

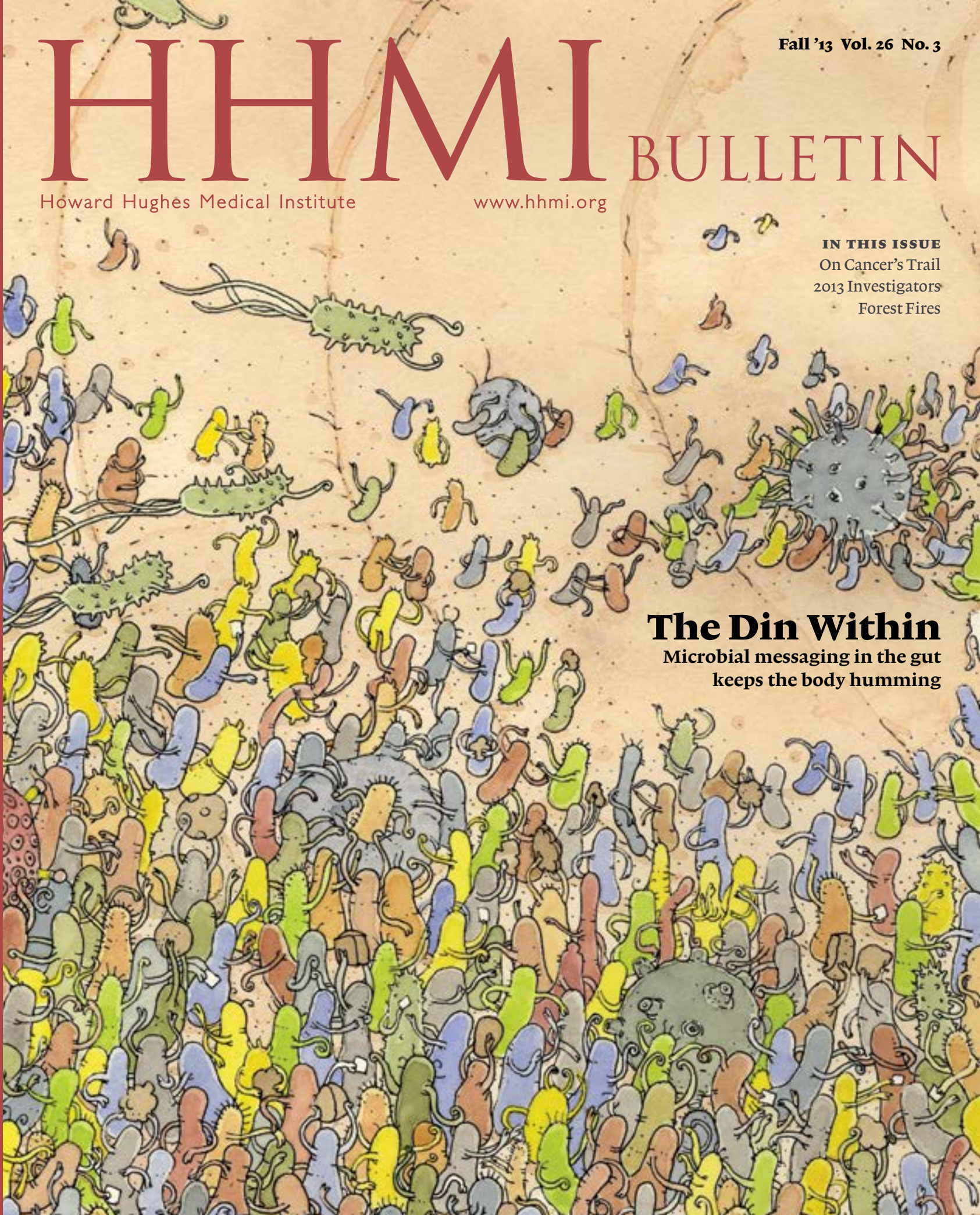
HHMI BULLETIN

Howard Hughes Medical Institute

www.hhmi.org

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2013 Investigators
Forest Fires

The Din Within
Microbial messaging in the gut
keeps the body humming





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Interstellar dust cloud?
Distant galaxy? Not quite,
though it is a vista into the
brain. This is a collection
of neurons, labeled in
a unique way to track
voltage changes that help
the cells communicate.
Adam Cohen developed the
technique using microbial
rhodopsins—bacterial
proteins that convert
sunlight into electricity.
When the rhodopsins
are run in reverse, they
fluoresce each time a pulse
of electricity races through
a neuron, producing an
otherworldly glow.

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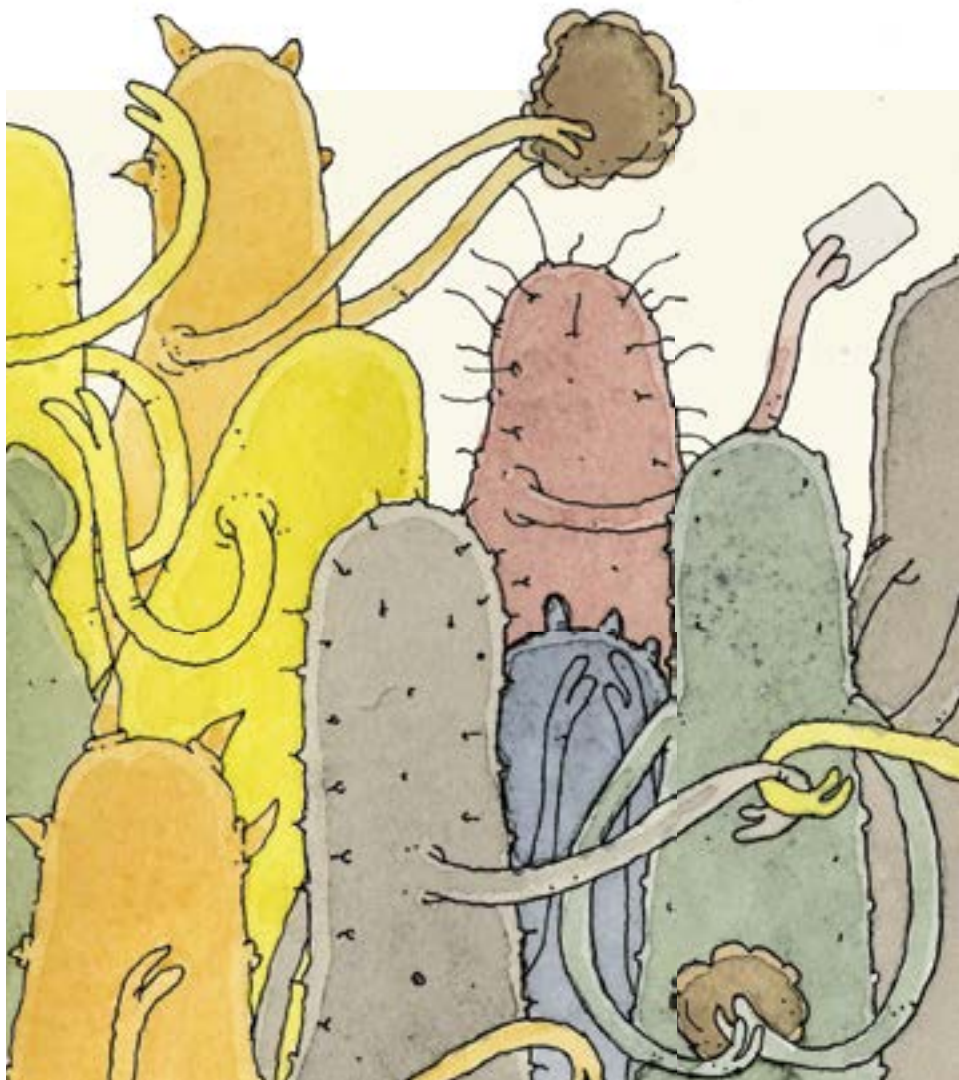
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- Join several new HHMI investigators as they discuss the draw of science, the role of creativity in research, and learning on the job.
- Take a journey through a transparent mouse brain.

- See students get a hands-on-and feet-in-experience that teaches them about the biology of a stream.
- Watch male fruit flies turn to alcohol after their sexual advances are rejected.
- Learn how HHMI finds creative scientists with the potential to make groundbreaking discoveries.

www.hhmi.org/bulletin

Cover image:
Mattias Adolfsson

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Contributors



Mattias Adolfsson (cover and “Microbial Social Network,” page 12) is a freelance illustrator operating from Sigtuna, outside Stockholm, Sweden. In addition to sketching bacteria and viruses, he makes other commissioned works but always finds time for moderate walks in the forest and personal drawings.



Sarah C. P. Williams (“Microbial Social Network,” page 12) can’t seem to cut her ties to HHMI. She’s been associated with the Institute first as a budding scientist—through an undergraduate research fellowship a decade ago—then as a staff member of the *HHMI Bulletin*, and now as a freelance writer. Though she grew up in New England, today she makes her home near the much warmer shores of Hawaii, where she enjoys kayaking and traipsing up mountains with her husband and dogs.



Scottish-born photographer **Graham MacIndoe** (“Driven,” page 18) lives and works out of New York City. He shoots for editorial, corporate, and advertising clients, including *The New York Times Magazine*, *The Guardian Magazine*, and *W* magazine, among others. Current personal projects include one about addiction and recovery and another on immigration detention in the United States.



Al Murphy (“Intoxicating Research,” page 6) is a British illustrator who loves to draw freehand. When not busy adding faces to inanimate objects, he is busy worrying how long it will be before he has to get a real job. He makes his home in Brooklyn, NY, with his partner Annie and their son Casper.

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President's Letter

Investing in the Basics

NOT LONG AGO, we had a health scare in my family. My sister was diagnosed with a rare disease. To make matters worse, her treatment caused a cascade of side effects that landed her in the hospital. No one in my family could understand why this was happening. So I went to Los Angeles and met with her doctor. After a lengthy discussion, I could finally piece together the trail of events that led to her illness and explain what was making my sister so sick.

Faced with serious illness, most people struggle to comprehend what seems like a foreign language. But understanding the situation, and being able to connect the dots, is critical to making informed health care decisions. That kind of deep understanding—for health care providers, as well as those of us on the receiving end—can't happen without foundational knowledge about how the body works. You've got to understand how something works before you can fix it.

At HHMI, our goal is to make that basic knowledge, that understanding, possible. We do that by finding and supporting the most talented scientists, giving them the

“If we want others to understand the value of basic scientific research, we've got to let them know why and help them appreciate what science is about.”

—ROBERT TJIAN



freedom to follow their instincts and apply creative approaches to hard problems. We have long described our strategy as funding “people, not projects.” In this issue of the *HHMI Bulletin*, you will meet 27 such people: the latest group of researchers to be named HHMI Investigators.

Each of these scientists is building on an already impressive body of work, and we want to share those stories. Today, more than ever, HHMI is working to raise the visibility of basic research. We believe deeply in supporting quality science and science education, and we are eager to discuss strategies for doing that with other like-minded organizations and individuals. Ultimately, we hope to dramatically increase investments in science.

To achieve this goal, we are collaborating with public and private partners, to raise awareness of the importance of foundational or “basic” research. If we want others to understand the value of basic scientific

research, we've got to let them know why and help them appreciate what science is about. If we succeed, people may be better informed. They may know the right questions to ask in a tough medical situation, they may be motivated to support biomedical research, or they may understand how to inspire students toward careers in science.

I'm happy to say that my sister is doing fine now. I'm grateful that I was able to interpret her doctor's information, so that my family understood what was happening. I wish the same for every family in our situation. Over time, I believe, our work at HHMI contributes to the basic knowledge we all need to make better decisions, stay informed, and understand the world around us.

A handwritten signature in black ink, which appears to read "Robert Tjian".

Centrifuge



Camp Normal

CAMP LORE HOLDS that Fireman Fred haunts the piney environs of Camp Conrad-Chinnock in the San Bernardino Mountains outside Los Angeles. Having perished while rescuing campers from a raging inferno, the ghostly firefighter wanders the woods in anguish, driving his hatchet into cabin doors at night.

“It’s absolutely true that a cabin once [had] a small fire. Fireman Fred? He never existed,” laughs Dylan Wolman, an HHMI medical fellow working on neuroimaging

in the labs of Davi Bock and Karel Svoboda at Janelia Farm Research Campus. “His legend, however, served as the basis for one of the more epic pranks at camp.”

Each summer for the past 6 years, Wolman has worked at Camp Conrad-Chinnock. Like many sleep-away camps, it offers rock climbing, mountain biking, archery, canoeing, and swimming. What’s different is that, mixed in with the typical fun, campers learn to manage their type 1 diabetes and thrive in an environment where having diabetes is the norm: most counselors and medical staff have the disease as well.

“It’s an amazing community and everybody gets something out of it,” says Wolman. He attended the camp as a depressed and angry young teen.

Diagnosed at age 11, Wolman rebelled against his disease. He didn’t want to deal with it. Because Wolman was resistant to learning to manage the disease, his doctor suggested camp.

“I really wasn’t in the right mental state to accept diabetes when I went to camp so I was bitter and rebellious the whole time,” recalls the young man who now has the chemical structure for glucose tattooed on his left forearm.

With time, Wolman started accepting his diabetes and moved from “couch potato” to avid runner. He began to understand the importance of knowing he wasn’t alone. That realization drives his commitment to work at the camp every summer.


“I really like dealing with the angry teenagers because I know how they feel,” he says. “At the very least, I can tell them I was in exactly the same spot.”

As camp counselor, Wolman participates in all activities and makes sure campers stay engaged and happy. He also carries sugar tablets and a blood sugar testing kit. “As medical staff I get to have a more direct role in management and teaching the logic behind dosing relative to diet, environment, and activity,” he says. “As a counselor I take part in more of the campers’ activities and [act as a] role model. They are different but equally fulfilling roles.”

Being part of the camp team and getting to know campers over multiple years gives Wolman his greatest satisfaction. “I see kids who first came to camp depressed and unhappy, and now they are doing great!” he says. “Camp would function just fine without me, but it’s great to be a truly competent part of something that is so much bigger than me.”

Besides, if he skipped camp, he’d miss out on the “Fred” hijinks, like that epic prank that still makes Wolman grin. Employing the skills of Hollywood make-up artists, full firefighter regalia, and a fog machine, one night the older boys fogged up the older girls’ cabin, and sent Fireman Fred inside.

“He came out covered in silly string,” Wolman laughs. “The girls knew about it the whole time—that poor guy was utterly defeated.”—*Lisa Chiu*

 Watch Strobel transform a slab of wood into a beautiful bowl at www.hhmi.org/bulletin/fall-2103.

Perennial Treasure

AFTER HURRICANE SANDY smashed into the Atlantic seaboard in October 2012, Scott Strobel, an HHMI professor and biochemist at Yale University, expected the call. No, his laboratory wasn't underwater. Sandy had toppled a five-story white oak tree (*Quercus alba*) on the New Haven, CT, campus's Science Hill. University arborists phoned to tell him to get his chain saw ready.

That day Strobel drove his pickup truck away groaning under the weight of a bed heaped high with cross sections of oak trunk. He took them back to his house in nearby Hamden. The oak tree, once a majestic landmark for Yale students and faculty, was gone. Strobel would give its wooden heart a second life, however, transforming it into campus landmarks of a different sort. He

would use his woodworking skills to carve the raw wood into unique sculptured bowls and pens.

Out of his two-bay garage woodworking shop, Strobel handcrafts hardwood objects, some for use, most for display. All are made from wood salvaged from fallen campus trees. He gives away many of his creations to friends and colleagues; others can find them in a few New Haven shops and at yalebowls.com, a Web business that plays on the name of the Yale Bowl, the university's National Historic Landmark football stadium.

Each numbered piece comes with a certificate identifying the campus location and history of the tree from which it was fashioned. Yale shares in a portion of Strobel's sales, which goes toward planting new trees on campus. The rest, he says, "pays for my woodworking tool habit."

In a turner's smock and face shield, Strobel stands up to his ankles in shavings hollowed from chunks of wood spinning on his lathe. To exit his shop, he weaves his way among the woodworking benches, power tools, cabinets of chisels and other hand tools, plus slabs of raw cherry, oak, elm, and maple. Scattered about are stacks of roughed-out bowls and pen shafts that must air-dry for months before he gives them a finishing turn on the lathe.

Picking up a roughed-out walnut bowl and noting its mother

tree's former location, he says, "It's wood with an address. If you know the place where the tree stood, well, here's something that gives you a memory of where you were."

Duke University President Richard Brodhead lived at the actual address of the bowl Strobel sent him. As Dean of Yale College in the 1990s, Brodhead had occupied a campus house shaded for nearly a century by a towering oak. He hadn't heard, but after he moved to Duke, the tree came down. Looking back on it, Brodhead recalls, "Scott saw its sentimental interest [for me]... So, presto! Using a skill I never knew he had, he turned a fallen tree into a beautiful object."

Strobel's love for wood and what it harbors extends to what he calls his "day job." He directs an HHMI-funded undergraduate course on rainforest bioprospecting and his lab group studies RNA biochemistry and the molecular chemistry of fungi. "Woodworking," he says, "provides a tangible way for me to express an alternative creative side, different from designing experiments and training students but in some ways the same. I start with materials that are often as raw as they can get and make something beautiful, enduring, and, I hope, meaningful from them."

—Marc Wortman

Scott Strobel gives trees a second life.



Bench Report



Her experiments are providing insights into the genetics of addiction and connections between environmental factors, such as rejection and stress, and alcohol abuse.

The fruit fly is a surprisingly good model for human alcohol addiction. In nature, flies live on rotting, fermented fruit and have done so for millions of years. “They live, court, mate, and lay their eggs around alcohol,” Heberlein says. “Their progeny grow in this fermenting fruit that can contain up to 5 percent alcohol.” And, more importantly, the tiny creatures exhibit many of the same behaviors after ingesting alcohol that people might display after a few strong drinks. First they become hyperactive, and then they lose coordination and fall over; eventually, they pass out.

Heberlein recently showed that, very much like humans, flies exhibit gender-specific responses to ethanol intoxication. That is, to put it bluntly, female flies get drunk faster than male flies. Part of this is because female flies metabolize ethanol more slowly than males. But another component, as Heberlein and her student Anita Devineni reported December 2012 in the *Proceedings of the National Academy of Sciences*, involves sex differences in the brain, some of which are controlled by a fly gene named *fruitless*.

