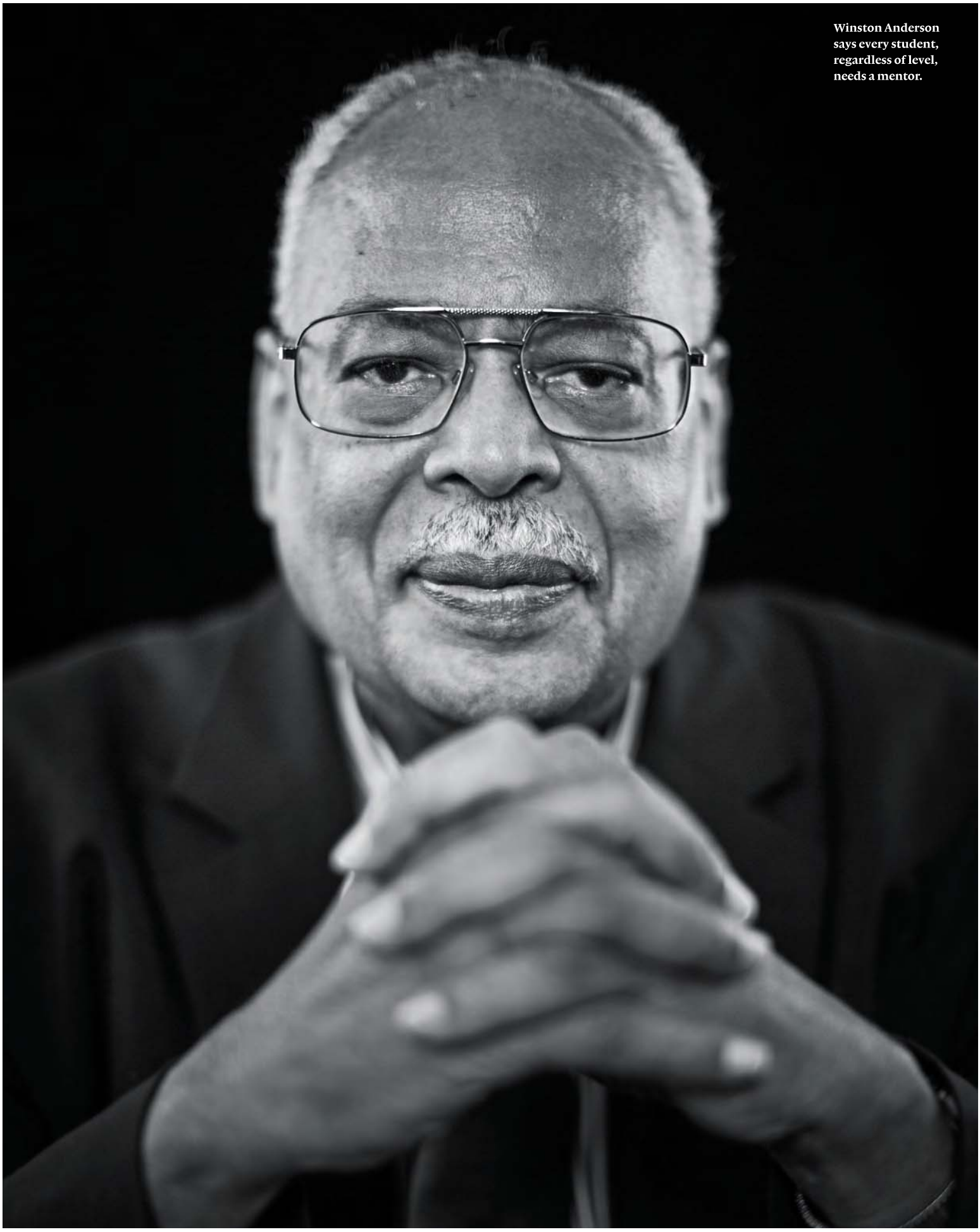
I gave a talk at the Massachusetts Institute of Technology, where they are turning out brilliant, fantastic scientists. One student asked, "What if I can't meet those expectations of being a great scientist?" I told her there are so many other ways to contribute to greatness: by being a good researcher and communicator, by working with students, by being a compassionate scholar. I've suggested that the PhD be viewed as equivalent to a law degree. A science background can open doors to a variety of career paths, just as law sets the foundation for myriad fields, such as politics.

To mentors: Don't train students to be scientists above all. If you teach them to be scientists and teachers, you've prepared scholars.

To students: Don't be afraid to get mentors. Find mentors with compassion; that is what will bind you. You are not a servant to these individuals; you are a colleague. Know that you can be adaptable and choose your mentors based on ideas over accomplishments. Be ready to reach back into your community in turn. We all need someone to lean on.

—Interview by Cori Vanchieri

Paul Fetters



Winston Anderson says every student, regardless of level, needs a mentor.

Perspectives & Opinions

Q&A

Which mentor had the biggest impact on your career and why?

Most of us can point to at least one person in our lives who helped shape who we are. Perhaps it was an enthusiastic track coach or a nurturing physics teacher. Below, four scientists discuss some of their favorite mentors and what made them so significant.

–Edited by Nicole Kresge

Sarah C. R. Elgin
HHMI Professor
Washington University in St. Louis

I've had several great mentors, including my thesis advisor, James Bonner, and my postdoc advisor, Leroy Hood. But my high school chemistry teacher, George Birrell, had the biggest impact. He helped us do experiments demonstrating that the physical laws really do work and that the world has some predictability. And he provided my first opportunity to do research. At his suggestion, I started collecting rainwater every time the Soviets or Chinese exploded a nuclear bomb above ground. I was delighted to find that I could use a Geiger counter to detect when the resulting cloud of radioactivity passed over Oregon!

Hopi E. Hoekstra
HHMI Investigator
Harvard University

I've had three great mentors. My undergraduate advisor, Robert Full, helped me fall in love with the research process—his enthusiasm is contagious and his support unrelenting. My graduate advisor, Scott Edwards, gave me freedom to pursue my interests and forge my own research path. And my postdoctoral advisor, Michael Nachman, taught me to think clearly and write efficiently, and by example, prepared me to be an independent investigator. All three gave me space to explore my interests, encouraged me to try new approaches, and allowed me to make mistakes. At the same time, they gently steered me back on track when I veered too far off course. It's a delicate balance, and one I try to achieve with my own students.

