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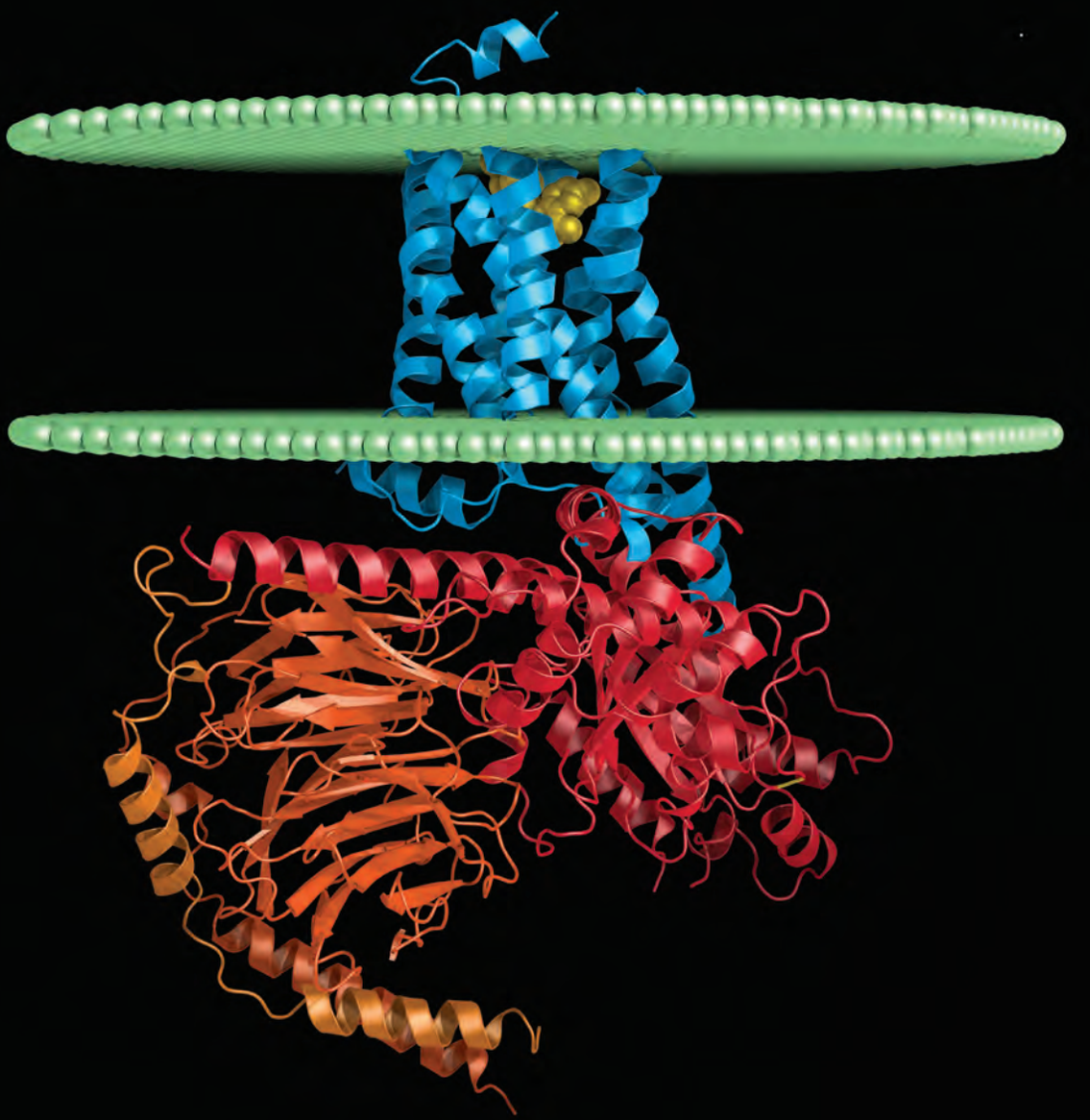
HHMI BULLETIN

Howard Hughes Medical Institute www.hhmi.org

In This Issue:

FIRST RESPONDERS

Robert Lefkowitz revealed a family of cell receptors involved in most body processes—including fight or flight—and earned a Nobel Prize.



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Not long ago this impressive structure was just a notion, a hypothesis for how a cell receives information from the outside. In the 1970s, Robert Lefkowitz set out to confirm its existence. His team eventually isolated three subtypes and cloned nine receptors in what is now known to be a ubiquitous family of G-protein-coupled receptors, including this beauty, the beta-adrenergic receptor. Here, the receptor (blue) sits within the lipid bilayer of the cell membrane (green) to respond to a hormone (yellow). The red, orange, and copper ribbons are three subunits of the G protein. Last October, Lefkowitz and former postdoc Brian Kobilka were recognized for this work with the Nobel Prize in Chemistry.