In a typical experiment in her lab, Heberlein’s team creates fruit flies with random genetic mutations and then runs them through a series of tests using tools like the inebriometer and the booz-o-mat to see how they react to alcohol. Heberlein created the booz-o-mat, a series of horizontal glass tubes that measure how fast and straight—or slow and crooked—inebriated flies can walk.

Intoxicating Research

Can fruit flies reveal the genetic and environmental underpinnings of alcoholism?

PERCHED IN THE CORNER of Ulrike Heberlein’s office is a four-foot-tall glass column crisscrossed with slanted platforms. It’s a fruit fly inebriometer. Although a relic now, Heberlein used it to gauge how long it takes flies to get drunk. Flies are introduced into the top of the column, which is perfused with ethanol vapor. As the flies become inebriated, they slip from the first platform to the one below, and then the one below that, and so on, until they pass out and tumble to the bottom.

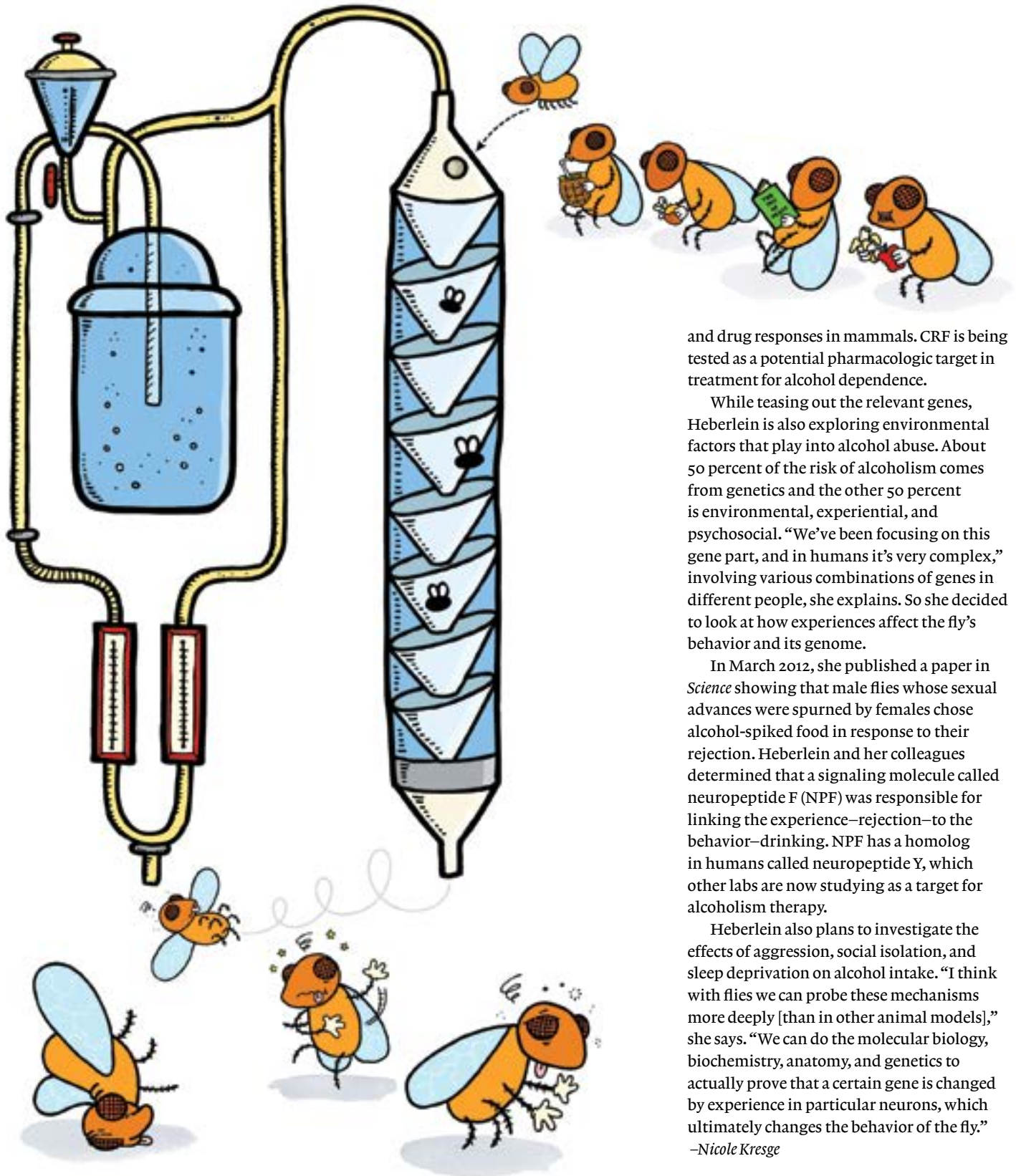
Heberlein, who became a lab head at HHMI’s Janelia Farm Research Campus in 2012, developed the fruit fly *Drosophila melanogaster* as a model to study alcoholism.

Over the past two decades, Heberlein and her colleagues have identified some 30 genes involved in the fruit fly’s response to alcohol. One mutant gene produced an extremely alcohol-sensitive fly her lab christened *cheapdate*, and, at the other end of the spectrum, another gene was responsible for a mutant called *happyhour*, with a higher than average tolerance for alcohol.

Studies detailed in a February 2013 paper in *The Journal of Neuroscience* showed that a gene called *apontic* (*apt*) plays a role in the sedative effects of alcohol. The Apt protein acts on a small subset of neurons that express Corazonin (Crz), a neuropeptide likely involved in the body’s response to stress. The Crz neurons also contain receptors for proteins similar to the mammalian corticotropin-releasing factor (CRF), which has been implicated in stress

Heberlein also plans to investigate the effects of aggression, social isolation, and sleep deprivation on alcohol intake. “I think with flies we can probe these mechanisms more deeply.”

➤ See male fruit flies turn to alcohol in response to their rejection at www.hhmi.org/bulletin/fall-2013.



and drug responses in mammals. CRF is being tested as a potential pharmacologic target in treatment for alcohol dependence.

While teasing out the relevant genes, Heberlein is also exploring environmental factors that play into alcohol abuse. About 50 percent of the risk of alcoholism comes from genetics and the other 50 percent is environmental, experiential, and psychosocial. “We’ve been focusing on this gene part, and in humans it’s very complex,” involving various combinations of genes in different people, she explains. So she decided to look at how experiences affect the fly’s behavior and its genome.

In March 2012, she published a paper in *Science* showing that male flies whose sexual advances were spurned by females chose alcohol-spiked food in response to their rejection. Heberlein and her colleagues determined that a signaling molecule called neuropeptide F (NPF) was responsible for linking the experience–rejection–to the behavior–drinking. NPF has a homolog in humans called neuropeptide Y, which other labs are now studying as a target for alcoholism therapy.

Heberlein also plans to investigate the effects of aggression, social isolation, and sleep deprivation on alcohol intake. “I think with flies we can probe these mechanisms more deeply [than in other animal models],” she says. “We can do the molecular biology, biochemistry, anatomy, and genetics to actually prove that a certain gene is changed by experience in particular neurons, which ultimately changes the behavior of the fly.”

–Nicole Kresge

Bench Report

The Guide on the Side

Hal White steers students to attack a problem, figure out how to find the answers, and communicate their results.

STUDENTS IN Harold “Hal” White’s sophomore biochemistry course don’t lounge in their seats, taking notes while he lectures from the front of the classroom.

They cluster in small groups, heads bent over a real-world problem based on classic research papers. They discuss the science, the historic background, and ethical implications while working to solve the problem before them.

White, meanwhile, floats around the room, like a “guide on the side,” as he puts it, asking probing questions to keep his students on the right track. He swears by this simple but powerful method, known as problem-based learning, or PBL, as a more effective way than lecture and discussion to engage students and stimulate curiosity to discover scientific concepts on their own.

“With PBL, the students ask the questions when presented with a relevant problem,” White says. “They identify and articulate what they don’t know, then go out and find the answers.”

Supported by a series of HHMI education grants since 1998, he and his colleagues have woven PBL through undergraduate science education at the University of Delaware (UD) and beyond. He has held workshops for

hundreds of visiting teachers—including more than 100 from foreign countries. Not long ago, he received an email from an instructor at Victoria University in Australia, saying she uses his strategy “with much success” in her art history courses.

“Hal has elevated teaching reform and the use of active learning strategies,” says Murray Johnston, chair of Delaware’s Department of Chemistry and Biochemistry. “Students give him glowing marks, and they are very well prepared to go on and are very successful in graduate school.” He earned the 2014 award for exemplary contributions to education from the American Society for Biochemistry and Molecular Biology.

One of White’s problem-based assignments hooks students with the hypothetical case of a researcher who contracts bubonic plague in the deserts of New Mexico. The case description includes information on how selective pressure in the wake of the Black Plague in the Middle Ages led to widespread—but not universal—resistance to the dreaded disease.

He then poses the problem: Assuming you could return via time machine to the Middle Ages with all the tools of modern molecular genetics, what changes in allele frequency resulting from the plague would you expect? And why?

PBL, like White himself, is a product of the 1960s. The method was developed at McMaster University as a model for medical education, like case-method instruction in law, to replace the system of force-feeding massive amounts of factual information to medical students. White was a graduate student at politically active Brandeis University near Boston, where educational and social change was in the wind. It was “a formative experience,” he says. As a volunteer, White tutored Upward Bound high school students in chemistry and math in a Boston neighborhood torn by racial riots.

These influences converged at Delaware, where after a period of research and teaching, White turned to instructional reform with PBL as the cornerstone. He has paid special attention to the academic challenges facing minorities, working with minority students in the university’s NUCLEUS program (funded by HHMI until 2010), which aims to recruit and retain graduate students majoring in science disciplines. “It provides a social



Hal White pushes college students to follow their curiosity.

network for minority students, who tend to feel isolated at UD,” he says.

In addition to coursework, each summer about two dozen sophomores—many with HHMI support—work in university research laboratories, supervised by faculty sponsors. “We usually have 8 to 10 research publications a year with at least one undergraduate author,” says White. Along with the experimental procedures they learn, the students meet weekly to explore issues like career paths, scientific misconduct, and communicating about science.

White serves on the leadership team of the university’s Institute for Transforming Undergraduate Education, which is directed



“With PBL, the students ask the questions when presented with a relevant problem. They identify and articulate what they don’t know, then go out and find the answers.”

—HAL WHITE

by English professor Stephen A. Bernhardt. Bernhardt is impressed with students who’ve come out of White’s sophomore biochemistry course. “They’re fearless when it comes to approaching a difficult research article from a scientific journal. They talk about how Hal has influenced their learning—teaching them how to ask questions, how to seek answers, and how to write and speak clearly about their knowledge.”

As he begins to think about passing the baton to younger faculty, White continues to guide Delaware’s approach to science teaching. He serves on a steering committee that is giving more weight to math and quantitative methods in biology teaching at UD, to better prepare

students for careers in life sciences research. The university now offers a bachelor of science in quantitative biology. “This degree provides an option for students trained in math to apply it to biological problems,” he says.

White is also brokering discussions among chemistry and biology faculty to integrate instruction and labs in the two historically separate fields; this movement is supported by HHMI funding in its latest grant and will affect an estimated 400 to 700 students a year. The plans include smaller classes, more team-based learning, and “a more active, problem-focused approach,” says Bernhardt. “Hal has provided both the vision and strategy to make this happen.”—*Richard Saltus*

Bench Report

Time to Sprout

Researchers discover how burning plants tell seeds to start growing.

A FOREST FIRE can devastate swaths of land, felling centuries-old trees and anything else in its path. Researchers have come to learn that this kind of destruction is not all bad, and can signal rebirth. Now a team at the Salk Institute for Biological Studies has revealed how a protein in dormant seeds gets a wake-up call from smoke and ash.

“Fire is an important way for an ecosystem to replenish itself every now and again,” says HHMI Investigator Joanne Chory, “because when you get unchecked undergrowth, these opportunistic plants reduce nutrients available to the large stands of older trees that create the forest ecosystem.”

As it burns, this undergrowth releases smoke and ash, which contain chemicals that remain in the soil after the fire dies down. One group of those chemicals, known as karrikins, tells dormant seeds that it’s time to germinate. “The ash left over includes not only these natural chemicals,” says HHMI Investigator Joseph Noel, “but also fantastic natural fertilizers to spur the growth of the older trees and ensure the next generation of trees.”



After a burn, chemicals left in the soil awaken dormant seeds.

To dig deeper into karrikins, Chory and her postdoc Zuyu Zheng teamed up with Noel and his postdoc Yongxia Guo as well as James La Clair, a chemist at University of California, San Diego. It helped that Guo and Zheng are a married couple and that the groups had collaborated in the past. Chory, a geneticist, studies how plants adapt their growth rate and body plan to their environment; and Noel, a biophysicist and biochemist, is interested in how plant metabolic pathways evolve and adapt to local ecosystems.

For this study, they wanted to know

“If we could modify the protein to keep seeds sleepy until the right time for each situation, it would do a lot to establish the seedling in the best possible environment.”

—JOANNE CHORY



exactly how karrikins, named after the aboriginal word for smoke, awaken dormant seeds. Using x-ray crystallography to elucidate the structure of the protein that binds to karrikins, they were able to see how dormant seeds sense karrikins.

Genetic studies from other groups identified plants with *kaiz* mutations that are insensitive to karrikins, implying a role for the KAI2 protein in sensing karrikins. The Noel-Chory team mapped out how karrikins, once they enter seeds, stick to the KAI2 protein, causing KAI2 to change its shape.

They hypothesized that seeds know that karrikins are in their midst because KAI2's shape changes, likely serving as the signal to other resting proteins in the seed to power up for seed germination. The team published its findings May 14, 2013, in the *Proceedings of the National Academy of Sciences*.

Curiously, KAI2 looks like a type of protein known as a hydrolase enzyme that would normally orchestrate chemical reactions in plant cells. However, the team's structural and biochemical studies show that KAI2 doesn't function as an enzyme in response

to karrikins; instead, evolution seems to have shaped it for another function: it acts as a receptor that binds karrikins and relays critical information to a network of proteins in plant seeds.

To verify their results, the scientists changed the shape of KAI2 slightly to see how karrikins would slip into the restyled KAI2 lock. They also looked at another essential group of plant compounds known as strigolactones (SL) that are similar to karrikins, but are involved in a chemical change instead of a shape change. By modeling SLs into their karrikin-bound KAI2 structures, they also gained a clearer understanding of how the two related systems operate to transmit distinct environmental signals in seeds and plants.

Plant evolution adapted the shape and function of ancestral receptors whose signatures remain as vestiges in the contemporary KAI2 family of proteins. Noel calls them "imprints of molecular fossils," because they allow researchers to peer into plants' storied pasts. At some point in the history of plants, it appears likely that only one protein receptor bound to chemicals that share the atomic structure of both strigolactones and karrikins. Through hundreds of millions of years of evolution, however, one became two.

Understanding plants' deep evolutionary past may help scientists shape a more sustainable and environmentally friendly future, Noel says. "We can learn from nature and harness the tools of synthetic biology to develop plants that can respond to changing times, ultimately creating hardier and more nutritious plants."

Chory also hopes their research findings may lead to better ways to sprout seeds when needed to meet the needs of Earth's growing population. "If we could modify the protein to keep seeds sleepy until the right time for each situation—say when it was warm enough or there was enough rain—it would do a lot to establish the seedling in the best possible environment," she says.