Thumbi Ndung'u
International Early Career Scientist
University of KwaZulu-Natal

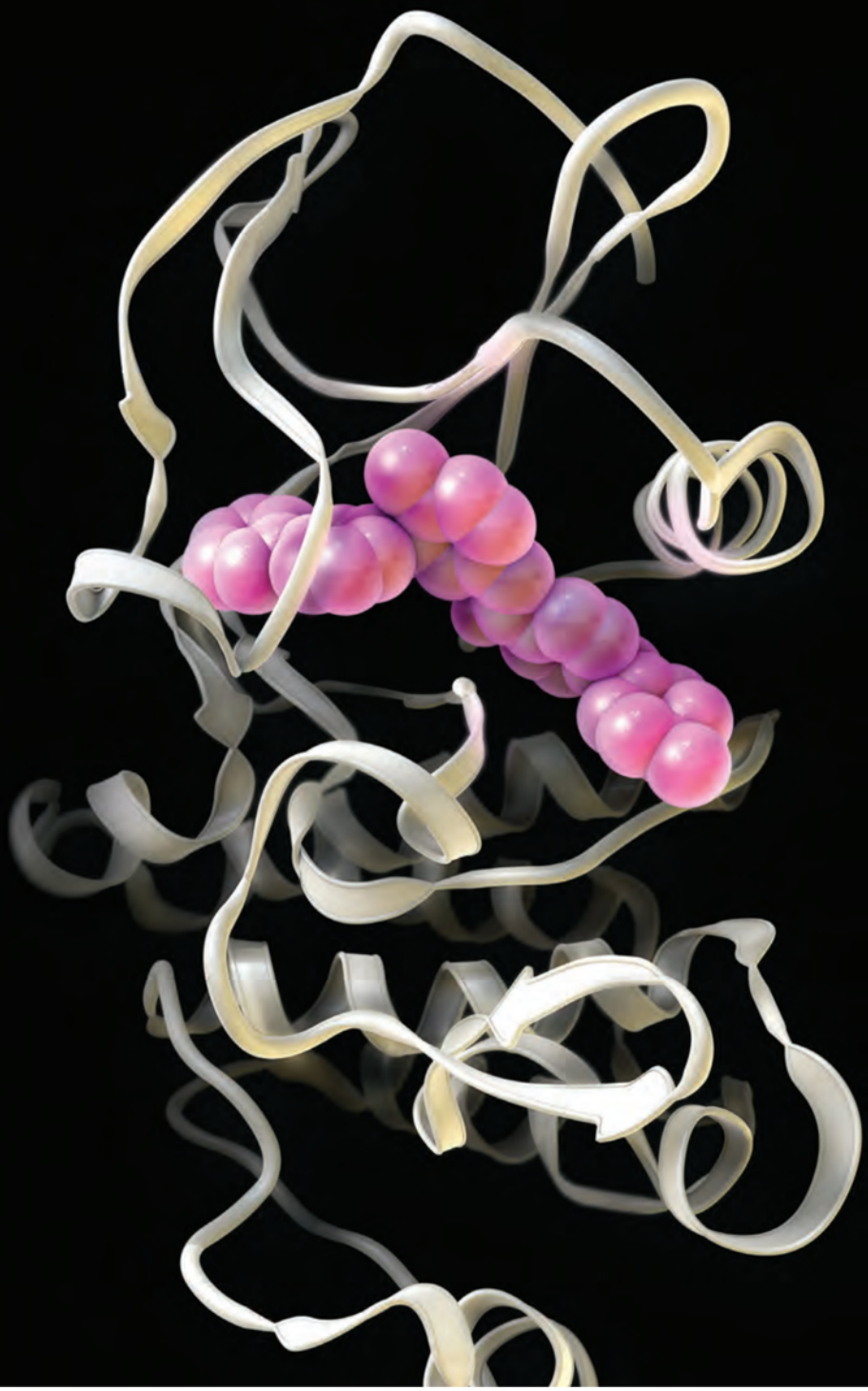
If I had to make a choice, it would be Professor George Kinoti of the University of Nairobi, in Kenya, where I started my research career. A tough but competent leader filled with integrity, he taught me to believe in myself and in Africa's great potential. George cared deeply about the people that science could impact. He set me on a path of inquisitiveness and service that has also been keenly influenced by Max Essex, Jerry Coovadia, and Bruce Walker. Their insights and doggedness in scientific investigation, along with their application of science for the greater good of humanity, have been refreshing and energizing.

Thomas R. Cech
HHMI Investigator
University of Colorado Boulder

I entered Berkeley as a physical chemistry grad student, but as I surveyed my research options I realized they didn't fit my temperament. Lucky for me, I found John Hearst. This young, bearded professor had been trained at Caltech as a physical chemist, but was now volcanically enthusiastic about eukaryotic chromosomes and repetitive DNA sequences. Hearst was really smart, solving differential equations in a flash, but more important were his personal characteristics. He expected his students to be independent, really listened to them, enjoyed yielding to a student's idea if it seemed better than his own, and was exuberant about good data. And he was generous: There was always room for one more at his Thanksgiving table.

Elgin: Paul Fetters, Ndung'u: Kevin Wolf, Hoekstra: Bizuayehu Tesfaye, Cech: Paul Fetters

Chronicle



Fabian de Kok-Mercado/BioInteractive

More than 90 percent of chronic myelogenous leukemia (CML) patients have one thing in common: they all experienced a gene mutation that produced a hybrid molecule called BCR-ABL (white). This hyperactive enzyme is the culprit behind the uncontrolled production of white blood cells in CML. HHMI Investigator Charles Sawyers helped discover a drug called Gleevec (pink) that shuts the enzyme down, putting an end to white blood cell overproduction. Gleevec's success launched a wave of drug research aimed at specific gene products implicated in cancer. In HHMI's 2013 Holiday Lectures on Science, Sawyers and HHMI Investigator Christopher Walsh discussed these and other examples of how advances in genomics are leading to targeted treatments for disease. *You can find the lectures at www.holidaylectures.org.*

Chronicle / *Science Education*

Learn more about flipped classrooms and blended learning at www.hhmi.org/bulletin/spring-2014.