Wayne Decatur

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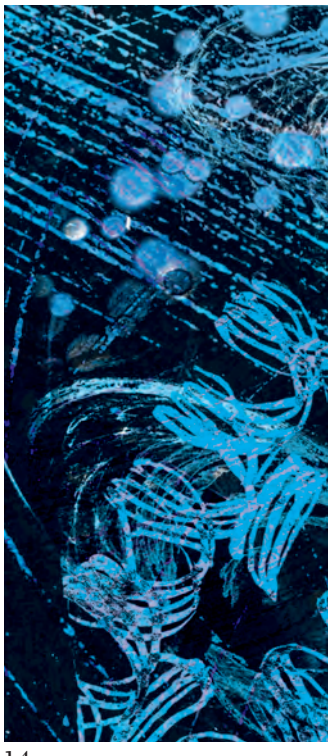
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- Read about an enzyme that is so unstable, almost any mutation will cause it to break apart.
- What do honey, barley, raisins, 15-gallon drums, and an old microscope have in common? They are all things you'll see on a tour of Janelia scientist Jasper Akerboom's garage brewery.
- Meet some of the proteins that journey along the cell's highways, hauling molecular cargo to various destinations.
- Learn how scientists are using blue light to target, and kill, nerve cells in worms.

GO GREEN

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An illustrator and photographer, **VIVIENNE FLESHER** (cover) also loves adventure travel. She has swum in Venezuela's Orinoco River surrounded by pirhana, spent a month on a coffee plantation in the highlands of Papua New Guinea, and hot-air-ballooned over the troglodyte villages of Turkey's Cappadoccia region. She currently lives in San Francisco, California, with her husband, artist Ward Schumaker. (1)

After graduate school, **JOHN CAREY** ("Model of Success," page 32) turned down a job with the Forest Service in Idaho to work at *Newsweek* magazine in New York City. Now, after three decades as a science journalist at *Newsweek, National Wildlife,* and *Business Week*, he's gone freelance. No regrets, he says, except for losing the bike commute to the office. (2)

JASON GROW ("A Happy Oasis," page 26) is a displaced Californian living in Gloucester, Massachusetts, with his wife, three daughters, dog, and one chicken. Specializing in photographing exceptionally accomplished people, his clients include *Time, Reader's Digest, Barron's,* and *Forbes,* among others. When not wrangling photo subjects or taxiing his children, he can be found impersonating a surfer at a nearby beach. (3)

KATHARINE GAMMON ("Trash Is Treasure," page 36) lives in Santa Monica, California, where she writes about science for *Wired, Popular Science, Nature,* and *FastCompany.* Her hunt for science stories extends around the globe, where she has chased sheep, photographed bats, and ridden ostriches—the meanest creatures she has ever met. (4)


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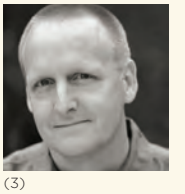
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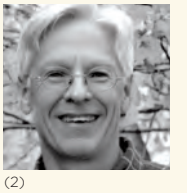
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Taking the Long View

IN ANYONE'S LIFE, THERE ARE SINGULAR MOMENTS THAT STAND OUT: graduation from college, marriage, the birth of a child. And in the life of an institute such as HHMI, there are exceptional events that hold particular significance. This October, I was honored to be present at one such event—the official opening of the KwaZulu-Natal Research Institute for Tuberculosis and HIV, or K-RITH, in Durban, South Africa. The culmination of sustained efforts by many individuals, in this country and in South Africa, this initiative brings a new dimension to HHMI's commitment to international research.

It was particularly moving for those of us—HHMI Trustees and officers included—who had the opportunity to visit clinics and see firsthand the devastating impact that coinfection with TB and HIV is having on the people of South Africa. It was an eye-opener to see hundreds of infected patients waiting for treatment. Knowing that the scenario was likely repeated in many other clinics and hospitals around the province brought home the importance of the mission of K-RITH. If we can actually make an impact on drug-resistant TB—devising a better diagnostic, for example—that could be as big as developing the Salk polio vaccine. In the process, we will have helped establish a center for research excellence in South Africa, training African scientists on their home soil. With recruitment of scientists well under way and the state-of-the-art laboratory building now open, the work begins in earnest.

While in South Africa celebrating K-RITH's milestone, we were thrilled to hear the news that the 2012 Nobel Prize in Chemistry was awarded to HHMI investigator Robert Lefkowitz of Duke University Medical Center and Stanford University's Brian Kobilka, who trained in the Lefkowitz lab and is an HHMI alum. An investigator for a whopping 36 years, Bob Lefkowitz exemplifies the HHMI investment: big thinkers who continuously produce good science and who inspire the next generation to do the same.

This Nobel was awarded for work confirming the physical existence of G-protein-coupled receptors—a family of cellular signaling receptors now known to be ubiquitous—and solving the structure of one such receptor, for adrenaline. Solving a protein structure is no easy task. Indeed, the technical challenges sometimes require years to overcome, as they did for Brian Kobilka. A quarter century ago, the leaders of HHMI took note of those challenges and launched, in 1986, a structural biology program to lend much-needed support for a field they believed held great potential (see "A Structural Revolution," page 14).