—Katharine Gammon

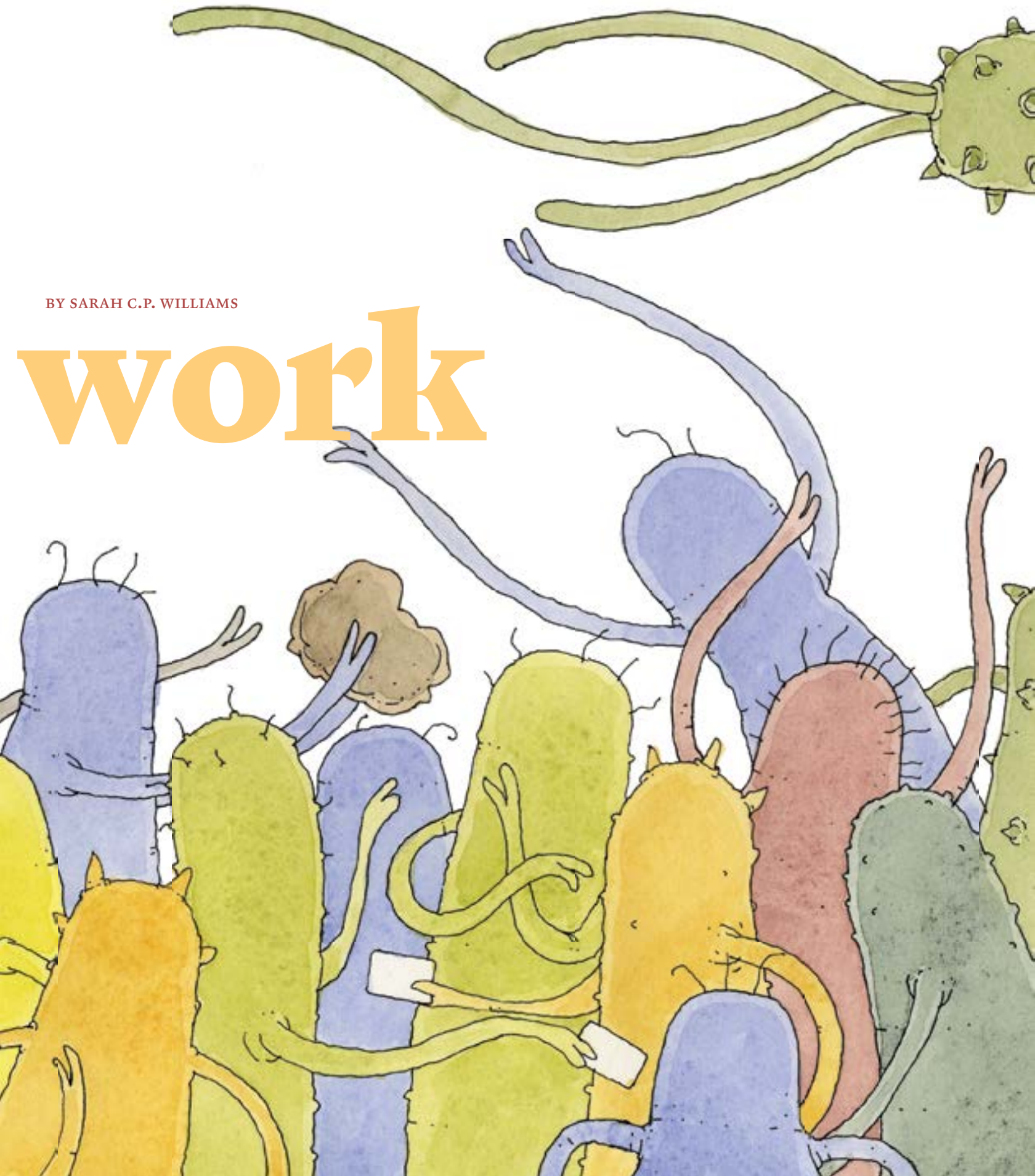


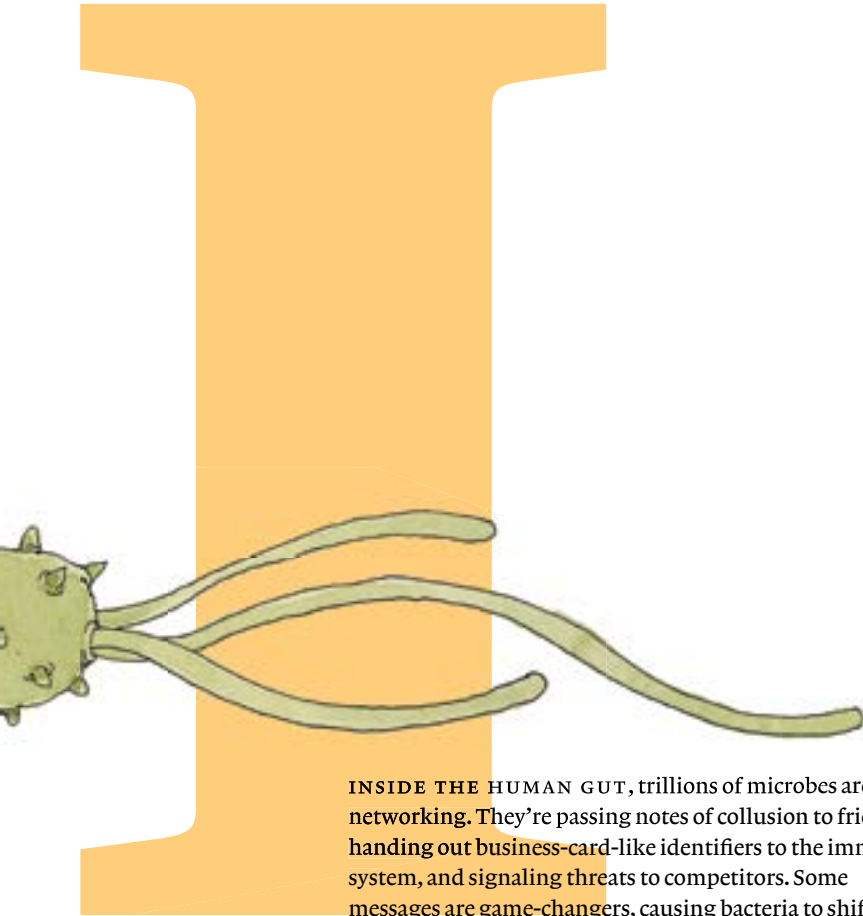
Microbial Social Net

Decoding bacterial chatter may clarify our relationship to their vast communities.

BY SARAH C.P. WILLIAMS

work





INSIDE THE HUMAN GUT, trillions of microbes are networking. They're passing notes of collusion to friends, handing out business-card-like identifiers to the immune system, and signaling threats to competitors. Some messages are game-changers, causing bacteria to shift their behavior, produce a new compound, or abandon the gut altogether. Other signals confirm that all is well.

In the late 1990s, scientists began to appreciate the extent and importance of the microbes that live in the human body—on the skin; in the mouth, stomach, and intestines; even inside ears and eyelids. Using modern genetic techniques, they began taking a census of the body's microbes, collectively called the microbiome, and discovered that they varied based on diet, health, and environment.

"At the beginning, we in the field had this idea that we'd be able to create one single reference data set for a healthy microbiome," says microbiologist David Relman of the Stanford University School of Medicine. "Now we've tempered our expectations as we've realized the complexity. There will be no one answer to what makes up a healthy microbiome."

Correlations have emerged between unique patterns of bacteria in the gut and everything from inflammatory bowel disease and obesity to asthma and depression. But the associations, even when based on large populations and strong data, do little to explain the mechanism by which changes in these microbial communities can alter human health.

"I think many of us are becoming a little bit frustrated with having correlations alone," says Akiko Iwasaki, an immunobiologist and newly named HHMI investigator at Yale University. "It ultimately doesn't prove anything."



Ruslan Medzhitov focuses on the immune system's response to invaders' subtle and not-so-subtle cues.

Researchers like Iwasaki are focusing on how bacteria and viruses in the gut communicate, and how they interact with each other, with the physical barriers of the body, and with the human immune system. Understanding both the structure of the microbial social networks and the nature of the messages reverberating around the gut, they say, are key to making connections between the microbiome and its effect on the human body. "What we're interested in now is the full spectrum of communication between the human host and the microbes we house," explains HHMI Investigator Ruslan Medzhitov, Iwasaki's husband and frequent collaborator at Yale.

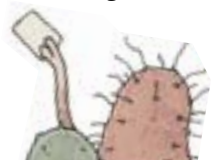
In the process of decoding the microbes' chatter, scientists have discovered a variety of new molecules—compounds that protect the gut's lining from the microbiome, chemical signals produced by gut bacteria to communicate with each other, and unique clusters of immune molecules whose job is to interact with the bacteria. Slowly, they're learning how to make sense of the language of the microbiome.

Passing Notes

The very idea that bacteria communicate with each other—rather than acting only as individuals—was discovered in experiments on the marine bacterium *Vibrio harveyi*. When a small throng of *V. harveyi* sloshes around in a glass of nutrients on a lab bench, the slurry is unremarkable at first. Over time, though, the mixture begins to glow as the bacteria multiply. In the late 1990s, HHMI Investigator Bonnie Bassler discovered that *V. harveyi* produce a molecule, AI-2, that the bacteria use to sense each other's presence. They luminesce only when they sense enough of the AI-2, indicating that they've reached a critical mass. Karina Xavier, who joined Bassler's lab as a postdoctoral researcher, wondered whether this so-called quorum sensing worked only within that bacterial species, or whether messages were also passed between species.

So Xavier mixed *V. harveyi* and *Escherichia coli* in a test tube and measured the glow. She found that the *V. harveyi* produced only 18 percent of the light they typically emit. The *E. coli* was slurping up the AI-2, so the *V. harveyi* bacteria never sensed the concentration required to initiate luminescence.

Xavier and Bassler went on to show that almost every type of bacteria—not just *V. harveyi* and *E. coli*—produces and consumes AI-2 at different levels. The AI-2 acts as a general "here I am" flag for bacteria. Communities of bacteria use levels of AI-2 not only to decide when to fluoresce, but also to coordinate more aggressive actions, like the production of antibiotics that kill competing bacteria. So by consuming the AI-2, *E. coli* and other bacteria can counter these tactics using the same strategy to protect themselves from offensive attacks.



“Once I figured out that this molecule mediated changes when you mix bacteria, I wanted to increase the number of species in my experiments,” says Xavier, now an HHMI international early career scientist at Gulbenkian Science Institute in Portugal. But when she mixed three, four, or five species of bacteria, the culture failed—one species would kill off the rest and the community would never reach a state of equilibrium suitable for studying AI-2.

When Xavier launched her lab in Portugal, she turned to a stable, multispecies bacterial home she didn’t have to create from scratch: the gut. “It was exactly what I needed to study the communication among many species.”

In the gut, Xavier hypothesizes, AI-2 is just as important as it is in the marine environment of *V. harveyi*. She believes that it’s one of the signals that bacteria use to launch, or sync, the production of other self-serving molecules, such as disease-causing virulence factors, antibiotics that target competing bacteria, and substances that suppress the immune system. A species of bacteria can coordinate such actions once levels of AI-2 have reached a threshold. The higher the level of the molecule being made, the greater the likelihood of its having an impact—whether that means spurring a change to the immune system, infecting the wall of the gut, or killing off a neighbor.

Xavier has developed ways to increase or decrease the amount of AI-2 in the mouse gut so she can observe how changes to the signal alter the makeup of the microbiome. Her results are forthcoming.

“I really think that cell-to-cell communication between bacteria is incredibly important when it comes to the homeostasis of the gut microbiota,” she says. There are likely to be as many types of messenger molecules as there are bacterial species in the gut, she adds. Some, like AI-2, are passed between many species, while others are unique to one or a few.

Immune System Messaging

As bacteria mingling in the gut use signals like AI-2 to decide when to change their behavior, they’re also communicating with the immune system of their human host. Typically, when a bacterium or a virus sneaks into a host, the immune system recognizes a physical component of the microbe—its cell wall or tail-like flagellum, for

example—as foreign. But this recognition isn’t sufficient to differentiate between pathogenic bacteria—those that cause disease and should be destroyed—and commensals—the harmless, or even beneficial, bacteria that should be left alone by the immune system. Particularly in the gut, pathogenic and commensal bacteria can appear outwardly identical. And a commensal bacterial species can even become pathogenic if conditions change. How does the immune system know?

Medzhitov likens the situation to a high-rise security system. “Detecting a person in a building does not necessarily mean they’re an intruder, since not all people are intruders,” Medzhitov says. “But if someone comes into the building through a window at night, then that might indicate the person is a burglar.”

The immune system could, for example, recognize behaviors such as the obvious destruction of human cells in the intestines as being a sign of a pathogen. But it also could detect more subtle behaviors, like the production of bacterial metabolites.

“There are certain metabolites that are only produced by certain types of bacteria, or bacteria under certain conditions,” explains Medzhitov. By detecting these unique end products, the immune system can sense which bacteria are there.

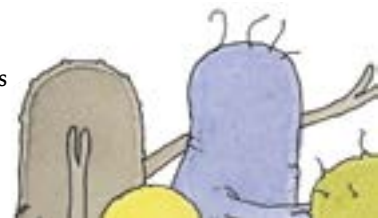
The immune system has adapted to recognize these byproducts as signals to act. One such metabolic product, butyrate, has been linked with bowel and digestive health; low levels of butyrate are a sign of colon problems. Associations like this—between signaling molecules or metabolites and human health—are likely to be more accurate than associations between particular bacteria and health, Medzhitov believes.

So what does the immune system do when it recognizes a particular metabolite or combination of metabolites in the gut?

“Some immune responses are like a sniper, others are like a nuclear bomb,” Medzhitov says. One pattern of metabolites, he explains, could be specific enough that the immune system can systematically pick out one particular bacterial species to kill off. Other patterns, though, might suggest a completely out-of-whack gut microbiome and elicit a massive wiping clean of the bacterial communities so they can be re-colonized in a more balanced way. In both cases, the bacterial byproducts signal a class of molecules called Toll-like receptors (TLRs), which Medzhitov discovered in 1997. TLRs act as a middle man in the body’s alarm system against intruders.



Katrina Xavier uses the mouse gut to observe interactions among bacterial species.



Not all messages produced by the microbiome will lead to an immune response, however, a fact that is critical to the body's ability to harbor the trillions of microbes. And Medzhitov has discovered that metabolites—and their interactions with TLRs—could be important to how commensal gut bacteria stay under the immune system's radar. Metabolites produced by some gut bacteria suppress TLRs rather than activate them, ensuring that the bacteria are not recognized as pathogenic.

The body keeps the microbiome balanced by using physical barriers as well. They help provide a safe environment—free of immune system attacks—for commensal bacteria to thrive in the human digestive system.

"If you have a hundred trillion bacteria in your gut, how do you keep them where you want them?" HHMI Investigator Lora Hooper says. "That's been a major question."

In 2011, Hooper, at the University of Texas Southwestern Medical Center, discovered a microbe-free zone along the edge of the gut. A protein called MyD88, she found, helps enforce the territory, turning on components of the immune system—such as TLRs—if bacteria enter.

This June, Hooper published results from a collaboration with HHMI Investigator Beth Levine, also at UT Southwestern. Levine studies autophagy, which enables cells to self-destruct by degrading their internal parts. The researchers discovered that if invading pathogenic bacteria reach the cells lining the intestines, these infected epithelial cells have a unique autophagic pathway that kicks in and destroys them, thus preventing the microbes from spreading. Like the enforcement of the microbe-free zone, the pathway requires MyD88. When Hooper engineered mice that lacked MyD88, or proteins vital to the autophagy process itself, more bacteria traveled out of the gut to other sites in the body.

"We're at a point now where we have lots of descriptive experiments confirming that the microbiome can affect the rest of the body," says Hooper. "But we now need to translate that into cause and effect."

By launching experiments focused on specific molecules, such as MyD88, that may play a role in this communication, Hooper aims to move beyond description and understand how molecular pathways function.

Inflammatory Remarks

While some researchers forge ahead with molecular biology approaches to decoding the cross-talk, others study the clinical implications of altering the microbiome. Their observations often lead right back to the biology of the microbiome-immune system interplay.

Immunologist Richard Flavell, an HHMI investigator at Yale School of Medicine, studies how multiprotein complexes called inflammasomes detect microbes when they invade the gut lining. In 2011, he and his

colleagues reported in *Cell* that the absence of a particular inflammasome known as NLRP6 led to a profound change in the composition of the gut microbiota. Many microbes increased or decreased in abundance, and some began invading the gut lining. Those changes, in turn, led to increased susceptibility to inflammatory bowel disease (IBD). These same alterations, they reported in *Nature* in 2012, also caused increased susceptibility to obesity, type 2 diabetes, and fatty-liver disease—factors associated with metabolic syndrome, a condition that affects one-third or more of the population in the United States and other developed countries.

Flavell also knew that prolonged IBD is a major risk factor for colorectal cancer. Since NLRP6 plays a leading role in causing IBD when the microbiome is out of balance, he wondered whether there might be a link between the inflammasome and colorectal cancer. He decided to test that out in mice.

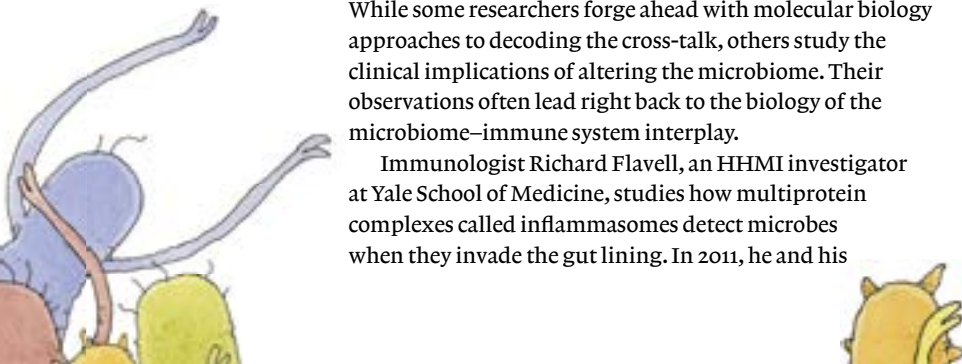
When mice with precancerous colon cells lacked NLRP6, the animals developed many more cancerous tumors than mice that had the protective inflammasome, according to a report by Flavell's team in the May 21, 2013, *Proceedings of the National Academy of Sciences*. Like MyD88, the inflammasome protects the gut lining from bacterial invasion. If a bacterium can get through these defenses and colonize the gut lining, the immune cells in the gut are stimulated to release a molecule called IL-6. The scientists found that IL-6 acts directly on colorectal cells to cause cell division. In the case of precancerous cells, a sudden signal to divide can be the push toward cancer. The increased cancer burden was transmissible to genetically normal mice as an infectious disease—through an imbalance in the microbiome.

"All these organisms interact with each other; they talk to each other," Flavell says. "Once we can understand better how these interactions lead to human diseases, we can start trying to prevent those diseases."

Of course, bacteria aren't the only microbes in that equation. At Yale, Iwasaki has discovered a link between multiple components of the inflammasome and how the body responds to the influenza virus. She's shown that mice without the full set of inflammasome molecules can't launch an effective fight against the flu. When Iwasaki



Lora Hooper studies the molecules that keep bacteria inside the gut, where they are less likely to cause trouble.



“What we’re interested in now is the full spectrum of communication between the human host and the microbes we house.”

—RUSLAN MEDZHITOV

went a step further and gave healthy mice a cocktail of antibiotics to wipe out their gut bacteria, these mice also were ineffective at fighting off the flu virus.

“What the bacteria seem to be doing is turning on genes that induce the inflammasome response,” Iwasaki says. So having a gut full of commensal bacteria helps the immune system remain alert, not just to protect the body from pathogenic bacteria, but to detect invading viruses. Those TLRs discovered by Medzhitov are also vital to this link: without TLRs, Iwasaki’s lab group found, the inflammasome was no longer activated.

Crowd Connections

The challenge with studying the microbiome is that—as Xavier discovered before she turned to mice—it’s hard to keep bacterial cultures in the same balance seen in the body, where they interact with immune molecules and the lining of the gut. And mice don’t always mimic human gut conditions accurately. Pellets of mice chow don’t go down the same as a salad or a burger and fries.

“It’s like trying to study tiger behavior by watching one pacing around in a cage at the zoo instead of studying one in the wild,” says Rob Knight, an HHMI early career scientist at the University of Colorado Boulder.

Knight is a project leader for an initiative called American Gut, which aims to genetically sequence the microbes found in fecal samples from 10,000 adults around the world. By July 2013, the effort had raised \$500,000

from more than 4,000 online donors, which Knight says is enough to start sequencing the microbiomes.