Flipped Classrooms

Instructors are sending lectures home to use class time for interactive learning.

ELIZABETH MCCORMACK WANTED more classroom time to work on group problem solving with her students. But lectures, as is tradition, ate up most of their time together. So two years ago she flipped her sophomore electromagnetics class on its head. Instead of sitting during class while she lectured, McCormack's students at Bryn Mawr College watched a series of short videos on their own time that covered the lecture material. Only one of three classes each week featured lectures. That freed up the other two classes for working through problems with each other on the board.

At first, McCormack's students weren't thrilled with the new format. They even showed up en masse at her office door to make the case for more lectures. They weren't confident in their grasp of some of the material, they told her. The videos were missing that immediate dialogue with the professor to answer their pressing questions.

"What they were really telling me is that they needed more opportunity to talk about the material, to talk about the challenging pieces," says McCormack. In response, she added some lectures back in, and then included "co-reading" homework assignments where students had to work in small groups and bring three questions that the group couldn't sort out to the next class. It pushed them to discuss what they were reading, she says.

Flipping, an idea that gained momentum around 2010, makes room for applied learning in class. Students take in the lecture material on their own time—often through videos made by the instructor or an outside source—then come to class prepared to ask questions and do hands-on work.

Instructors can assess whether students are actually watching the videos by using online surveys, quizzes at the beginning of class, or questions the students answer in class with a hand-held remote, or clicker.

According to a September 2013 special report in the *Chronicle of Higher Education*, the National Center for Academic Transformation has helped redesign about 300 college courses since 1999, many in a flipped format.

Hard data on the impact of flipping are hard to come by, but some professors who've tried the flipped format see improvements among their middle- to lower-performing students. Emily Niemeyer, a chemistry professor at Southwestern University in Georgetown, TX, introduced "Flipped Fridays" in her introductory chemistry class in spring 2013 as part of an HHMI grant. With those flipped classes, she says, she's had fewer students performing in the C and D range, and many more in the B range.

Diane O'Dowd, HHMI professor and chair of developmental and cell biology at University of California, Irvine, and her co-instructor, Adrienne Williams, have compared a small, fully flipped introductory biology course with a larger, partially flipped class in 2012 and 2013. Singling out the students with low SAT scores, O'Dowd's preliminary data show that the students in the flipped class outperformed by 7 percent the students in the partially flipped class on identical final exam questions.

O'Dowd says that beyond test scores, flipped classes can be rewarding for faculty who like a challenge. "Most of us became scientists because we want to do things that are intellectually stimulating," says O'Dowd.

Physics Nobelist Carl Wieman, now teaching at Stanford University, has been experimenting with alternatives to the traditional lecture format for 15 years. He doesn't lecture and offers pre-class reading assignments. Wieman calls his classes "an ongoing problem-solving activity where the students are regularly getting feedback and interactions along the way, both from their internal discussion and from the instructor." Before moving to Stanford, he compared the results of two Introduction to Modern Physics courses in 2011 where he taught at the University of British Columbia. One had a traditional lecture format. The other had pre-class reading assignments, peer discussion, clicker questions, small-group activities, and minimal lecturing. Both had about 60 students. Those in the latter class, with the problem-solving format, scored 18 points higher—85 percent versus 67 percent—on a standard test of knowledge of quantum mechanics.

Working it out

McCormack, chair of Bryn Mawr's physics department, wanted to give her students a better conceptual and operational understanding of the topics, provide them more opportunities to practice the mathematical framework in class, and lock in their learning with more writing and problem-solving skills. She's been learning as she goes.

After experimenting with the flipped format for two years, McCormack found that the videos have to be short—less than 10 minutes. Sometimes, she makes them up on the fly if questions come up or if students are having

R Kikuo Johnson

trouble grasping certain material. "You have to let go of being rigidly prepared," she says.

At University of Maryland College Park (UMCP), flipped classes in life sciences and introductory physics have almost no in-class lecturing, says Katerina Thompson, UMCP's director of undergraduate research and internship programs and a leader of HHMI's National Experiment in Undergraduate Science Education (NEXUS). Students watch videos or complete assigned readings on their own time, and then answer online questions that give the instructor a sense of how well they've grasped the material. From there, the instructor decides what to highlight when the class meets again.

In the physics courses, "we developed almost everything from scratch," including the videos and online textbook, Thompson says. Students are given dry-erase boards and markers to work through a conceptual question requiring them to apply what was in their reading. In biology, some flipped courses use custom created video lectures, while others make use of videos that are freely available on the Internet.

Using clickers, students can see a distribution of answers from their classmates, Thompson says. "Then we get into a discussion of 'Why did you choose this one and why did you choose that one?'" They then break into small groups and start rethinking their answers.

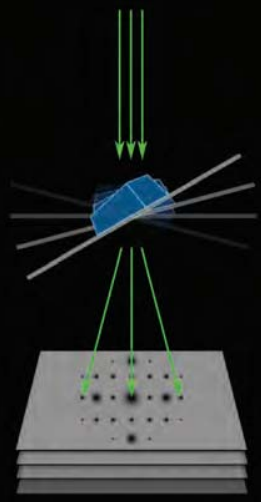
For the flipped concept to work, Thompson says, faculty have to buy in. It can require more prep time and interaction with students than the traditional lecture format.

"There are some faculty who are very interested in adopting new things and jump in with both feet, and then there are some who are more hesitant." Right now, she says, "It's a coalition of the willing."

To build that coalition, UMCP is offering small grants to faculty to revamp their courses, along with multi-day training institutes, short workshops, and faculty learning communities.
–Laura Putre

For the flipped concept to work, faculty have to buy in. It can require more prep time and interaction with students than the traditional lecture format.
—KATERINA THOMPSON

Chronicle / *Toolbox*



Size Matters

New technique uses small crystals to solve big structures.

TAMIR GONEN HAD a problem. He wanted to produce big, resilient crystals from the membrane proteins he was studying. The crystals would allow him to use x-ray crystallography to see the proteins' three-dimensional structures and learn how they form pores in cell membranes. But the proteins weren't cooperating. He could only coax them to form tiny crystals.

So Gonen decided to make use of what he had. He devised a technique with the potential to help the structural biology community by radically increasing the number of proteins whose structures can be solved.