“This is very exciting because we now get to see what microbes look like out there in the wild,” he says, “rather than in a few carefully defined cohorts.”

In 2011, Knight published data showing that people could be classified, with 90 percent accuracy, as lean or obese based solely on their gut microbial populations. Of course, there are other ways of determining whether someone is obese, but such correlations—even if they don’t have immediate biological explanations—will lead to many early clinical utilities of the microbiome, Knight says. By testing particular aspects of the microbiome, clinicians may be able to predict who is at risk for a condition, how a disease will progress, or whether a particular diet or drug will be effective for someone.

Knight was part of a team of scientists who analyzed the microbiomes of 317 pairs of young twins in Malawi, a country in southeast Africa that is among the poorest and least developed in the world. The microbiomes, which differed even between twins, indicated which children were plagued by kwashiorkor, a form of protein-deficient malnutrition, according to their February 1, 2013, paper in *Science*. The microbiomes also predicted whose symptoms—including an enlarged liver and skin problems—would be reversed by a particular nutritional supplement.

“There’s a difference between what information can be useful and what information provides us with a complete understanding of the science,” says Knight. “And I think it’s important for the field to not worry about whether we have every bit of the microbiome system completely connected before we move toward applications.”

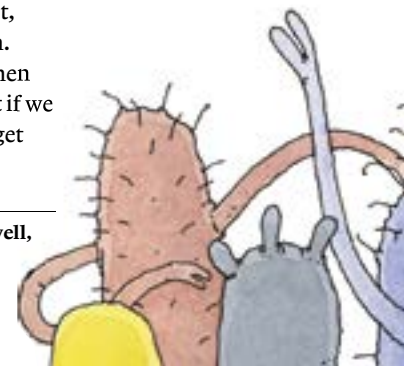
Knight expects that tests to diagnose patients or shape their treatment plans will become available relatively soon. Creating personalized microbe-based treatments to change someone’s microbiome will take longer, he says.

To move toward personalized treatments, the field will need both correlative studies that link microbiome states to health—like Flavell’s research on metabolic syndrome and cancer and Knight’s on malnutrition—and mechanistic studies on how this connection plays out at a molecular level, Knight says. Each piece of research informs the rest of the field and helps shape new directions for researchers. “This is a time when the bench scientist has got to be talking to the clinician, who has got to be talking to the chemist, who has got to be talking to the ecologist,” says Relman. “If we just have a willy-nilly collection of lots of data, then understanding the microbiome will be slow going. But if we collaborate to design thoughtful experiments, we can get data that answers questions.” ■

For more information: See the perspective by Richard Flavell, “Hypothesis: Unknown,” on page 30.



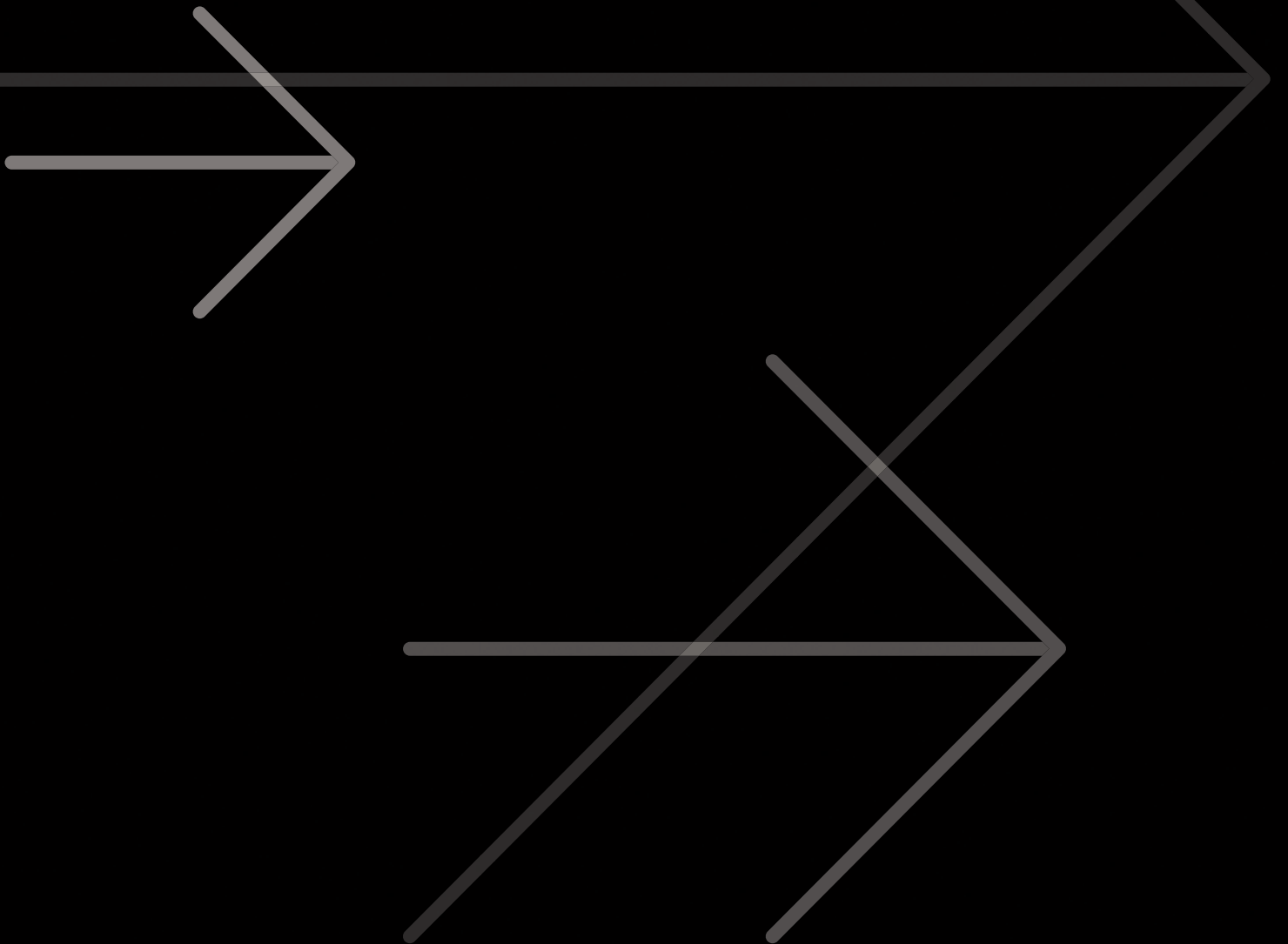
With help from the public, Rob Knight will sequence the microbiomes of thousands of people.



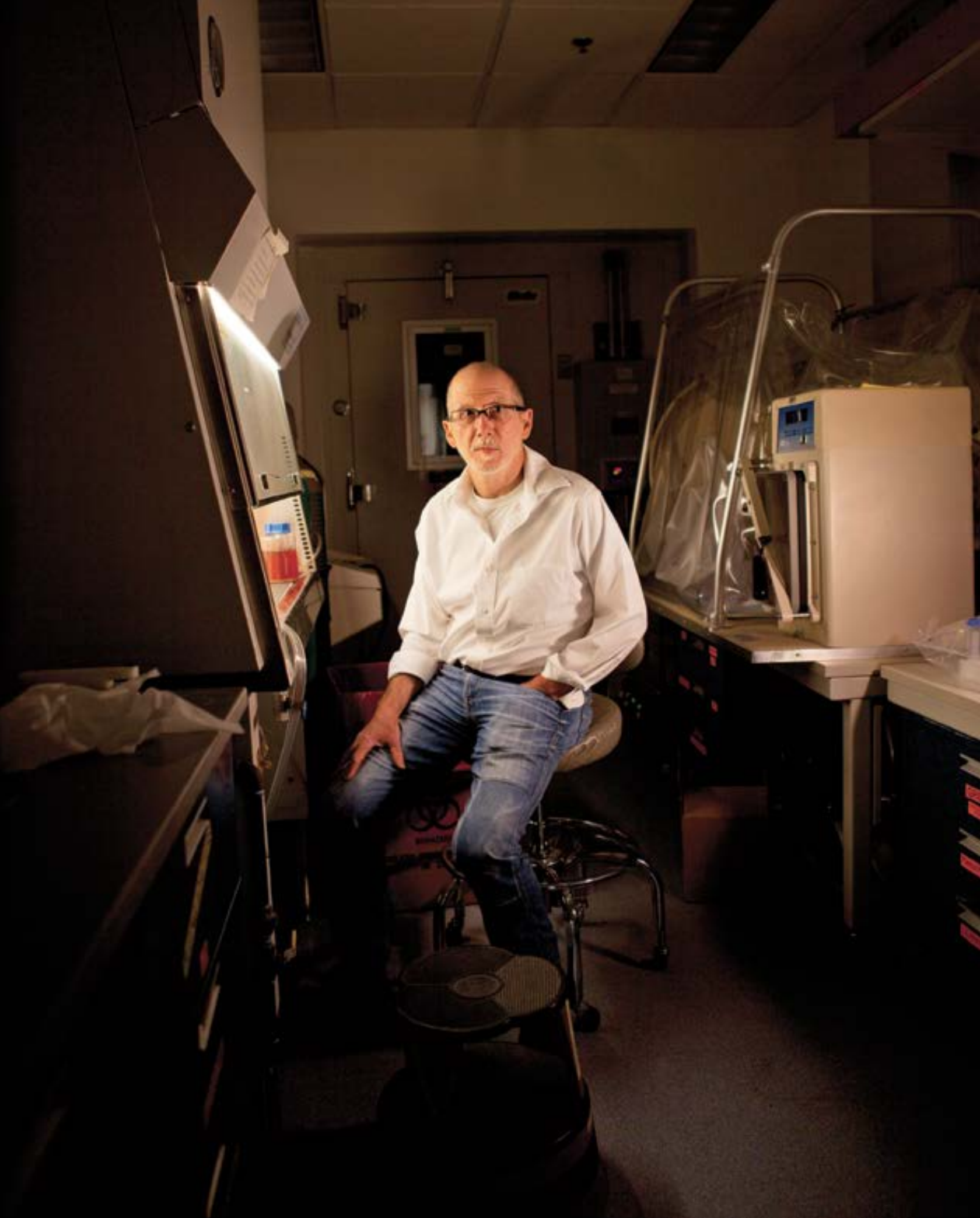
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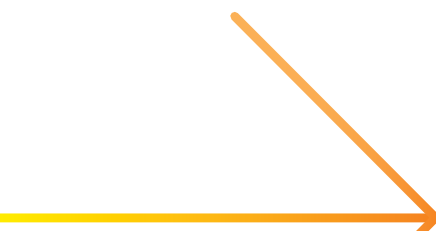
Bert Vogelstein is on
a mission to change
the outlook for people
with cancer.

BY SARAH GOFORTH



PHOTOGRAPHY BY GRAHAM MACINDOE





ON FRIDAY AFTERNOONS, unarmed visitors to Bert Vogelstein's laboratory enter at their own risk. Vogelstein and four other faculty members at Johns Hopkins Medical School in Baltimore gather with two dozen trainees in the lab's central space. Presentations begin with a mandatory joke and end, if they dare go long, with rapid-fire assault by Nerf gun. No one is safe from friendly fire.

Vogelstein, a lean 64-year-old, is equally merciless when the time comes for Q&A—probing trainees when a premise lacks sufficient evidence or a proposal falls short of actionable. For while the mood in the lab is light, the subject matter at hand is serious. The real battle here is against cancer, the arsenal a broad range of disciplines and experimental approaches.

Having spent three decades uncovering the molecular pathways that allow tumor cells to multiply and spread, Vogelstein is now focused on using that knowledge to save lives. To that end, he and his faculty colleagues—with whom he jointly manages the lab—are very deliberate when it comes to selecting new lab members and creating an environment that brings out their best work.

“People have to care more about the final goal than individual credit,” says Vogelstein of the team, which includes medical students, interns, and research trainees in disciplines from chemistry to pathology to computational biology. “This is the rule in industry, which long ago recognized that you need people with diverse talents working on projects together, but it's uncommon in academia. We try to be our own harshest critics. We also try to have fun.”

In addition to lab meeting antics and friendly billiards battles, the lab shares a Delaware beach house that members reserve in weekly slots. For years the lab even had its own band, called Wild Type, with Vogelstein at the keyboard, laboratory codirector Ken Kinzler on drums, and vocals delivered by a rotating lineup of musical postdocs.

The work-hard, play-hard model clearly works for this team. Studies from Vogelstein's lab on the genetic roots of cancer are among the most widely cited in all of science. Through painstaking genomic analyses of human tumors, he and his team demonstrated that cancers usually result from the slow accumulation of key mutations over years. Their work formed a paradigm upon which much of modern cancer research is based.

The group was also the first to decipher the molecular mechanisms underpinning a common human cancer. Working with colorectal tumors, they helped prove the existence of tumor suppressor genes, which prevent the growth of tumors by keeping cell division in check. Mutations in the genes have been implicated in many different types of cancer malignancies.

Biology for Mankind

A Hopkins-trained MD and HHMI investigator since 1995, Vogelstein, typically dressed in jeans and a crisp white button-down, is director of the Ludwig Center for Cancer Genetics and Therapeutics at Hopkins. His work has earned him multiple prestigious awards, and he was one of 11 to win the inaugural Breakthrough Prize in Life Sciences established in 2013 by Facebook founder Mark Zuckerberg and others. You wouldn't know it, looking at Vogelstein's orderly office, where the walls are mostly bare except for family photos and a postcard depicting a famous Jack Russell terrier, with whom Vogelstein shares a nickname (to his three grandchildren, he is “Uggie”).

“He hasn't sought out any of that attention,” says Vogelstein's colleague Ken Kinzler. Kinzler, who first joined the lab in 1983 as a graduate student and is now among the group that manages it, was trained in toxicology and pharmacology, and he shares Vogelstein's sense of purpose. “We're not here because we love biology,” he says. “We're here because we love biology as it serves mankind.”

That ethos has taken the lab in a practical new direction in recent years. Despite great gains in understanding the biological basis of cancer, long-term survival rates for most cancers are little different than they were in the 1970s, when Vogelstein entered the field. There are exceptions for a handful of cancer types where new treatments have been particularly successful or early diagnostic tests have allowed clinicians to find and treat tumors before they spread. But by and large, says Vogelstein, the outlook for cancer patients remains unnecessarily grim.

“The situation has dramatically changed in our understanding of cancer. It wouldn't be accurate to say, however, that the situation has dramatically changed in terms of what we can *do* for a patient with cancer,” says Vogelstein, who began his career as a pediatrician with a special interest in oncology and transitioned to full-time research out of frustration that he could do so little for his patients. “We clearly don't understand the entire disease, but at what point do you understand enough to spend your time trying to exploit that knowledge? Over the last decade we decided that time has come.”

Roughly two-thirds of the lab's energy, he estimates, is now devoted to developing early diagnostic tools. A quarter goes to creating new treatments; the rest is spent on questions in basic biology the group deems most relevant to saving lives.

A Matter of Time

The eldest of five children in a close-knit Jewish family, Bert Vogelstein's capacity for academic success was not always obvious. As a child, he was so often absent from school that he was nearly expelled—twice—for underperformance.

“Sometime in middle school, I decided I wasn't learning as much as I wanted,” he says. Most days his father, an attorney, would drop his son off at school on his way to work. Instead of heading to class, Vogelstein

would walk to the Enoch Pratt Public Library, a downtown Baltimore landmark. He would spend all day reading—science fiction, histories, biographies, whatever captured his interest. “I didn’t know I was doing some delinquent thing. I was just learning in my own way,” he says.

Today, Vogelstein prefers reading the scientific literature over attending conferences. Unlike most senior scientists, he rarely travels for work, preferring to generate ideas within his lab and a small circle of frequent collaborators. Part of that is simple time management. Vogelstein’s higher priorities include meeting with his trainees at least weekly, designing experiments, and working at the bench—sometimes to an uncommon degree. (Soon after joining the lab, postdoctoral researcher Margaret Hoang was surprised to learn that when the lab ran out of a glycerol buffer used to dye DNA in many experiments, Vogelstein mixed the new batches himself. “I asked the technicians where I could get more buffer, and they were like, ‘Oh, Bert always makes it.’ Imagine my nerves, asking my new boss to make my buffer,” she remembers with a laugh.)

By the time he entered the University of Pennsylvania as an undergraduate, Vogelstein was serious about his studies. He majored in mathematics and excelled at it, briefly pursuing a master’s degree before he was lured into medicine for the opportunity to improve people’s lives. As an intern and resident in pediatric oncology, however, he grew increasingly frustrated with how little he knew about his young patients’ disease. “In the 1970s, cancer was a black box,” he says. “It just seemed to come from outer space and hit people without any rhyme or reason. It was bleak.”

Wanting to use his time for maximum impact, he redirected his attention to research. At the time there were several prevailing hypotheses explaining the origins of cancer: Scientists knew cancer cells were variants of normal cells that had gone rogue, but some thought the cause was a defect in the immune system or an infectious agent. Swayed by evidence suggesting that exposure to environmental contaminants or radiation correlated with cancer incidence, Vogelstein was among a minority of scientists who suspected the roots were in genetic alterations. But he could only prove that by finding the affected genes and showing that, in cancer cells, they were mutated. In 1978, Vogelstein applied for a junior position in a new cancer research program at Hopkins, under the supervision of Donald Coffey, a scientist known for questioning dogma and encouraging his trainees to do the same.

“From the very beginning, it was important to me to study human tumors.”