For the past 30 years, x-ray crystallography has been the go-to method for figuring out a biological protein's three-dimensional shape. The process is fairly straightforward: Grow a protein crystal, shoot it with an x-ray beam, and collect data on how the x-rays are diffracted by the crystal. Analyze the data using a computer, and out pops a structure. Surprisingly, the first–and least technical–step in this process is often the hardest. Only about one-third of all complex biological molecules can form crystals, and only a tiny fraction of those are large enough for x-ray crystallography.

"These days, most of the easy proteins–the ones that readily form large crystals–have been solved," says Gonen, a group leader at HHMI's Janelia Farm Research Campus. What are left are the difficult ones. "If [scientists are] really, really lucky, they can still get some crystals, but they're very, very small," he says. And unfortunately, these microcrystals are useless for x-ray crystallography. The intense x-ray beams destroy them before meaningful data can be collected from the proteins.

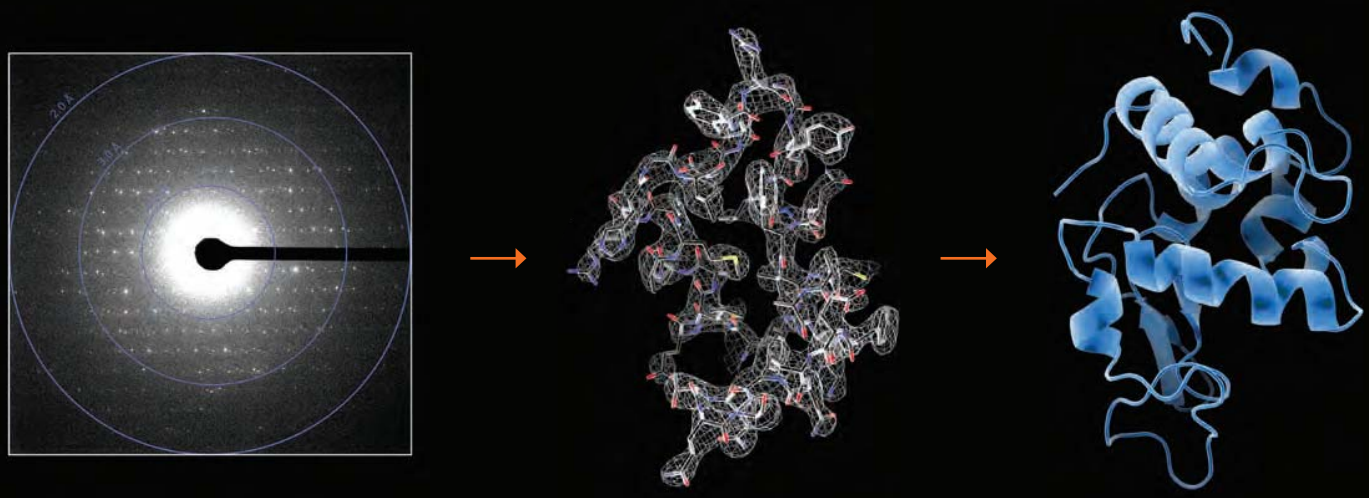
Gonen conceived of a new method as an HHMI early career scientist at the University of Washington, where he was working with cryo-electron microscopy. Because electrons interact with atoms more strongly than with x-rays, Gonen thought an electron microscope might be able to get data from small crystals.

"X-rays are pretty lousy at interacting with matter," explains Gonen. "Electrons are about a thousand times better, and that means you should be able to get meaningful data from very small crystals."

Gonen had to wait a few years to see if his idea worked. "I'd been thinking about this for a long time, but only after coming to Janelia did I have the time, as well as the right people and the right equipment, to try things out," he says.

At Janelia, Dan Shi, a microscopist in Gonen's lab, started working on the technique. He was joined by postdoctoral fellow Brent Nannenga, an engineer who wanted a challenge. The third team member, Matt Iadanza, joined the effort by happenstance, or what Gonen likes to refer to as "the magic of Janelia." In Gonen's lab, the desks are all together in one room. One day, Nannenga was lamenting that the commercial software he was using couldn't handle the electron diffraction data. Iadanza, a desk away, couldn't help but overhear. "It turns out that Matt's hobby is writing programs," says Gonen. "He said, 'If you guys work out the math, I will try to code it.' And out came this program that allowed us to solve the structure."

Typical electron microscopy experiments expose samples to 20 electrons per angstrom



squared (an angstrom is roughly equal to the diameter of an atom). But this dose destroys a crystal pretty quickly. The team learned it could cut the dose substantially—down to 0.01 electrons per angstrom squared—and still collect enough data to solve a protein's structure.

The scientists christened the technique "MicroED" and published their results November 19, 2013, in the journal *eLife*. The "Micro" refers to the submicron size of the crystals; "ED" is short for electron diffraction.

The equipment for MicroED is exactly the same as that used for cryo-electron microscopy, making it very accessible to structural biologists. And the fact that MicroED uses crystals one million times smaller than those needed for typical x-ray crystallography means that the structure of many hard-to-crystallize proteins may now be attainable.

That is, once the technique is ready for prime time. For one thing, Gonen's team has used MicroED only with lysozyme, a widely used test protein known for its ease of handling and great diffraction; it has yet to work on other proteins. To help the technique go mainstream, Gonen's group also needs to optimize data collection to capture as much diffraction information as possible. And, there needs to be an easy way to process the resulting data—the program Iadanza wrote was customized for lysozyme.

Richard Henderson, an electron microscopist at the MRC Laboratory of Molecular Biology in Cambridge, UK, agrees that the technique could have broad applicability for other protein crystals. However, he adds, "Before the value of MicroED can be fully evaluated, Gonen and his colleagues will need to make some technical improvements," including the use of an energy filter to remove extraneous scattered electrons, better software, and a more efficient electron detector.

Gonen's team is working out all these kinks. They're trying the technique on other protein crystals and collaborating with several groups to tweak existing x-ray crystallographic software to process MicroED data.

"I think that once these challenges are met, solving structures by MicroED is going to become routine," says Gonen. And then, maybe Gonen's membrane proteins will finally reveal how they form biological pores.

–Nicole Kresge

"I'd been thinking about this for a long time, but only after coming to Janelia did I have the time, as well as the right people and the right equipment, to try things out."
—TAMIR GONEN

Tamir Gonen

Chronicle / *Lab Book*

Neanderthals' Lasting Legacy

Early encounters with Neanderthals left marks on human genes.