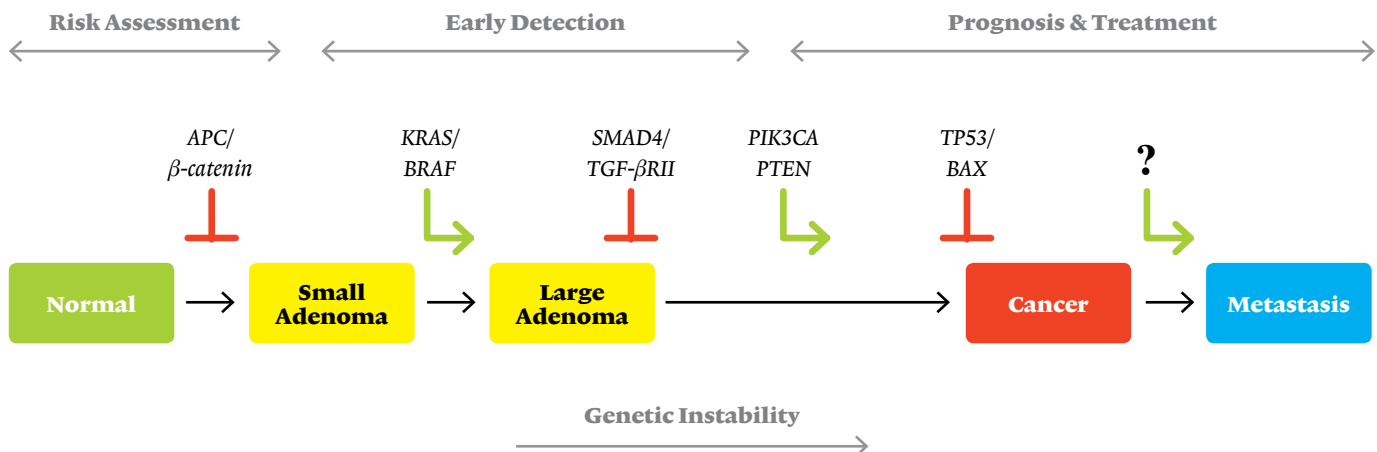
—BERT VOGELSTEIN

“From the very beginning it was important to me to study human tumors,” Vogelstein says. Many senior scientists discouraged him from taking that approach, arguing that it was impossible to conduct effective experiments without relying on animal models. “I thought that, even though your ability to do some types of experiments is limited, if you choose what to examine carefully and get the right kinds of tissue samples, you could get at things that you couldn’t learn any other way,” says Vogelstein. He joined a group of young scientists at Hopkins who had gained access to colon tumor samples in various stages of progression and began looking for disease-causing genes.

Laying a Foundation

In 1989, Vogelstein and a handful of trainees showed that a gene called *p53* was mutated in colorectal cancers and a large number of other tumors. Though several groups had identified the *p53* protein 10 years earlier, its gene was not known to be important in human cancer. Moreover, Vogelstein’s team found that *p53* was not an oncogene, or tumor promoter, as had been thought. Rather, it is a tumor suppressor whose protective function goes haywire in the mutated form. That

The Long Path from Normal Tissue to Colon Cancer’s Spread





Bert Vogelstein hopes his work will lead to simple blood or urine tests to detect early genetic changes in cancer.

discovery, and his group's subsequent elucidation of the biochemical mechanisms behind how p53 works, led to a surge of research that continues today.

Over the next several years, he and Kinzler discovered a string of other colon cancer genes, developing a new model for cancer progression by examining human tumors for mutations. In the following years, the group uncovered dozens of genes and mutations that play a role in other cancers, including breast and pancreatic. Their lab, and its influence, grew quickly.

By the turn of the millennium, their ambitions had grown too. In 2005, Vogelstein and Kinzler set out to sequence the first "cancer genome" in 22 breast and colorectal tumor samples. To find the most important genes, they limited their search to the areas of the genome thought to harbor most disease-causing mutations. These are the protein-coding regions, or exomes, of each tumor's DNA. But even this focused approach would still require hundreds of thousands of experiments to extract and amplify these 13,000 genes. Most of their peers thought the goal was far too difficult for a single lab to undertake,

says Charles Sawyers, an HHMI investigator at the Memorial Sloan-Kettering Cancer Center. This being before the era of high-throughput sequencing, Vogelstein's team would need to accomplish the work by brute force and find millions of dollars to fund it.

"Everyone knew we needed to know the sequence of all the genes in cancer cells," says Sawyers. But even the most successful labs were targeting 100 genes or fewer at a time, for practical and financial reasons. "That was a huge compromise because you didn't know which genes were most important to sequence, so you had to hedge your bets. Bert wasn't willing to do that," says Sawyers. "It was the most audacious thing to take on as an individual lab, but he wasn't going to let anyone stop him from doing the right experiment." To supplement his funding from HHMI, Vogelstein raised money from other philanthropic sources and formed a partnership with a sequencing company to industrialize the process.

In 2006, his group published the first genome-wide study of breast and colorectal cancers in a landmark paper in the journal *Science*. The analysis revealed several new cancer genes and many that were already



known, in a mind-boggling array of patterns that varied from tumor to tumor. They later tackled a form of brain cancer called glioblastoma, uncovering cancer-causing mutations in the gene *IDH1* that a much larger government-run sequencing project had missed.

The lab's approach to genomic analysis was given a boost by new, faster computing tools to sequence cancer DNA that emerged over the next several years. With the help of that new technology, his team completed 88 of the first 100 whole exomic sequences of human cancers. These detailed maps were an important step toward personalized medicine, in which clinicians look for the characteristics of each person's cancer and tailor therapies to guide their treatment.

Breaking Rules and Barriers

In a long-standing collaboration with Martin Nowak of Harvard University, Vogelstein is using mathematics to predict the most effective tailored treatments for multiple forms of cancer. Most recently, Nowak and Vogelstein used a mathematical model of melanoma to understand how resistance emerges to a commonly prescribed drug, vemurafenib.

Their work, published in June 2013, in the journal *eLife*, showed that targeted therapies are often only effective when used in combination with others. The medical community faces many barriers—mostly financial or imposed by the pharmaceutical industry—to taking that approach, says Sawyers, a senior editor at the journal. “Bert isn’t so much someone who wants to march on Washington, but the *eLife* paper provides unbelievable ammunition for those of us who are convinced of the value of combination therapies,” he says.

“That’s the challenge, to take this incredible intellectual triumph of understanding this group of diseases and actually do something about them in people,” says Vogelstein. “And that is starting to happen. But it will take time and it will take a lot of effort.”

Increasingly, his time is devoted to creating diagnostic tools that detect cancer mutations in the blood before symptoms appear. In a collaboration with Sawyers, who helped develop the targeted therapeutic Gleevec that changed the outlook for patients with chronic myeloid leukemia, Vogelstein is designing screens for prostate cancer that detect cancer-causing mutations in DNA from blood plasma. He is developing similar assays to detect pancreatic and colorectal cancers.

The dream, he says, would be for cancer screening to be a routine part of annual medical exams. A doctor would order standard cancer biomarker analysis along with DNA sequence screening for genetic alterations in blood or urine samples. The trick is to design tests that are inexpensive and easy to administer.

“There are many reasons I’m excited about early detection,” says Vogelstein. “It’s not just reducing deaths by early detection or prevention, although that is of course important. It’s the suffering and the misery that you can preclude when you detect somebody’s cancer early enough that they can be cured by surgery alone.”

If transforming his lab from a basic research powerhouse to a more clinically focused enterprise was an unconventional move, it didn’t surprise anyone who knew him well. “Many rules exist just because that’s the way it’s always been. My dad was never a fan of that,” says R. Jacob Vogelstein, the eldest of Bert Vogelstein’s three children and a neuroscientist at the Johns Hopkins Applied Physics Laboratory. “We were encouraged to ask, ‘Why is it like this?’ If there was no good reason, we didn’t follow the rule.” For instance, on late-night trips to the grocery store, Vogelstein would let his kids ride around the store in motorized carts intended for the disabled. “All a kid wants to do is ride that cart,” says Jacob, “and the answer is, you can’t because it’s against the rules. But when there are six carts and absolutely no one in the store, my dad had no qualms. Why shouldn’t I have my own motorized cart?”

Had Vogelstein been born with a stronger tie to tradition, he might have become a rabbi—the 14th generation of such on his mother’s side of the family.

“I broke the trend, but it’s still in my genes,” he says with a wry smile. “There’s an old expression: If you have two rabbis you have three opinions. They can argue against themselves if they have to. And one of the things that is critical in science, I think, is to not accept the status quo. We try to teach that to our trainees, to think Talmudically; to try to prove themselves wrong.” ■



DYCHE MULLINS



JOHANNES C. WALTER



HOPHI HOEKSTRA



ARDEM PATAPOUTIAN

Explo

After an intense competition, 27 scientists had the right stuff to be selected as HHMI investigators. Where will their curiosity take them?

BY JENNIFER MICHALOWSKI



CHUAN HE



DAVID REICH



AKIKO IWASAKI



RACHEL I. WILSON



PETER W. REDDIEN



RUSSELL E. VANCE



KARL DEISSEROTH



AVIV REGEV



TIRIN MOORE



CHRISTOPHER D. LIMA



NICOLE KING



EVGENY NUDLER



VAMSI K. MOOTHA

Directors



PETER BAUMANN



MICHAEL A. DYER



HARMIT S. MALIK



MARC R. FREEMAN



ADAM E. COHEN



MICHAEL S. BRAINARD



JEAN-LAURENT CASANOVA



YUKIKO YAMASHITA



NEIL HUNTER



MICHAEL RAPE

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ILLUSTRATION BY GLUEKIT



“I get a real kick out of applying physical principles that have not been fully understood or applied before, to find new ways to visualize what’s going on in biological systems.”

—ADAM COHEN

PEOPLE THINK SCIENCE is linear, but there are so many ways to approach a problem,” says biophysicist Adam Cohen. “Creativity is the key to everything.”

That is to say, the road to discovery may depend less on technical expertise than on a researcher’s ability to choose the right questions, design an elegant experiment, follow unexpected leads, and turn failures into opportunities. HHMI scientists are known for these qualities, and in May, the Institute selected Cohen and 26 other highly creative scientists to join the ranks as new investigators.

Chosen from among 1,155 applicants and based at 19 host institutions nationwide, the new investigators are exploring a broad swath of biology, asking how songbirds learn to sing, how cells sense mechanical forces, and what we can learn about human evolution from ancient DNA, among other questions. Once officially on board, these new HHMI investigators will have the freedom to stretch their imaginations in unexpected directions.

It’s impossible to know where these scientists’ instincts will take them, but HHMI is confidently investing \$150 million in the group over the next five years to support their exploration.

Bioelectrical Phenomena

When **Adam Cohen** reversed his thinking about how microorganisms carry out a life-sustaining transformation, he turned a looming research failure into a boon for the neuroscience community. Cohen had been puzzling about a member of the protein family known as microbial rhodopsin. Single-celled microorganisms called archae living in the Dead Sea use this form of rhodopsin like a personal photovoltaic cell to convert sunlight into electricity. His research group at Harvard University spent two years trying to figure out the mechanism of the reaction, but they didn’t get far. “I learned a lot, but it wasn’t really anything people didn’t already know,” he recalls. It was time to abandon the pursuit, but Cohen was determined to find some way to salvage the knowledge and rhodopsin-specific research tools he had developed.

Bacteria mastered the conversion of sunlight into energy long ago, but nature seems to have found the reverse reaction—converting biological voltage changes into light—less useful. That’s frustrated neuroscientists, because neurons communicate with one another through electrical impulses, and researchers have longed for a way to directly visualize that activity. Cohen, who says he was vaguely aware of this need, wondered if it might be possible to coax the rhodopsin protein into running in reverse.

At the simplest level, the task was easier than Cohen expected. “It turns out that without any modifications, the protein is a little bit fluorescent, and the fluorescence is sensitive to membrane voltage,” he says. But “that’s entirely a side effect of the protein’s natural role, so there’s been no selective pressure to make the effect large or good.” That’s where Cohen came in.

“I get a real kick out of applying physical principles that have not been fully understood or applied before, to find new ways to visualize what’s going on in biological systems,” Cohen says. He has already distributed his rhodopsin-based voltage indicator to more than 100 labs, and the chemists and physicists in his lab are quickly learning how to work with living cells so they can investigate bioelectrical phenomena.

Some will use rhodopsin to compare neuronal activity in induced pluripotent stem cells derived from healthy patients to activity in cells from people with neurodegenerative disease; others are growing stem cell-derived cardiac cells that pulse in a dish, generating a flash of light from the voltage indicator with each beat. Now, Cohen's team is trying to make the protein more useful as a research tool, engineering it to be brighter, more sensitive, or less likely to perturb cells. The more broadly applicable his tools are, the better, he says.



Secrets of the Past

Like Cohen, **Nicole King**, an evolutionary biologist at the University of California, Berkeley, has developed a tool whose full potential she intends to exploit—again, from a microscopic organism with some extraordinary talents. Choanoflagellates float and flit about as single cells in oceans, rivers, streams, and puddles—pretty much anywhere there's water. They can also divide and cling together, balling up to form multicellular colonies that resemble embryos of some animals. To King, the process is reminiscent of the kind of cellular cooperation that must have happened when the first multicellular animals evolved from their single-celled ancestors more than 600 million years ago.

King first learned of choanoflagellates as a graduate student at Harvard University, where her curiosity about developmental biology brought her to work in the labs of microbiologist Richard Losick, an HHMI professor, and paleontologist Andrew Knoll. Microscopists discovered the organisms in the 1840s and proposed that they might be closely related to animals, but molecular biologists completely ignored the simple organisms, according to King. She was convinced that choanoflagellates could unlock important secrets of the past, but there were no genetic tools to study them. So she took it upon herself to develop the tools.

For her postdoctoral research, she joined the lab of HHMI Investigator Sean B. Carroll at the University of Wisconsin–Madison, who was intrigued by King's unconventional and ambitious proposal. There were no choanoflagellate experts to turn to for advice, so King first spent time in the lab of an “old-school protistologist” at the ATCC, a nonprofit biological resource center in Virginia, to learn how to grow and care for related organisms. Fortuitously, marine ecologists visiting the ATCC happened across some colony-forming choanoflagellates in their water samples, so King packed them up and took them back to Madison—where they immediately stopped forming colonies. “I would look at these cultures day and night, and do various things to them hoping they would form colonies,” she says. “They wouldn't. Actually, they would form a few colonies sporadically—just often enough to break my heart.”

King was staking her research program on her ability to study colony formation in this finicky little protist, so eventually she decided she needed a different approach. She would sequence the genome of the organism, compare it to the genomes of better-studied organisms, and see

what she learned. Because choanoflagellates are grown in the presence of prey bacteria, she and her lab group at Berkeley first treated her cultures with antibiotics to reduce the amount of bacterial DNA. “Suddenly and unexpectedly,” she says, “we observed tons of colony formation.”

The antibiotics altered the community of bacteria coexisting with her choanoflagellates, killing off one species and giving another an opportunity to thrive. Boosting the population of that second species triggered colony formation. King was thrilled. Not only did she now have a straightforward way to coax choanoflagellates into colonies, she also had an easy-to-manipulate system for studying how bacteria influence the biology of other organisms. “We know that the first animals evolved in a sea of bacteria,” she says. “So there's this exciting idea that multicellularity might be a response to chemical cues produced by bacteria.” At the same time, her lab's recent discovery that bacteria isolated from the guts of mice and humans also have a colony-promoting effect on choanoflagellates has King planning a new line of research on how microbes influence animal biology.



Search for Truth

The origins of **Johannes Walter's** research program were no less compelling, but somewhat less deliberate. As an undergraduate at the University of California, Berkeley, Walter was energized by discussions in his art history and

literature classes. But he met with an unexpected struggle. “I didn't realize that in the humanities, the idea is to put forth a reasonable argument and then defend it. I thought you were supposed to find an ultimate truth,” he says. “So I'd get halfway through writing a paper and see a way someone could make a counter argument to my idea, and I'd have to start over. I'd be up all night.”

Meanwhile, his quest for truth aligned perfectly with the culture Walter found on the science side of campus, so he focused his energy there and earned a biochemistry degree. Today, Walter's lab group at Harvard Medical School is searching for answers about how cells repair damaged DNA. DNA repair is a crucial response to toxins and environmental insults, and to errors introduced during routine cellular processes. With errors of many types lurking within a long and winding DNA code, cells need an assortment of tools to find and correct them to maintain the integrity of their genome.

Contemplating the orchestration demanded by that vigilance is more absorbing than Walter ever imagined, he says, but it's a field he stumbled into. In 2006, he was setting up biochemical experiments to investigate the enzyme responsible for unzipping a DNA molecule's twisted strands, exposing the information inside so that replication can begin. To investigate how the enzyme, a helicase, traveled along the double helix, he created a physical obstruction by linking the two DNA strands together—reasoning that if the circular enzyme tried to slide along one strand, it would collide with the crosslink and stall, unable to continue. Walter's team added the modified DNA to an extract from frog cells that contained the molecules necessary for DNA replication, and waited to see how the helicase would handle the obstacle. To their surprise, although the helicase stalled temporarily, it eventually moved forward.

The researchers learned that the frog extract contained repair proteins that removed the obstacle, known as an interstrand crosslink, enabling the replication machinery to proceed. Researchers knew that cells repair these lesions; when they don't—as occurs in patients with the inherited disease known as Fanconi anemia—cancer often develops. But no one had been able to recreate interstrand crosslink repair in a simple, easy-to-manipulate system.

Walter saw this unexpected result as an opportunity to investigate the process more deeply, and has since worked out the details of how the helicase reorganizes itself after it binds DNA, and how swapping of DNA between chromosome strands contributes to the repair. Further, his team has identified the specific repair defect responsible for Fanconi anemia. With this system, as well as a technique he devised for monitoring individual DNA molecules during replication, Walter plans a much more comprehensive description of DNA repair mechanisms, which he says will lay the groundwork for rational cancer therapies.

An Original

Yukiko Yamashita grew up in Japan with a father who valued originality above all else. He was eccentric, she says, but he instilled in her an untempered imagination that has led her, more than once, to discoveries that have surprised her colleagues and changed the way biologists think about how stem cells divide.

“Your conclusions shouldn’t be influenced by anyone else,” her father taught her. “You have to come up with your own uniqueness.” Today, when she thinks about a scientific problem, she says, “I don’t care what people in the field are proposing. It’s not that I think they’re wrong—they’re probably right. But I don’t start from that place.”

As a graduate student in biophysics at Kyoto University in Japan, Yamashita loved experimenting, but struggled because she had no female role models. Her motivation waned. It wasn’t until she began a postdoctoral position in Margaret Fuller’s lab at Stanford University that Yamashita found herself in a supportive environment where she could let her imagination run wild. “I could feel my cells breathing,” she recalls.