NEANDERTHALS MAY HAVE died out tens of thousands of years ago, but they live on in our DNA. When early humans migrated out of Africa, they encountered, and mated with, some of their Neanderthal cousins. As a result, many people today have about 2 percent Neanderthal DNA in their genomes. David Reich, an HHMI investigator at Harvard Medical School, recently led a team that sleuthed which modern genes can be traced back to these ancient trysts.

The researchers compared DNA from a Neanderthal woman's remains discovered in Siberia to DNA from 1,004 present-day people. "The goal was to understand the biological impact of the gene flow between Neanderthals and modern humans," says Reich. "We reasoned that when these two groups met and mixed, some new traits would have been selected for and remained in the human genome, while some incompatibilities would have been selected against and removed."

Some of the DNA that endured the test of time left its mark on our hair and skin. Reich and his colleagues discovered that a number of the Neanderthal genes that exist in people today are involved in making keratin, a fibrous protein that lends toughness to skin, hair, and nails. Reich speculates that the Neanderthal versions of these genes may have helped humans adapt to non-African environments by producing thicker hair and skin to withstand a colder climate or to shield the humans from pathogens.

The study, published January 29, 2014, in *Nature*, also indicates that Neanderthals and early humans were "at the very edge of being biologically compatible," Reich says. The team found little Neanderthal DNA in the human X chromosome or in genes that are normally highly expressed in testes. This pattern is often linked to a phenomenon called hybrid sterility—when two organisms are distantly related, their male offspring can be rendered infertile. Thus, modern males who inherited a Neanderthal X chromosome may not have passed along that X chromosome to offspring.

Reich and his colleagues also discovered that some genes associated with a risk of lupus, diabetes, and Crohn's disease most likely originated in Neanderthals. The group's findings may help scientists glean more information about human disease genes, Reich says. – *Nicole Kresge*

About 2 percent of the human genome can be traced back to encounters with Neanderthals.

IN BRIEF

MALARIA HITS THE HIGHLANDS

When British colonists came to Africa in the nineteenth century, they would often seek refuge from heat and disease in "hill stations." These towns, built in the cool tropical highlands, were less likely to harbor the heat-loving mosquitoes that carry malaria. However, these and other high-altitude locations may soon be prone to malaria as well. According to HHMI Investigator Mercedes Pascual, climate change is increasing the risk of malaria transmission in these regions.

"There has been ongoing, heated debate on the role of climate change in the increased incidence of malaria observed from the 1970s to the 1990s in the East African highlands," explains Pascual of the University of Michigan. "One challenge has been to isolate the effect of a trend in temperatures from that of many other changing factors."

She and her colleagues sifted through malaria records dating to the 1980s from densely populated areas in the highlands of Ethiopia and Colombia, South America. They found that as temperatures increased, more cases of malaria at higher elevations occurred. When temperatures cooled, the disease retreated to lower elevations. Because the team focused on how malaria cases shift in altitude in response to yearly temperature changes, other variables that influence malaria trends, such as drug resistance and fluctuation in rainfall, were discounted.

The findings, reported March 7, 2014, in *Science*, underscore the need for sustained and increased intervention, including mosquito control, to mitigate the effect of climate change in these areas. Otherwise, Pascual and her colleagues have estimated, future temperature increases could result in millions of additional cases of malaria in Ethiopia alone.

A THERMOSTAT FOR MUCUS

Our guts are constantly exposed to bacteria, some helpful, some harmful. If gut defenses aren't strong when unfriendly bacteria attack, the pathogens can lead to increased susceptibility to diseases such as colon cancer and type 2 diabetes. Fortunately, humans and other animals have a thin lining of intestinal mucus that helps keep the bad guys at bay. Recently, three scientists—Richard Flavell, an HHMI investigator at Yale University, Eran Elinav at the Wiezmann Institute of Science, and Brett Finlay at the University of British Columbia—collaborated to show that this entire defense system depends on a protein complex called the NLRP6 inflammasome.

Inflammasomes are collections of proteins responsible for turning on immune responses that result in inflammation. In mice engineered to lack the NLRP6 inflammasome, no intestinal mucus shield was produced. Without that layer, the team reported on February 27, 2014, in *Cell*, bacteria began to attack the lining of the mouse gut, causing infection.

The scientists believe that the NLRP6 inflammasome acts as a thermostat that opens and closes the mucus faucet. The protein complex senses the amount of mucus needed and tells mucus-producing cells how much of the antimicrobial liquid to make. In mice without the NLRP6 inflammasome, the faucet stays closed and there is no mucus shield. This finding is surprising, Flavell explains, because "it was thought that the mucus layer was maintained in a constitutive fashion—in other words, it was essentially present at all times."

They next plan to test whether the inflammasome-mucus system works the same way in humans and to figure out how to "dial up" the protective shield.

CHANNEL CHECKPOINTS

Every second, more than one million calcium ions must squeeze through individual calcium channels into cardiac muscle cells to keep the heart

Photo: AquilaGib (Wikimedia Commons); Illustration: Leif Parsons

To watch the Cas9 complex in action, go to www.hhmi.org/bulletin/spring-2014.

CRISPR's Little Helper

Bacteria use a tiny signal motif to save time when detecting foreign DNA.

BACTERIA HAVE A secret weapon for dealing with viral invaders: a library of genetic mug shots. These bits of DNA, collected from previously encountered viruses, help the bacteria target and destroy their invaders. HHMI scientists recently showed that this defense mechanism—known as the CRISPR-Cas system—gets some of its accuracy and speed from a tiny sequence of DNA that is just three nucleotides long.

The workhorse of the CRISPR-Cas immune system is an enzyme called Cas9. Each Cas9 molecule carries a 20-base pair "guide" RNA sequence that matches one of the DNA mug shots. When a repeat offender invades, it's up to the Cas9 complex to find the complementary DNA sequence on the pathogen and to cut it.

Scientists also use the CRISPR system in their research labs to make precise changes in the genomes of animals and plants. "One of the concerns for people who are using this as a tool has been whether there are off-target effects, in which sites are mistakenly recognized by Cas9 and perhaps cut or modified in experiments," says HHMI Investigator Jennifer Doudna from the University of California, Berkeley.

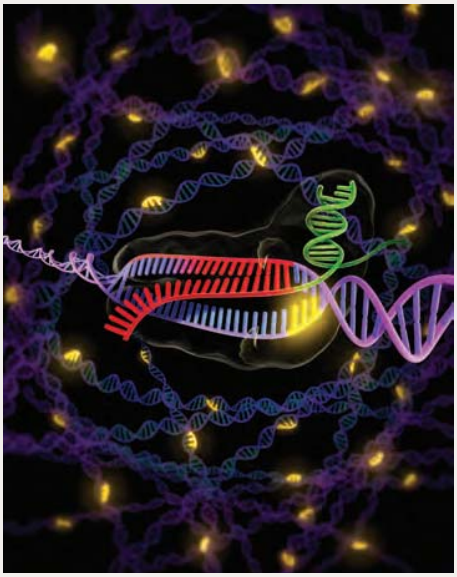
Doudna teamed up with HHMI Early Career Scientist Eric Greene of Columbia University to figure out how Cas9 does its job. Using a technique called a DNA curtains assay that was pioneered by Greene, the scientists were able to watch Cas9 interact with individual DNA molecules. Their findings were published on March 6, 2014, in *Nature*.

Instead of inspecting the entire genome of an invading virus, Cas9 bounces onto and off of the viral DNA while looking for a specific three-letter sequence of nucleotides, called a PAM. "The enzyme really only spends time at sites that have this motif," explains Doudna.

Cas9 unzips the DNA at each PAM site, looking for sequences that match its own guide RNA. There may be thousands of PAM sites, but only one sits next to the DNA pattern that Cas9 is looking for. If there's no match, Cas9 falls off, and the search continues.

Knowing that Cas9 relies on a PAM sequence in addition to its RNA guide molecule will help reduce concerns about the technique and will guide efforts to make Cas9 a better tool in genome engineering.

–Nicole Kresge



Short DNA sequences known as PAMs (yellow) enable bacteria to recognize and destroy foreign DNA.

Cas9 illustration: K. C. Roeyer; Chromosome illustration: Leif Parsons

beating, among other functions. This is an impressive feat—especially because sodium ions are the same size as calcium ions and are 70 times more plentiful outside the cells, yet few of them pass through the tiny pores. Using x-ray crystallography, HHMI Investigator Ning Zheng and his collaborators in William Catterall's group at the University of Washington recently caught a calcium channel in action and revealed the secret of its selectivity.

As they reported in *Nature* on January 2, 2014, the key to this filter is a series of three checkpoints that ions must pass through. Each of these three sites selectively binds to hydrated calcium and rejects other ions. The first site, near the mouth of the pore, recognizes calcium and admits it into the channel. Once inside, the calcium ion binds to the second site, where it remains until it's pushed out by the next calcium ion entering the channel. The final checkpoint, near the end of the channel, helps move the ion into the cell's interior.

Next, Zheng would like to figure out how drugs disrupt these calcium channels. "The mammalian voltage-gated calcium channels are the molecular targets of therapeutic drugs in the treatment of hypertension, angina pectoris, and cardiac arrhythmia," he explains. By understanding how the drugs interact with calcium channels, scientists can create new compounds that work better and have fewer side effects.

THE ROOTS OF A DISEASE

French Settlement, LA, is an unassuming little town just northwest of New Orleans. It is also the place where a group of genetic disorders known as hereditary spastic paraplegia (HSP), or French Settlement Disease, was first discovered. Unlike the town, the disease is anything but modest: its main features are stiffness and involuntary contractions in the legs. Thirty-five years after the disease's discovery, a study led by HHMI Investigator Joseph Gleeson at the University of California, San Diego, has almost doubled the number of genes associated with HSP.

Scientists had already linked 22 genes to HSP, but mutations in those genes explained less than 50 percent of the cases. To find more genes, Gleeson and a team of 51 scientists from around the world recruited 55 families with HSP. The scientists sequenced every gene in 93 family members and discovered 18 genes newly linked to the disease. They then created an "HSPome"—a genetic map showing how all the HSP-associated genes interact with each other.

The effort, which took 10 years, allowed the researchers to link HSP to other common neurodegenerative diseases, such as Alzheimer's. "This told us that common neurodegenerative diseases share similar networks and cellular vulnerabilities," says Gleeson. "Maybe we need to think about these less as individual diseases and more as collective problems of neuronal susceptibility."

The findings, reported January 31, 2014, in *Science*, may help Gleeson and his colleagues develop new treatments for HSP. They're already pursuing several promising targets.

DNA DOESN'T HAVE THE LAST WORD

During transcription, the RNA polymerase enzyme reads information from a strand of DNA and uses it to create RNA. It's often assumed that the RNA is an exact copy of its DNA template. Three years ago, Vivian Cheung, an HHMI investigator at the University of Michigan, showed otherwise. She found widespread sequence differences between RNA transcripts and their template DNA in human cells. In her latest research, Cheung has pinpointed when these mismatches, or RNA-DNA sequence differences (RDDs), occur.

Cheung and her collaborator, John Lis at Cornell University,

Chronicle / *Lab Book*

Hidden Killers

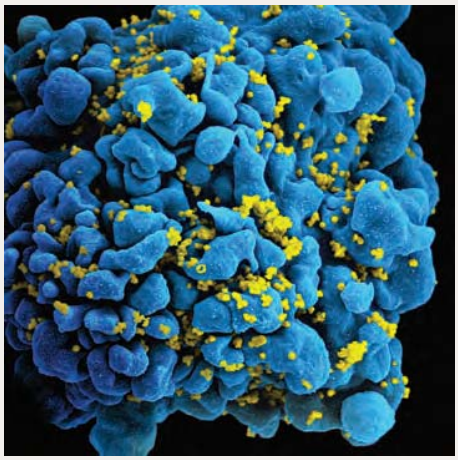
A larger-than-imagined reservoir of HIV is evading current antiretroviral therapies.

THERE WAS A time when a positive test for human immunodeficiency virus (HIV) meant certain death. Today, the future is much brighter for people with HIV, thanks to advances in drug treatments. But even the most powerful therapies can't flush out the dormant virus that lurks in a patient's body—a reservoir that, according to HHMI Investigator Robert Siliciano at the Johns Hopkins University, may be up to 60 times larger than previously imagined.