She turned her attention to the asymmetric way in which stem cells divide, generating one cell that remains a stem cell and another that becomes more specialized. Working with fruit fly cells, she showed that the fate of stem cells after division depends on inheritance of a cell structure called the centrosome. The centrosome works with components of the cell skeleton to ensure that daughter cells receive the appropriate allotment of chromosomes when a cell divides. A second centrosome is produced prior to cell division, and Yamashita discovered that the cell inheriting the mother cell’s original copy remains a stem cell. The daughter cell that receives the duplicate will differentiate into a more specialized cell. Other labs went on to show that this surprising mechanism also regulates asymmetry in cells from other organisms.


In her lab at the University of Michigan, Yamashita remains focused on asymmetric cell division, but recent findings have her thinking about the phenomenon much more broadly. “We used to think that asymmetry is a special case,” she says, explaining that even for stem cells, divisions that give rise to two identical cells are thought to be the norm, and only under certain circumstances does the balance shift. “But I’m starting to think it is inevitable. Every cell division is asymmetric, whether the cells mean to use that asymmetry or not.” That shift in her view comes from the recent discovery in her lab that every time a stem cell divides, there is a strong bias as to which cell inherits the mother cell’s original X and Y chromosomes.

“It completely flipped my thinking,” she says. The generally accepted model is that cells activate certain signaling to trigger asymmetric division. “But if every single cell division is asymmetric, when you have to make it equal, what do you have to do?” Finding out, she says, could take her a long way toward understanding how an organism develops from a single cell.



“I don’t care what people in the field are proposing. It’s not that I think they are wrong—they’re probably right. But I don’t start from that place.”

—YUKIKO YAMASHITA

 Go online to learn more about these 27 scientists and the competition selection process. www.hhmi.org/bulletin/fall-2013



Shifting His Gaze

Tirin Moore grew up in a family that embraced a broad curiosity. He says his father, a clerk at the *San Francisco Chronicle* who read voraciously and taught himself hieroglyphics, electronics, and computer programming, instilled in him some of the core qualities that fuel his research. “I learned from him the fun of asking important questions, even if you weren’t going to use the answer for any particular purpose,” Moore says. “We’d have long discussions about all sorts of things, including the brain—or at least our version of what we thought the brain was. It was all just guesswork and talk,” he says, but the casual debates captured his imagination.

When he was exposed to the foundations of neuroscience for the first time as an undergraduate at California State University, Chico, everything was exciting and new. He was suddenly equipped to ask the right questions. “It was incredible to be able to begin to explore,” he recalls.

Since then, Moore has kept his curiosity focused on the fundamental problem of how the brain uses sensory information. In his lab at Stanford University, he wants to understand the neural circuitry that enables us to focus our attention and ignore irrelevant information, as we do when we tune out others’ chatter in a crowded room or ignore visual distractions to focus more keenly on an object of interest. “There’s a huge bottleneck of information that comes in from the senses,” he says. “So what determines which information gets processed completely, and remembered, and acted upon?”

When he began to investigate how the brain integrates visual information with movement, Moore discovered that the motor-related neurons that control eye movements—shifting the gaze four or five times a second and smearing the visual scene across the retina each time—actually help focus attention. Experiments with rhesus monkeys have shown that, before the gaze shifts to a target of interest, neurons in the brain’s frontal eye field fire more strongly. These signals serve to produce the movement and to enhance the strength of sensory information in the visual cortex corresponding to the visual target. Importantly, Moore says, the enhancement occurs even when movements are planned, but not executed.

When Moore became an HHMI early career scientist in 2009, he saw the appointment as an opportunity to deepen his research question. He devised experiments that led him to the discovery that mechanisms related to simply planning a gaze shift to a visual target, without actually moving the eyes, is enough to improve attention and sensory processing in the visual cortex. “When you prepare an eye movement to a location every few hundred milliseconds, you’re mustering up information about the target to help plan the movement,” he explains. “When you do that, you are necessarily filtering out information about the location—and that filtering really is attention.”

Moore has already identified some of the neurons and circuits involved in this cognitive function, but there’s still a lot to learn about the neural circuitry that controls attention—including what goes wrong when it fails, such as in people with attention deficit disorders. “There’s still a lot of low-hanging fruit that we can pick with some elegant experiments and a lot of hard work,” Moore says. “It’s very exciting that we can now go after them. I can’t wait.” ■

2013 HHMI Investigator Competition Winners

Peter Baumann, PhD

HHMI Early Career Scientist
Stowers Institute for Medical Research

Michael S. Brainard, PhD

University of California,
San Francisco

Jean-Laurent Casanova, MD, PhD

The Rockefeller University

Adam E. Cohen, PhD

Harvard University

Karl Deisseroth, MD, PhD

HHMI Early Career Scientist
Stanford University

Michael A. Dyer, PhD

HHMI Early Career Scientist
St. Jude Children’s Research Hospital

Marc R. Freeman, PhD

HHMI Early Career Scientist
University of Massachusetts Medical School

Chuan He, PhD

University of Chicago

Hopi Hoekstra, PhD

Harvard University

Neil Hunter, PhD

HHMI Early Career Scientist
University of California, Davis

Akiko Iwasaki, PhD

Yale University

Nicole King, PhD

University of California,
Berkeley

Christopher D. Lima, PhD

Memorial Sloan-Kettering
Cancer Center

Harmitt S. Malik, PhD

HHMI Early Career Scientist
Fred Hutchinson Cancer
Research Center

Tirin Moore, PhD

HHMI Early Career Scientist
Stanford University

Vamsi K. Mootha, MD

Massachusetts General Hospital

Dyche Mullins, PhD

University of California,
San Francisco

Evgeny Nudler, PhD

New York University

Ardem Patapoutian, PhD

The Scripps Research Institute

Michael Rape, PhD

University of California,
Berkeley

Peter W. Reddien, PhD

HHMI Early Career Scientist
Massachusetts Institute of
Technology

Aviv Regev, PhD

HHMI Early Career Scientist
Massachusetts Institute of
Technology

David Reich, PhD

Harvard Medical School

Russell E. Vance, PhD

University of California,
Berkeley

Johannes C. Walter, PhD

Harvard Medical School

Rachel I. Wilson, PhD

HHMI Early Career Scientist
Harvard Medical School

Yukiko Yamashita, PhD

University of Michigan

Perspectives & Opinions

Hypothesis: Unknown

Observation-driven studies have a place in science

Richard Flavell has actively shaped the field of genetics, devising new ways to ask questions about the role of genes in human biology. The HHMI investigator at Yale University does not start every experiment, however, with a hypothesis.

IN THE SECOND half of the 1900s, biology revolved around forming hypotheses and testing them. The prevalent view was that the only role of observation in this context was to attack or support the hypothesis. As this approach became engrained in biologists' minds, it led them to teach students a single formula to address scientific problems: First, ask a question and come up with a hypothesis that is the best educated guess as to the answer. Then, design an experiment that tests whether that hypothesis is correct.

My lab group certainly does that kind of work, lately focused on how gut bacteria affect human health through their interactions with the immune system. We hypothesized, for example, that mice lacking a particular set of immune molecules would be more likely to develop colon cancer than other mice. We tested this by genetically engineering mice to lack the immune molecules and determining which mice developed cancer. The data confirmed our hypothesis.

However, we now know that there's no one right way to do science. Especially in extremely

complex or fledgling fields, like the study of gut bacteria, many experiments stray from this formula. Often, we don't have enough information to formulate a useful hypothesis, or the complexity of the system is not suitable for reductionist approaches. In those circumstances we ask a question that we have no best-guess answer for, and we collect lots of data to find an answer. This is observation-driven science.

For instance, in my lab we might ask: What is the effect of deleting one or more immune molecules on metabolism or intestinal ecology? Then, we would engineer mice to lack these immune molecules and observe what happens next. We would record changes to the prevalence of gut bacteria, changes to the weight and body composition of the mice, as well as disease, life span, and anything else we can possibly measure. We have the computational power and statistical methods to deal with large amounts of data, so finding useful correlations in experiments like this is possible. And we may discover a connection that we never expected to find—and therefore never would have hypothesized or tested in a more focused way.

We need both observation- and hypothesis-driven research to get a full grasp of a field. At certain times—when a field is new or stuck on a question—observation may be more important. At other times, forming hypotheses and designing targeted studies may be more useful. The kind of observational work common in biology today—collecting large amounts of information—can help scientists see links between biological systems, like the gut and immune system, or genes and their transcripts in given cells, that they might otherwise have missed.

Research on the collection of microbes in the gut, the microbiome, is still relatively new and has taken a more observational approach. The ability to use high-throughput methods to describe the mix of microbes in the gut at any given time has researchers asking broad questions: What happens to the

microbiome when an animal's diet changes? What happens to an animal's health and behavior when we raise it in a microbe-free environment? These studies have yielded fascinating insights into all the body's systems and health conditions, including cancer, that might be related to the microbiome.

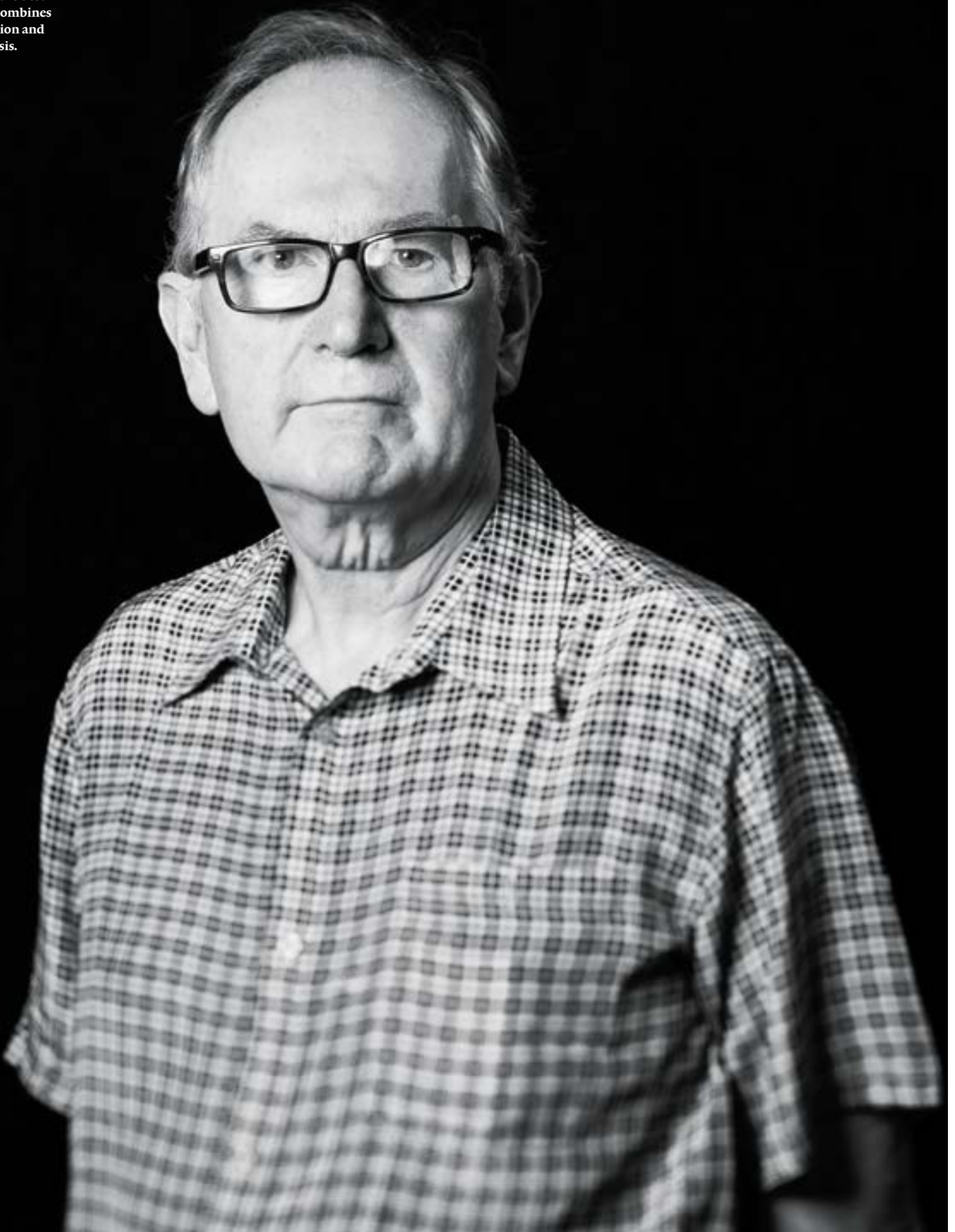
To shed light on the molecular mechanisms of those connections, we move from observation to hypothesis. If a large, data-driven study reveals that people with inflammatory bowel disease (IBD) have collections of bacteria in their gut that differ from people without the disease, we can begin to hypothesize the mechanism of the bacteria's pathological effects. For instance, we could hypothesize that the high prevalence of bacteria A in IBD patients explains their disease. Then, we would design an experiment to test that hypothesis, studying whether mice with extra bacteria A get IBD. Moreover, we can modify the bacteria to determine the molecular determinants that trigger disease in those individuals or change one component of the immune system at a time and ask whether it can causally recapitulate the health status we're interested in.

To me, the best science happens not when we're wedded to one way of doing things, but when we're wedded to a topic that fascinates us and we can follow the research wherever it leads. There's nothing wrong with a lab team doing observational study after observational study. They are still helping advance the science, and likely providing fodder for hypothesis-driven studies to come. After all, the best hypotheses always stem from a collection of important observations.
—Interview by Sarah C.P. Williams

Richard A. Flavell

—
Chair of the
Department of
Immunobiology
at Yale School of
Medicine

Richard Flavell
believes the best
science combines
observation and
hypothesis.



Perspectives & Opinions



Martin Cohn

*HHMI Early Career Scientist
University of Florida*

When investigating why limb development is arrested in python embryos, I was struck by the limb-like appearance of their genitalia. Wondering whether limb and genital development might involve similar processes, I decided to look into the mechanisms that build these appendages. I learned that, though defects in external genitalia are common in humans, almost nothing was known about the molecular processes involved. So my first graduate student compared gene expression in the limb and genital buds of mouse embryos, which are, thankfully, more tractable than pythons! Today, genital development is a major focus of my lab.



Elaine Fuchs

*HHMI Investigator
The Rockefeller University*

In the 1990s, my lab was studying a skin protein that we thought reinforced adhesion between the epidermis and underlying dermis. We created a knockout mouse that showed mild skin blistering, but as the mice grew, they began twisting and writhing, and soon their sensory nervous systems had degenerated. We discovered that the affected gene had both epidermal and neuronal promoters, encoding different forms of protein. As expected, the epidermal form strengthened the internal framework and adherent properties of the epidermis. The neural form, on the other hand, stabilized the nerve cells and helped with long-distance protein trafficking. This work shaped our current interests in how stem cells use the cytoskeleton to build and repair tissues.

Q&A

Tell us about an observation that sent your research in an unexpected direction.

Research doesn't always follow a set path. Sometimes a single observation will send experiments on an entirely new course. Here, four scientists reveal pivotal moments in their scientific careers.

—Edited by Nicole Kresge



David L. Stern

*Lab Head
Janelia Farm Research Campus*

I like to make bold predictions about experimental outcomes and a running joke in my lab is that I'm almost always wrong. Most of our significant observations have taken our work in unanticipated directions. For example, our observations that certain body parts in fruit flies evolved entirely through changes in gene transcription led us into the field of *cis*-regulatory evolution. We had no desire to enter the quagmire of transcriptional regulation; we were forced into it by the data! As we dug deeper, we were surprised by the complexity and redundancy of these regulatory regions. This led us to new ways to tease apart the contributions of single nucleotide changes to morphological evolution, and new ways of thinking about how *cis*-regulatory regions function.



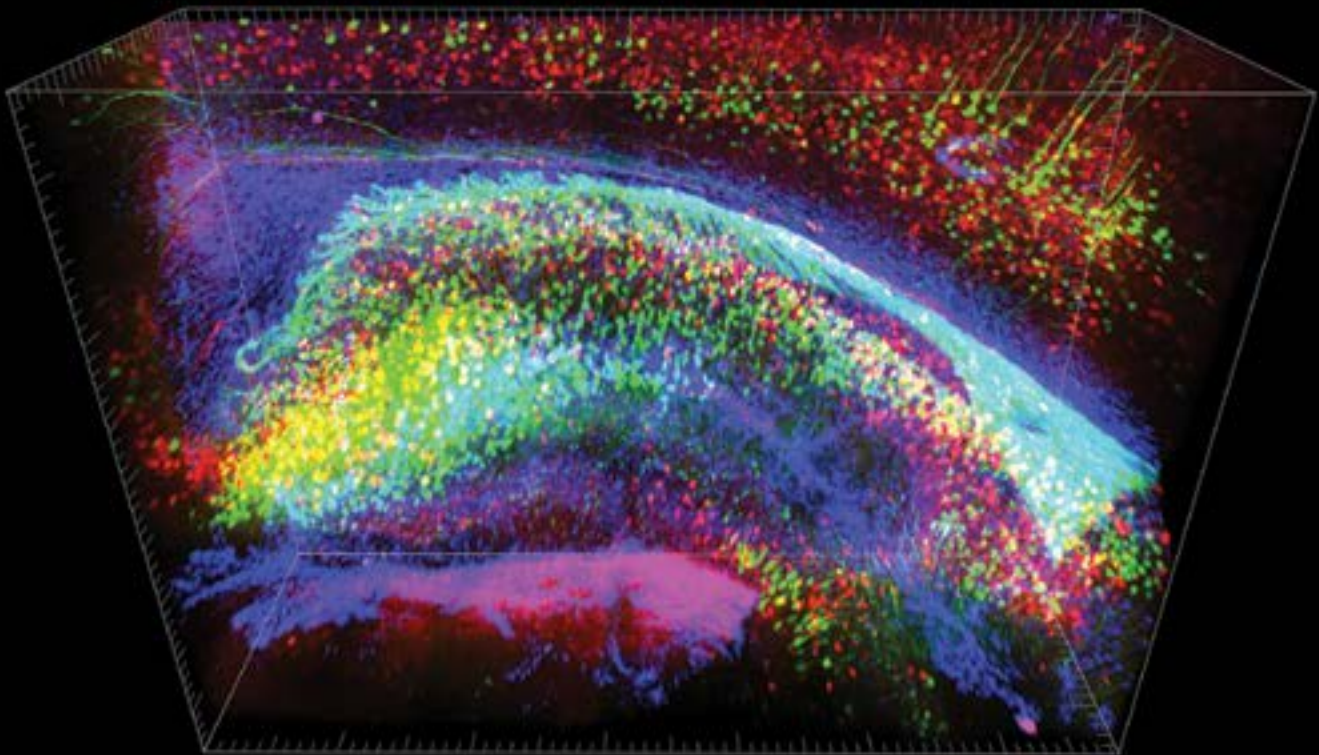
Luísa M. Figueiredo

*HHMI International Early Career Scientist
Institute of Molecular Medicine
Lisbon, Portugal*

As a postdoc, I showed that the enzyme DOT1B helps determine which glycoproteins appear on the surface of trypanosome parasites—protozoa that cause human disease. Trypanosomes have about 2,000 copies of the *variant surface glycoprotein* (VSG) gene, but only one is active at any given time. Because DOT1B adds methyl groups to the H3 histone—a nuclear protein that helps pack DNA into chromatin—I looked to see if the active VSG had more histone methyl groups than the silent VSGs. To my surprise, there were no histones on the active gene! This contradicted previous findings and led our research in a new direction: characterizing active VSG chromatin.