HIV wipes out the immune system by converting the very cells that are meant to help kill it into virus-producing factories. During an infection, HIV injects its genetic material into activated CD4+ T cells. The HIV DNA integrates into a T cell's genome, causing it to create more virus. Most of the infected T cells in this viral production mode die, but some survive and go into a resting state in which virus production is shut off. About 100 to 1,000 resting CD4+ T cells per million harbor dormant HIV sequences called proviruses. These sleeper viruses are invisible to circulating immune cells—and to current antiretroviral therapies.

Until recently, scientists were unable to get an accurate fix on the size of the provirus reservoir. Moreover, they didn't know how many of these stealthy invaders could wake up and mount an attack against the immune system. Siliciano and Ya-Chi Ho, an HHMI international student research fellow, published a technique in the October 24, 2013, issue of *Cell* that has revealed both the size and the composition of this reservoir. The good news is that about 88 percent of these dormant viruses have genetic defects that make them unable to reactivate. Unfortunately, the remaining 12 percent are fully functional and have the potential to reawaken and attack at any time. The findings suggest that previous calculations vastly underestimated the magnitude of the provirus population—by about 60-fold.

To effectively target HIV, future therapies need to consider these dormant viruses. "It doesn't mean that it's hopeless, but it does mean we need to focus on getting an even clearer idea of the scope of the problem," says Siliciano. Currently, he and his colleagues are working on simple clinical assays to assess reservoir size. – *Nicole Kresge*



HIV (yellow) attacks the immune system by infecting T cells like this one.

T cell: NIAID; Synapse illustration: Leif Parsons

IN BRIEF

isolated newly made RNA from human cells and compared the sequences to the DNA in the same cells. They discovered that RDDs are not formed when RNA polymerase creates an RNA copy of the DNA template. Instead, the changes often occur right after the synthesis, when the new RNA folds back on its DNA template, forming a DNA-RNA hybrid called an R-loop. These structures are thought to play a role in gene regulation. The group published its findings on March 13, 2014, in *Cell Reports*.

Now that Cheung knows when RDD formation occurs, she's looking for the processes that form these mismatches. "The knowledge that RDDs are not as rare as previously thought tells us that genetic studies need to give RNA variation some attention," she says. Figuring out the mechanisms behind RDD formation will further the understanding of how RNA processing contributes to genetic diversity.

REPROGRAMMING CANCER

Glioblastomas are the most common brain tumors. They are also the most lethal, in part because of a small population of stem cells that live inside each tumor. Although the stem cells comprise only a small portion of the tumor—just a few percent—they are not trivial. They cause aggressive tumor growth as well as resistance to radiation and chemotherapy. Recent findings by HHMI scientists point to a way to disarm these cells by focusing on what makes them so different from the rest of the tumor.

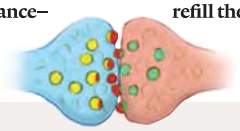
HHMI Early Career Scientists Bradley Bernstein, at Massachusetts General Hospital, and Aviv Regev, at Massachusetts Institute of Technology, collaborated to examine the circuits that regulate genes in both stem cells and non-stem cells from glioblastoma tumors. The researchers found four transcription factors—proteins that turn genes on and off—that were present only in the stem cells. When they expressed a cocktail of four transcription factors in the non-stem cells from the glioblastomas, those cells turned into stem cells. The experiments, published April 24, 2014, in *Cell*, show that transcription factors can override a glioblastoma cell's programming and drive it into a more aggressive state.

Bernstein and Regev hope to use this information to target these aggressive stem cells with small molecule inhibitors.

NEURONAL RECOVERY

In the body, neurons use molecule-filled sacs, called vesicles, to communicate. When it's time to send a message, a neuron's vesicles fuse with its membrane, releasing their contents into the synaptic space between the cells. If there is a lot to communicate, the neurons burn through their vesicles quickly.

Rapid replenishment of vesicles is of the utmost importance—the neurons need to be ready to send the next batch of signals.