Chronicle

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Partners for the Environment
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Now You See It
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Community Building /
Dialing Back a Disease /
A Helpful Hormone



For scientists studying the brain, fat is a big problem. Light bounces off the lipid molecules, obscuring researchers' views when they look at brain tissue under microscopes. Fortunately, HHMI Investigator Karl Deisseroth and his colleagues have figured out how to make this fat disappear. Their technique, called CLARITY, involves removing the fat from the brain and replacing it with a clear hydrogel. The resulting transparent sample offers an unobstructed view of the brain's fluorescently labeled circuitry. Deisseroth and his colleagues used this technique on a mouse hippocampus—part of the brain used for learning, memory, and emotion—to create the image shown here.

Learn more about CLARITY in Toolbox, page 36.

Chronicle / *Science Education*

Partners for the Environment *In Maryland and Virginia, every stream and park can be an outdoor classroom.*

THE CHESAPEAKE BAY, stretching from northern Maryland to the Virginia coast, is North America's largest estuary. Fed by 100,000 rivers and streams from as far away as New York and West Virginia, it is home to 3,600 species of plants and animals and has a rich ecological history. It offers endless opportunities to spark a love of science and environmental stewardship among students.

What does runoff from a new school parking lot mean for soil quality? Is there a link between algae blooms and nitrate levels in the bay? How do sunlight and water affect growth of salad greens in a school's container garden? Students are tackling these questions and posing their own about the watershed in their backyards.

Schools around HHMI's Montgomery County, MD, headquarters and its Janelia Farm Research Campus in Loudoun County, VA, are partnering with environment-focused nonprofits to get K-12 students and teachers outside to do research. Their goal is for students

to learn about the scientific process, and to develop an appreciation for their local streams, fields, woods, and the larger watershed. The school districts and foundations receive grants from HHMI to promote environmental education among schoolchildren.

"HHMI is pleased to provide support to these organizations so that students in our area can have the opportunity to engage in inquiry-based learning," says David Asai, HHMI's senior director for science education.

Plugging into Existing Systems

The Audubon Naturalist Society (ANS) and the Chesapeake Bay Foundation (CBF) offer expertise, onsite activities, and curriculum resources to build environmental literacy and develop a constituency for clean water among schoolchildren, teachers, and principals in districts including Loudoun and Montgomery counties.

"Because of these partnerships, we're getting professional development for our teachers plus curriculum resources," says Odette Scovel, instructional supervisor in science at Loudoun County Public Schools. "We're integrating content into daily instruction. In some cases, we're getting partners, like Audubon, in the classroom working with students."

Their efforts appear to be paying off. After participating in CBF field studies, students show gains in ability to articulate critical environmental issues as well as environmental stewardship and engagement, according to Tom Ackerman, CBF's director of teacher training. Teachers who went through the CBF training in 2012–13 gained significant confidence in their ability to teach about the watershed, he says, and their intentions to get their students outside for research projects spiked.

Ideally, students would have multiple opportunities to visit the Chesapeake or streams closer to home to collect specimens, tally bacteria and macroinvertebrates, or test the chemical levels in the water. But in reality, very few classes manage to make more than one trip per year, if that.

To meet this challenge, several groups, including CBF, are beginning to take advantage of shared databases and geographic information system, or GIS, technology. This way, a class can visit once and input its data into a master database, and then have continuing access to a more robust data set than they could maintain themselves. Students can measure stream health over time, for instance, by measuring levels of *Escherichia coli* in a stream at 3:00 p.m. on March 5 and then comparing their data to measurements taken by other groups on different afternoons.

CBF connects schools with National Geographic's FieldScope, a web-based interactive mapping tool, to monitor watershed health. Ackerman says he would like to see more classes participate in site visits and data input, to build a larger data set.

"These are great examples of how students can collect data that is relevant to their community," Asai says.

From the Top

GreenKids is a two-year ANS program that is free to Montgomery and Loudoun County elementary, middle, and high schools. A GreenKids naturalist visits classrooms multiple times, and ANS provides funding for container gardens, field trips, and teacher training. The kids—28,000 since the program began in 2005—learn how to plant school gardens, compost waste, save energy, recycle in the classroom, and monitor stream water quality. Students also perform student service learning by removing litter and invasive plants.

Along with building teacher capacity and confidence, ANS and CBF work together to gain buy-in among school principals. Ackerman notes that about 70 percent of principals who attend CBF retreats report launching new environmental watershed initiatives for their students.



➤ Go with students on a GreenKids stream clean-up by visiting www.hhmi.org/bulletin/fall-2013.

“Kids won’t be able to get away from [research]. I want them to get their hands dirty; get confused by the data; struggle.”

—ODETTE SCOVEL

“It makes all the difference to have principals who ‘get it,’” says Diane Lill, GreenKids director. Principals set expectations at the school that teachers will take kids outside for science-based lessons or plan environment-focused field trips. “The entire school culture is different.”

Lill met principal Lee Derby, from Cedar Grove Elementary, in Germantown, MD, on CBF’s boat, the skipjack Stanley Norman. “He personally cares so much about the environment and getting kids outside,” she says. “He understands the importance for [student] development.” Derby invited GreenKids to run a training workshop for all staff—teachers, administrators, specialists, and support staff. Everyone went outside, evaluated the schoolyard, walked to a nearby stream, and brainstormed ideas for getting kids out to explore nature.

“This only happens if the principal makes it happen,” Lill says. “It can sometimes be hard for us to get even 10 minutes at a staff

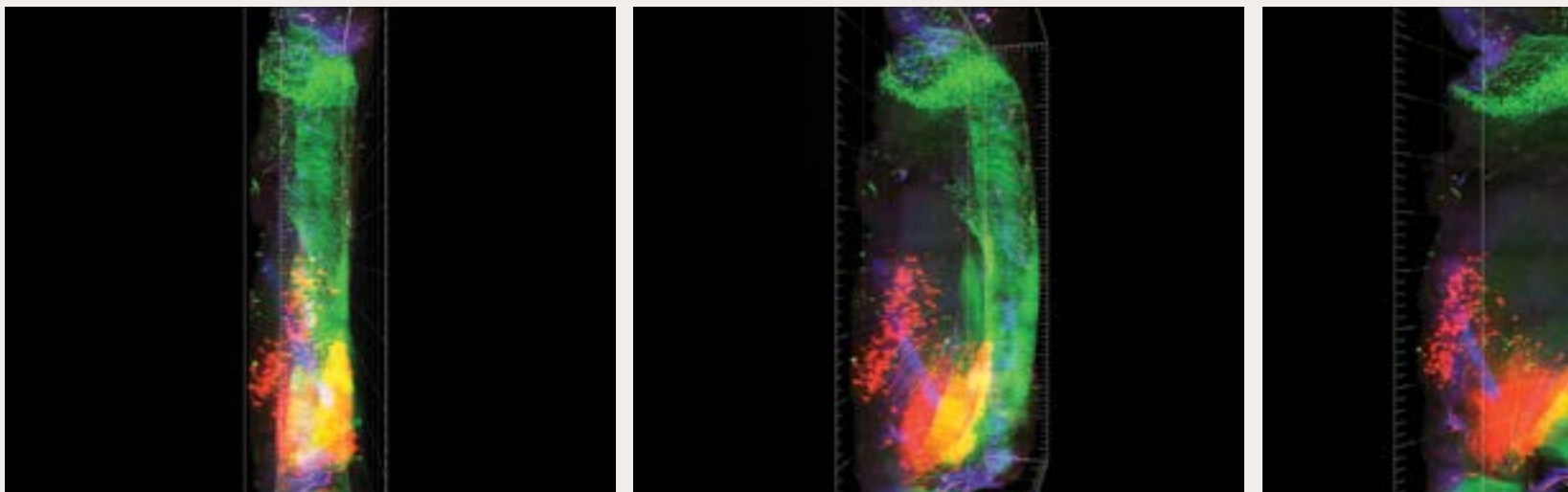
meeting.” Cedar Grove was one of two GreenKids schools to win a 2013 National Green Ribbon Schools Award from the U.S. Department of Education for “offering environmental education to boost academic achievement and community engagement.”

Montgomery County educators expect to see a boost in state support now that Maryland has become one of five states to adopt the Next Generation Science Standards (NGSS), a new set of voluntary K-12 standards that emphasize hands-on learning and critical examination of scientific evidence.

Scovel doesn’t expect Virginia to rush to adopt the NGSS, but Loudoun County isn’t waiting to include research in the curriculum. “Kids won’t be able to get away from it,” she says. She wants students to understand how science works and get beyond what she calls paint-by-number science fair research. “I want them to get their hands dirty; get confused by the data; struggle.”

—Cori Vanchieri

Chronicle / *Toolbox*



Now You See It *Replacing the lipid-rich infrastructure of the brain with a hydrogel makes it transparent, revealing details of its circuitry.*

IN NEUROSCIENTIST Karl Deisseroth's lab at Stanford University, samples of brain tissue—even entire brains—have a way of disappearing. There's nothing mysterious about this vanishing act: It's the result of CLARITY, a technology Deisseroth and his colleagues developed to bring the brain's complex neural circuits into better view by removing the neurons' fatty components, which distort images produced by even the most advanced microscopes.

"This is something we've wanted to do for a long time," Deisseroth says. His lab group studies the neural circuits that underlie behavior. Using light-based tools he developed in 2005 that give researchers precise control over specific nerve cells (an approach Deisseroth dubbed optogenetics), his team has identified cells and connections in the brains of mice that are involved in anxiety, drug abuse, social behavior, and depression. Still, these cell-by-cell studies offer too limited a view of brain function for Deisseroth, an HHMI early career scientist and practicing psychiatrist who wants to understand mental illness and improve treatments for it.


"That approach has been useful, but it hasn't allowed us to come to a deeper, circuit-level understanding of how physiology and behavior arise from the neurons that we target," he says. "That's because we don't know in detail how they're wired—how they're connected in tissue, both locally and globally. We can control cells, we can see behaviors—but to turn that into a deep understanding of how the circuitry works has been a challenge."

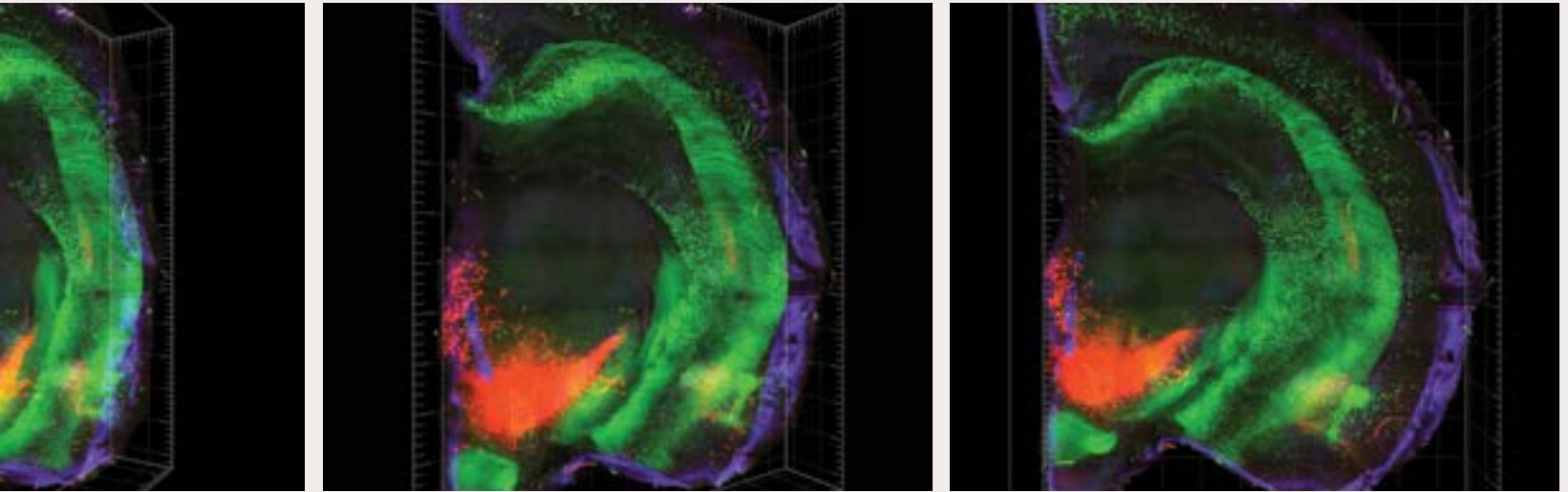
The wiring information Deisseroth hopes for has been difficult to come by in part because to examine the brain's cellular structure under a light microscope, scientists must first slice the tissue into thin, light-accessible sections. Today's imaging tools can reveal remarkably fine details about the individual sections, but reconstructing a

tissue's original three-dimensional structure is a labor-intensive and error-prone process. Contextual information, which Deisseroth says is essential to understanding the real consequences of a cell's activity within a functioning brain, is often lost.

The problem, Deisseroth says, is fat. Lipids in cell membranes provide structure and support for information-processing neurons, but because they bend and scatter light, they cloud the view of biological tissues. Furthermore, lipids interfere with a tissue's permeability to antibodies, which researchers use to label specific molecules and characterize cells. This is true for all biological tissue. But the brain, packed with elongated and elaborately branched membrane-bound cells, is particularly lipid-rich. Removing fat from the brain allows access by light and macromolecules like antibodies. But without it, the tissue loses its structure and stability.

"We knew we needed to build an infrastructure within the tissue," Deisseroth says. His team, led by postdoctoral researcher Kwanghun Chung, began experimenting with various molecular support structures a few years ago and finally settled on a hydrogel. They found that they could infuse

 Still frames captured from a 360-degree view of a CLARITY-processed mouse brain. See the complete video at www.hhmi.org/bulletin/fall-2013.



Under a microscope, [the samples] offer a never-before-seen view of the brain's circuitry—revealing connections between distant parts of the brain while allowing researchers to zoom in on fine details of the cellular structure.

a tissue with the building blocks of the gel (hydrogel monomers) and chemically link them to the proteins, DNA, and RNA inside the tissue. “It leaves the lipids out in the cold,” Deisseroth says. “They don’t crosslink with the polymer.”

When the infused tissue is heated, the gel monomers link together, forming a three-dimensional network that captures proteins and genetic material in their original positions. With the scaffold set in place, lipids can be removed with strong detergents and an electric current.

Tissue treated in this way emerges from the process transparent and accessible to biological labeling molecules. Deisseroth named the procedure CLARITY and his team described it in the May 16, 2013, issue of the journal *Nature*.

CLARITY can transform an intact mouse brain—a pale pink lump of tissue about the size of a pencil eraser—into a transparent form in about five days. Deisseroth acknowledges that the clarified samples are a little harder to keep track of at the lab bench, but under a microscope, they offer a never-before-seen view of the brain’s circuitry—revealing connections between distant parts of the brain

while allowing researchers to zoom in on fine details of the cellular structure.

With lipid barriers gone, labeling antibodies can permeate the porous hydrogel, enabling specific proteins and structures to be visualized under the microscope. Further, the hydrogel is stable enough to withstand the harsh treatments needed to remove labels, meaning that researchers can reanalyze the same tissue with a focus on different proteins.

Deisseroth says the ability to image an intact mouse brain will complement the optogenetic tools his lab has developed for manipulating neuronal activity, finally allowing them to directly link what they learn about activity patterns to a deeper understanding of brain anatomy. But that’s not CLARITY’s only application. Deisseroth says the technique should be applicable to any tissue from any organism.

His team has successfully clarified clinical samples of postmortem human brain and glimpsed neuronal connections that weren’t discernible in images reconstructed from a series of thin samples. Other labs are finding their own applications of the technology, such as visualizing cancer cells within the three-dimensional context of a biopsy sample. Deisseroth is encouraging widespread use of the technology, sharing resources online at clarityresourcecenter.org and hosting hands-on training courses in his Stanford laboratory.

—Jennifer Michalowski

Community Building

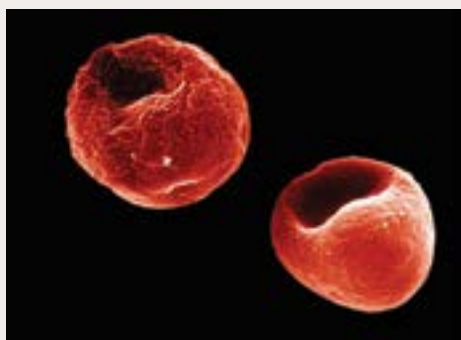
Malaria parasites help each other survive by passing messages between red blood cells.