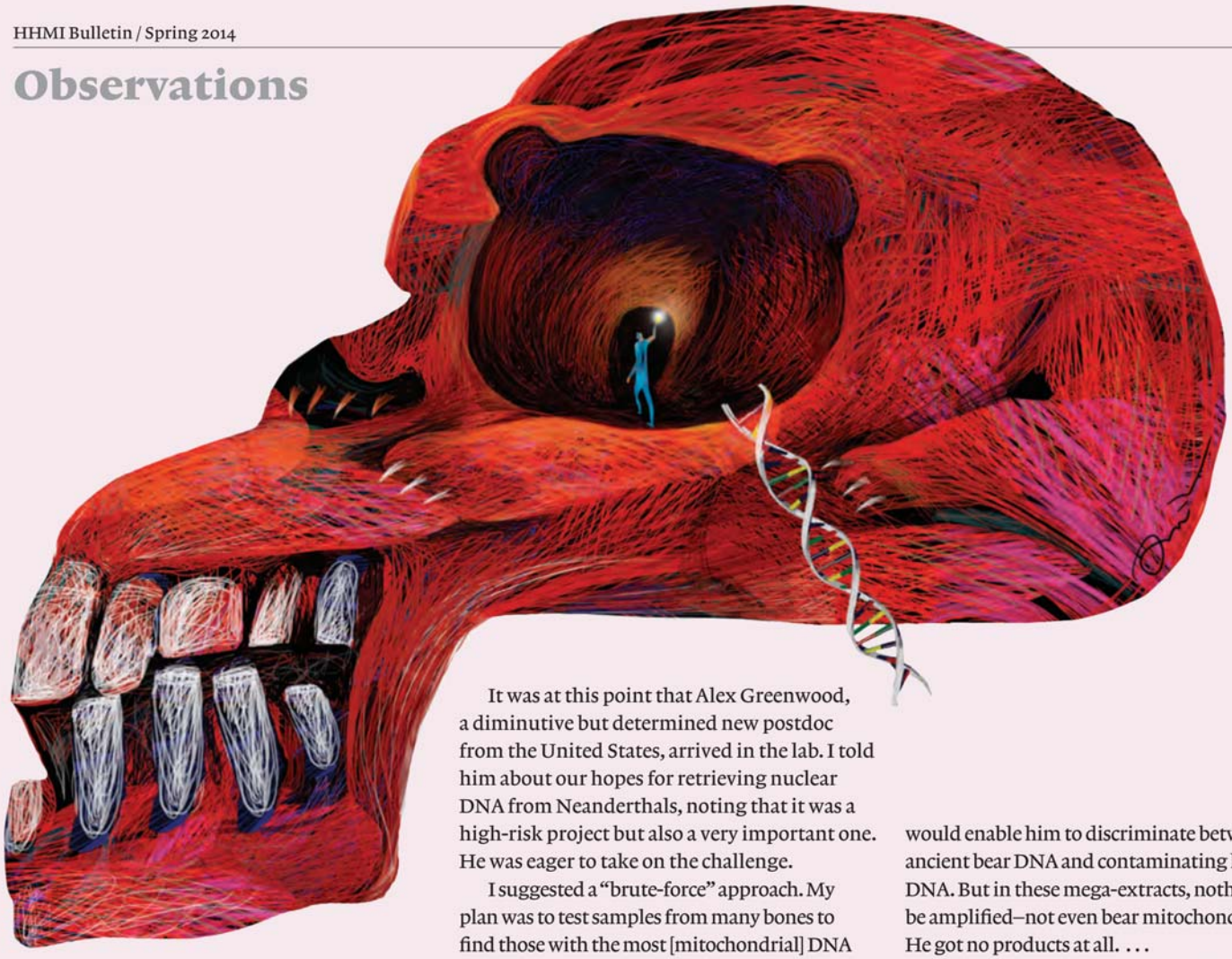


HHMI Investigator Edwin Chapman and his team at the University of Wisconsin recently showed that two calcium-binding proteins, calmodulin and synaptotagmin 7, work together to ensure that neurons have adequate vesicles for communication.

One route of restoring synaptic vesicle supplies depends on calcium and calmodulin. Chapman's team discovered that, in response to Ca^{2+}, synaptotagmin 7 binds to calmodulin, and this complex initiates the vesicle replenishment pathway. The study, published in *eLife* on February 25, 2014, clarifies a number of controversies about the function of synaptotagmin 7 in the nervous system.

Now that Chapman knows what's involved in the pathway, he wants to figure out how the components work together to replenish the vesicles. "Do they clear out release sites so incoming vesicles can dock and fuse? Do they refill the releasable pool of vesicles? Or do they do both?" he wonders. "At the moment, this remains a mystery."

Observations



Neanderthal Man

In 1856, workers clearing a quarry cave in Neander Valley, near Düsseldorf, Germany, uncovered the bones of what they thought was a bear. Instead, the skeletal remains turned out to be an extinct, possibly ancestral, form of human, dubbed the Neanderthal. Some 150 years later, paleoanthropologist Svante Pääbo found a way–using grit, creativity, and modern technology–to genetically define what makes us different from our Neanderthal cousins. It was a crowning moment for Pääbo, whose fascination with ancient history, sparked during a family trip to Egypt at the age of thirteen, led to a career exploring human genetic evolution by analyzing DNA extracted from ancient sources.

Ajubel

It was at this point that Alex Greenwood, a diminutive but determined new postdoc from the United States, arrived in the lab. I told him about our hopes for retrieving nuclear DNA from Neanderthals, noting that it was a high-risk project but also a very important one. He was eager to take on the challenge.

I suggested a "brute-force" approach. My plan was to test samples from many bones to find those with the most [mitochondrial] DNA and then extract DNA from yet larger samples in an attempt to retrieve any nuclear DNA. This approach meant that we could not perform our initial experiments with the uncertain technique on Neanderthal remains; they were too scarce and valuable to use when the risk of failure was so high. Instead we resorted to animal bones, which were both considerably more abundant and less valuable to paleontologists. . . .

Alex began by extracting DNA from the Croatian cave-bear bones, which were between 30,000 and 40,000 years old. . . . The problem he faced was a familiar one to me: because each cell in a living animal contains hundreds of mitochondrial genomes but only two nuclear genomes, any particular piece of nuclear DNA was present in 100- or 1,000-fold fewer copies in the extracts than any particular piece of mitochondrial DNA. So even if some nuclear DNA was present in minute amounts, the chances of amplifying it were 100- or 1,000-fold lower.

One obvious way to overcome this problem was to simply use more bone. Alex made extracts of ever larger amounts of cave-bear bone and tried amplifying ever shorter pieces of nuclear DNA using primers flanking nucleotides where bears were known to differ from humans. That would enable him to discriminate between ancient bear DNA and contaminating human DNA. But in these mega-extracts, nothing could be amplified–not even bear mitochondrial DNA. He got no products at all. . . .

Thwarted by the cave bears, and wondering whether the conditions in the cave may simply have been too unfavorable to preserve nuclear DNA, we decided to switch to material that we expected to show the very best preservation–permafrost remains of mammoths from Siberia and Alaska. These had been frozen ever since the animals died and freezing, of course, will slow down and even stop both bacterial growth and many chemical reactions, including those that degraded DNA over time. We also knew, from Matthias Höss's work, that mammoths from the Siberian permafrost tended to contain large amounts of [mitochondrial] DNA. Of course, no Neanderthals had ever been found in the permafrost–so switching to mammoths meant taking a step away from my ultimate goal. But we needed to know whether nuclear DNA could survive over tens of thousands of years. If we found no nuclear DNA in the frozen remains of mammoths, then we could forget about finding it in Neanderthal bones preserved under much less ideal conditions.

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Inner Diva

A chameleon by nature, the scalyhead sculpin adapts its coloring to the shallow reefs and rocky shorelines where it lives, leaving flamboyance to other creatures that share the intertidal zone. But strip the fish down to its skeleton and add a bit of color and out pops an important evolutionary link. Adam Summers, a University of Washington biologist who studies the mechanics of movement in animals, stained the sculpin's bones red and its cartilage blue, and then treated the fish with chemicals to make it transparent. The red-stained bones in the sculpin's fins are evolutionary predecessors to our arms and hands. You can learn more about our fishy past in the PBS documentary *Your Inner Fish*—the first production from HHMI's Tangled Bank Studios (www.tangledbankstudios.org).

Adam Summers