LIKE TINY NOMADS, malaria parasites move from human to mosquito and back again. But how do they know when to pack up and move? New research from HHMI Senior International Research Scholar Alan F. Cowman suggests that when it's time to leave their human hosts, the parasites, a type of protozoa, send each other dispatches saying it's time to head out.

A mosquito transmits the malaria parasite to a human host when it plunges its proboscis

through the skin for a sip of blood. The protozoa travel through the bloodstream to the host's liver, where they reproduce asexually and infect red blood cells. When it's time to go, the protozoa develop into gametes that are taken up during a second mosquito's bite. Inside the mosquito's gut, the protozoa reproduce sexually and the process begins again.

Cowman's postdoctoral fellow Neta Regev-Rudzki discovered that the protozoa were



The malaria parasites in these two infected red blood cells send each other packets of information to coordinate group activity.

talking to each other inside their human hosts, passing vesicles between the red blood cells they had infected. As the researchers reported in *Cell* on May 23, 2013, vesicle production increased when the protozoa were stressed—for example, when they were exposed to an antimalarial drug—and seemed to signal to the parasites to mature into their sexual form. The communication mechanism made sense: the protozoa need a way to broadcast environmental conditions and let the community know when it's time to catch a ride with the next mosquito.

Although Cowman and his team at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, have yet to determine the exact content of the vesicles, once they do, they could have some potential drug targets. "A big aim among malaria researchers is not only to develop ways to treat the disease but also to make compounds that inhibit transmission," says Cowman.

"Blocking the passage of the parasites into the form that can spread to mosquitoes is one way to do that." —Nicole Kresge

IN BRIEF

THE GEOMETRY OF (CELL) DIVISION

During development, a cell needs to know up from down and left from right. For example, a disoriented hair follicle could produce hair growing into, rather than out of, the body. HHMI Investigator Roel Nusse has shown that a localized source of a signaling molecule called Wnt3a coordinates orientation in a dividing stem cell.

Wnt proteins play key roles in embryonic development, tissue regeneration, bone growth, and stem cell differentiation. In the body, Wnt typically acts on receptors on just one portion of the cell. To recreate this "orientation effect" in the lab, Shukry Habib, a postdoctoral researcher in Nusse's Stanford University lab, attached Wnt protein to tiny beads and added the beads to dishes of growing embryonic stem cells. Habib then used a Bessel beam plane illumination microscope—a high-speed, high-resolution, three-dimensional imaging

device created by Janelia Lab Head Eric Betzig—to watch the cells respond to the beads.

The effects of the localized signal were clear, according to the team's March 22, 2013, report in *Science*. The stem cells all divided in the same way, with the plane of division occurring perpendicular to the location of the incoming Wnt signal. Furthermore, the daughter cell closest to the bead retained the characteristics of an embryonic stem cell while the more distant progeny cell began to differentiate into a specialized cell. "That makes sense," Nusse says, "because the Wnt signal is important for stem cell fate, and the lack of a Wnt signal would allow the distal cell to differentiate."

Next, Nusse and his team will try to uncover the molecules that help Wnt orient cell division.

NEWBORN HEART DISEASE CULPRITS

Each year, approximately 40,000 babies are born in

the United States with congenital heart disease. Many of these birth defects are fatal without surgical intervention. The causes of most cases are unknown, but new findings by two HHMI investigators indicate that spontaneous mutations are the culprit in some cases.

Although genetic factors contribute to congenital heart disease, many children born with heart defects have healthy parents and siblings, hinting that the responsible mutations might not be present in the parents. Richard Lifton of the Yale School of Medicine and Christine Seidman of Brigham and Women's Hospital led a team of scientists that analyzed the protein-coding regions of the genomes—exomes—of more than 1,800 individuals with and without the disease. The team discovered that about 10 percent of children's heart defects are caused by spontaneous, or de novo, genetic mutations. Strikingly, as they reported June 13, 2013, in *Nature*, many of these mutations affect a pathway that

regulates modification of proteins called histones, a pathway also implicated in children with neurodevelopmental problems.

The findings may help physicians provide better care for patients with congenital heart disease. "After we repair the hearts of these children, some children do great and some do poorly," Seidman says. This could be due to differences in the underlying causes of the disease. Understanding those variations might help doctors improve outcomes for their patients.

TOLERATE THINE ENEMY

When a pathogen attacks the body, learning to live with the invader is sometimes more important than getting rid of it. A recent study by HHMI Investigator Ruslan Medzhitov, at the Yale School of Medicine, showed that without immunological tolerance, infections can turn deadly.

Tolerance complements the immune system's fight against an infection by lessening pathogen-induced damage. To explore the



Dialing Back a Disease

A cellular signaling pathway affects the levels of a protein that kills nerve cells.


MORE IS NOT always better; it can be deadly. A neurodegenerative disease called spinocerebellar ataxia type 1 (SCA1) is caused by too much of a mutant version of a protein called ataxin-1. A recent discovery from a team led by Huda Zoghbi, an HHMI investigator at Baylor College of Medicine, hints at a way to decrease ataxin-1 levels and perhaps reduce the disease's severity.

SCA1 occurs when a specific stretch of

three nucleotides—the molecules that make up DNA—is repeated in the *ATAXIN-1* gene too many times. The altered gene produces a large, misshapen protein that can't interact normally with its protein partners. Instead, the abnormal protein piles up in nerve cells, eventually killing them. As a result, the person loses coordination and balance and experiences difficulty speaking, breathing, and swallowing.

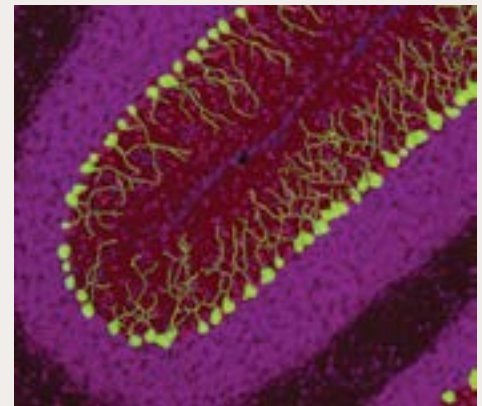
Although there is no treatment or cure for the disease, Zoghbi and her colleagues previously discovered that by decreasing levels of ataxin-1 in a mouse model of SCA1, they could reduce disease symptoms in the animals. With funding from an HHMI Collaborative Innovation Award, Zoghbi assembled a team of researchers with a collective goal: to speed up screening for genes and proteins that could be targeted with drugs to achieve the same effects.

The team focused on about 600 genes in both human nerve cells and fruit fly SCA1 models. The genes encode kinases—enzymes that attach phosphate groups to proteins and are often easily blocked with drugs. As they reported June 20, 2013, in *Nature*, 10 of the genes influenced ataxin-1 levels in both systems. All of them were part of a cell signaling pathway called RAS-MAPK-MSK1. When the pathway was inhibited in mouse models of SCA1, ataxin-1 levels went down

 To see Huda Zoghbi interview a patient with SCA1, go to www.hhmi.org/bulletin/fall-2013.

and neural damage was limited.

Zoghbi's team is searching for other genes that affect ataxin-1 levels as well. "We want to look for other relevant pathways now, because if we can find two or three pathways that are important in SCA1 and inhibit all of them just a little bit, we may be able to avoid toxicity," she explains. —Nicole Kresge



Reducing levels of the *Msk1/2* kinase decreases the amount of ataxin-1 and improves survival of Purkinje neurons (green) in mouse models of SCA1.

balance between tolerance and immune response, Medzhitov and his colleagues infected mice with influenza virus and *Legionella pneumophila*, the bacterium that causes pneumonia. The mice easily fought off infections from either pathogen alone. But when combined, the pair produced deadly results. Mice that received a dose of virus and a subsequent dose of bacteria were dead within a week. The team reported June 7, 2013, in *Science* that expression of genes involved in tissue repair and stress response in lungs—two important features of tolerance—was diminished in coinfecting mice. However, the researchers increased survival among the mice with the drug amphiregulin, a growth factor that promotes tissue cell growth.

"Something about the double infection prevented those genes from being engaged," Medzhitov says. "Mechanistically, we don't know what it is yet. We can imagine several scenarios, but it's something that we will be investigating in

the future." If boosting tissue repair can improve tolerance and rescue coinfecting mice, there may be promise in such an approach for patients who are particularly vulnerable to infections, such as infants and the elderly.

ADVANCE MALARIA ALERT

Want to know if there will be a malaria epidemic in northwest India this year? Just check the temperature of the South Atlantic Ocean. According to HHMI Investigator Mercedes Pascual, sea surface temperature in the South Atlantic is a big determinant of levels of monsoon rain in that part of India, which in turn is linked to the number of malaria cases.

Malaria outbreaks in these semideserts of India are directly related to the severity of monsoons, according to earlier work by Pascual and her team at the University of Michigan: the more rainfall, the worse that year's outbreak. Since monsoon season ends in September and malaria season

peaks in November, malaria outbreaks could be predicted one or two months in advance.

"We were interested in whether we could use other climate measures to predict monsoon patterns and develop an even earlier warning system," says Pascual. Her team looked at global monthly ocean temperatures over a 20-year period and compared them to rainfall patterns and the size of malaria outbreaks in India each year. They saw a clear link between abnormally cold temperatures in the tropical South Atlantic in June and July and an increase in malaria in northwest India four months later. The lower ocean temperatures led to changes in the atmosphere, which over time increased rainfall, providing the perfect breeding conditions for the malaria-carrying *Anopheles* mosquito. The scientists published their findings May 2013 in *Nature Climate Change*.


This is good news for public health authorities in India. Gaining

an extra month or two to predict the severity of a malaria outbreak gives them time to better prepare drug stockpiles and enhanced mosquito control interventions, and encourage prevention efforts.

LIGHTS, CAMERA, TRANSCRIPTION!

RNA polymerase has one job: to make RNA from DNA templates. Overall, it does a great job, except that it needs help with some small but important details, like figuring out where genes begin and separating the strands of a DNA double helix. Enter the preinitiation complex: an assembly of at least six different transcription factors that find a gene's start site, pull the DNA strands apart, and put the polymerase in the right spot. To help scientists understand how these molecules work together, Eva Nogales, an HHMI investigator at the University of California, Berkeley, created snapshots of the complex as it readied DNA for transcription.

Using cryo-electron microscopy—

 Listen to Douglas Melton discuss the work behind this discovery at www.hhmi.org/bulletin/fall-2013.

A Helpful Hormone

Betatrophin increases the production of beta cells, the body's insulin factories.

INSULIN INJECTIONS are a daily routine for many people with type 2 diabetes. The tide may be turning for them, however: HHMI Investigator Douglas Melton has found a hormone that dramatically increases the body's store of insulin-producing beta cells in the pancreas.

For more than 10 years, Melton has focused on turning stem cells into beta cells as a potential treatment for diabetes. With more of these insulin producers in their bodies, diabetics may be able to forgo the insulin injections that help regulate blood sugar levels. When Melton heard about a synthetic molecule called S961 that inhibits insulin

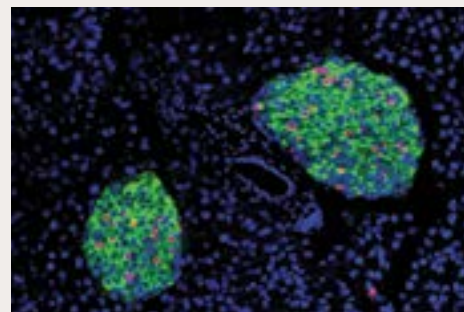
action, he wondered if mice given the molecule would compensate by producing more insulin, or even additional beta cells.

Peng Yi, a postdoctoral fellow in Melton's Harvard University laboratory, injected mice with S961, and to his delight, the rodents started making more beta cells. But, as the researchers reported May 9, 2013, in *Cell*, when they tried adding S961 to beta cells in a dish, it had no effect, suggesting that it acts indirectly on beta cells. The missing link, they discovered, was a hormone the investigators named betatrophin.

When the researchers turned on the *betatrophin* gene in the livers of mice, the number of beta cells in the animals' pancreases tripled within 10 days and their blood sugar returned to normal levels. "It boosts beta cell replication more than anything anyone has ever observed," says Melton. "It does it fast, and it does it specifically. The only cells in the body that

divide [as a result of *betatrophin* activation] are the beta cells." This makes betatrophin a promising potential therapy for people with type 2 diabetes.

Melton's group is now working to find betatrophin's receptor to figure out how it works. He also has partnered with companies Evotec and Janssen Pharmaceuticals to bring betatrophin to the clinic. "We're counting on our partners to make [the hormone] in large amounts for testing in animals and then humans," Melton says, adding that these first steps will take at least a year. —Nicole Kresge



The expression of betatrophin (red) in the mouse liver increases cell division in pancreatic beta cells.

IN BRIEF

a technique for viewing flash-frozen samples through a microscope—Nogales and her colleagues made the electron microscopy equivalent of a stop-motion movie. They first captured an image of the complex's core on a strand of DNA. Then they added the remaining factors one by one, taking pictures of the growing complex after each addition. The resulting short film, described in a paper published March 28, 2013, in *Nature*, reveals just how the transcription factors work together.

According to Nogales, this is only the beginning. "If we want to get at what is different between one gene and another, we have to start building up even larger complexes," she says. The next step is to add in the factors that allow the transcription machinery to recognize genes in a regulated fashion.

WITH TIME COMES DIVERSITY

The nervous system is incredibly complex, consisting of hundreds of

different types of neurons. So how does a neural stem cell know what kind of neuron to produce during embryonic development? Location plays a role—stem cells in different areas of the brain make different types of neurons. And now HHMI Investigator Chris Q. Doe and his doctoral student Omer Bayraktar have shown that timing is important too: as stem cells age, they produce different types of neurons.

Bayraktar and Doe studied a newly discovered *Drosophila* brain stem cell—the type II neuroblast—that generates a series of stem cells called intermediate neural progenitors (INPs). Each neuroblast makes about 50 INPs, and each INP makes about 10 neurons. The researchers discovered that both neuroblasts and INPs produce different transcription factors over time, and in INPs this leads to the production of different neurons. Neuroblasts are

known to change over time, says Doe, but having INPs also change over time vastly expands the number

of different neurons in the brain.

The results, published June 27, 2013, in *Nature*, help explain how a few stem cells can generate the huge diversity of neurons within the fly brain. Because humans have similar neural stem cells and INPs, explains Doe, this knowledge may help guide the development of stem-cell-based therapies for replacing damaged nervous system tissue.

ANOTHER ROLE FOR VITAMIN D?

Vitamin D is beneficial in many ways. It helps build strong bones, heals skin, aids in calcium absorption, and boosts the immune system. And now, thanks to research from Ronald M. Evans, an HHMI investigator at the Salk Institute for Biological Studies, it may decrease liver fibrosis as well.

When the liver is damaged, so-called hepatic stellate cells spring into action and start producing connective tissue known as collagen to stop the damage and heal the injury. However, excessive wound healing caused by chronic infections

or alcoholism commonly leads to fibrosis—too much accumulated collagen—and can result in cirrhosis or liver cancer. Currently, there are no drugs that effectively reverse the damage.

Evans and his colleagues noticed that stellate cells contain high levels of vitamin D receptors and wondered if these proteins played a role in liver fibrosis. When they gave mice a synthetic version of vitamin D called calcipotriol, stellate cells stayed dormant and collagen production was blocked. On the other hand, as they reported April 25, 2013, in *Cell*, mice lacking the vitamin D receptor gene spontaneously developed liver fibrosis.

Because the same biological mechanisms are at work in humans, these findings could lead to new treatments for patients with liver disease. Since the Food and Drug Administration has already approved calcipotriol for the treatment of psoriasis, the researchers hope to move quickly to test it in clinical trials.



Observations

For more information: See the feature profile of Bert Vogelstein, "Driven," on page 18.



The Road to Cancer's Abyss

In Siddhartha Mukherjee's bestseller on the history of cancer, HHMI Investigator Bert Vogelstein at the Johns Hopkins Medical School plays the role of mapmaker. Through his research, he begins to make sense of the mess of genetic changes that lead to a handful of cancers and maps out several long pathways that have become targets for early detection and new therapies.

Simon Pemberton

Knowing the heterogeneity of every cancer, one might naively have presumed that every patient's cancer possessed its own sequence of gene mutations and its unique set of mutated genes. But Vogelstein found a strikingly consistent pattern in his colon cancer samples: across many samples and many patients, the transitions in the stages of cancer were paralleled by the same transitions in genetic changes. Cancer cells did not activate or inactivate genes at random. Instead, the shift from a premalignant state to an invasive cancer could precisely be correlated with the activation and inactivation of genes in a strict and stereotypical sequence....

This was a relief. In the decade between 1980 and 1990, proto-oncogenes and tumor suppressor genes had been discovered in such astonishing numbers in the human genome—at last count, about one hundred such genes—that their abundance raised a disturbing question: if the genome was so densely littered with such intemperate genes—genes waiting to push a cell toward cancer as if at the flick of a switch—then why was the human body not exploding with cancer every minute?

Cancer geneticist already knew two answers to this question. First, proto-oncogenes needed to be activated through mutations, and mutations are rare events. Second, tumor suppressor genes need to be inactivated, but typically two copies exist of each tumor suppressor gene, and thus two independent mutations are needed to inactivate a tumor suppressor, an even rarer event. Vogelstein provided the third answer. Activating or inactivating any single gene, he postulated, produced only the first steps toward carcinogenesis. Cancer's march was long and slow and proceeded through many mutations in many genes over many iterations. In genetic terms, our cells were not sitting on the edge of the abyss of cancer. They were dragged toward that abyss in graded, discrete steps.

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Competitive Streak

Teeming bacteria of all stripes make their home in the gut. Like siblings sharing a bedroom, the microbes can get a little competitive for nutrients and other molecules they need to grow. Katrina Xavier and her research team aim to identify traits that neither help nor hurt *Escherichia coli* bacteria grown in pure cultures but are beneficial when the bacteria have to compete with other species in a complex environment like the gastrointestinal tract. The macro-colony seen here is a mixture of two mutant *E. coli* strains that differentially express fluorescently labeled proteins (yellow and blue) when grown in competition on an agar plate. The two markers indicate the fitness of each strain as they compete. To learn more about the team's work, see "Microbial Social Network," page 12.