A prescient decision, the infusion of funding from HHMI led to technologies and discoveries that have fundamentally advanced our understanding of the relationship between protein structure and function. Still a remarkably robust program, it now includes 36 investigators at 24 institutions. Over the years, three of our structural biologists—Johann Deisenhofer, Tom Steitz, and Rod MacKinnon—have themselves been awarded Nobel prizes.

Today, the field of structural biology is evolving, moving from crystallography of single polypeptides to characterization of large, macromolecular machines. Many of our researchers are tackling such projects, including some at Janelia Farm Research Campus. A major element of the work there is molecular imaging, which is a modern way to think about structural biology. We've recently made a big investment in a cryoelectron microscopy facility at Janelia—a powerful tool for visualizing macromolecular structures—and we are actively recruiting scientists with compatible research interests.

James Kegley

> "In the life of an institute such as HHMI, there are exceptional events that hold particular significance."
>
> ROBERT TJIAN

This kind of fresh, expansive thinking has been a hallmark of HHMI since its inception, guided in large part by its Board of Trustees. In November, we passed a particularly poignant moment as the last of the Institute's eight Charter Trustees, Hanna H. Gray, retired from the board. Her departure marks the end of her 39 years of unparalleled service to the Institute but not the end of her influence. Her thoughtful and wise counsel stands as a shining example for us all. Following in her footsteps, we welcome Susan Desmond-Hellmann, chancellor of the University of California, San Francisco, as the newest Trustee elected to the board. An oncologist and biotechnology leader, Susan brings a strong record of practical experience and innovative thinking to her role at HHMI.

As has been true for so many institutional decisions over the years—from bringing on new Trustees to launching pioneering programs—we owe our profound gratitude to Hanna Gray. One of her last official acts as a Trustee was to travel to South Africa to celebrate the opening of the K-RITH building, which she was so instrumental in making a reality. It's hard to imagine a better capstone for her remarkable tenure.

Rational Exuberance

A Nobelist shares the secrets to his longevity in the lab.

TRAINED AS A CARDIOLOGIST, ROBERT LEFKOWITZ IS THE FIRST TO ADMIT that his 39-year research career has been driven by more than a desire to treat coronary disease. He has always had an unshakeable urge to figure things out. Yet his work on a large class of signaling molecules known as G-protein-coupled receptors, which earned him the 2012 Nobel Prize in Chemistry, has led to drugs for conditions ranging from allergies to schizophrenia to yes, even coronary disease.

The receptors relay signals from hormones and other molecules to the cell's interior, allowing the cell to respond to changes in the body such as a surge in adrenaline. When Lefkowitz picked up the trail of these receptors in the 1970s, many scientists were unconvinced they even existed. With a growing army of trainees, Lefkowitz showed they did. He eventually isolated three subtypes of what is now known to be the largest and most pervasive family of receptors—there are 1,000 or more in all—and cloned nine receptors in that family. G-protein-coupled receptors play a role in virtually all known physiological processes.

An HHMI investigator since 1976, Lefkowitz shared the Nobel with Brian Kobilka of Stanford University School of Medicine, who was a fellow in Lefkowitz's lab in the 1980s and an HHMI investigator from 1987 to 2003. Two weeks after learning of the award, Lefkowitz spoke to the *HHMI Bulletin* from his office at Duke University Medical Center.

What was it like to get that early morning call from Stockholm?

Well, you hear rumors. For years I'd heard that I was in the running, but the talk was always about the prize in Medicine. That award came and went, so on the morning in question it was the last thing on my mind. But they don't keep you in suspense. You hear a Swedish voice saying they're calling with some good news. They're not calling about the weather in Durham!

A chalk-based high-five outside Lefkowitz's lab on the morning of the Nobel announcement.

Paul Vetters

There was never a moment when my heart rate jumped, but there was this wonderful feeling of satisfaction and delight. I could see my wife's eyes opening like saucers, and she was tearing up. It was one of those moments you'll never forget. There were about 45 minutes between the call and the first news conference. You know it's happened, nobody else in the world knows about it, and a storm is about to descend. It was surreal and wonderful, sitting there with my wife, drinking coffee, just trying to get our minds around the whole thing. I still haven't done that.

How did you react to hearing that your former student Brian Kobilka would share the prize?

I experienced it as being perfect; I cannot tell you how thrilled and proud I am. My 40-year career and his 30-year career tell one smooth, continuous story. Brian left my lab around 1989, and we kept in reasonable touch, as I do with many of my trainees. We talked at intervals about all manner of things, mostly scientific, but two or three years ago we started collaborating again. On October 8, Brian and I Skyped for an hour and a half about a paper we are writing together. Thirty-six hours later we win the Nobel Prize. Back together again, in more ways than one.

You've been an HHMI investigator for 36 years. How has HHMI support affected your work?

Challenging things take time. That's the definition of challenging. One of the nice things about being a Hughes investigator is it allows you to take a bigger risk because you don't have to have an immediate result.

At the time that I was entering the field, nobody was trying to study receptors directly. My vision was, boy this could be big. If there was really a way to get at these mythical receptors, it could be something I could build a whole career on. I realized that if we were going to move forward we would need to develop all kinds of techniques to study receptors—we needed to label them, bind things to them, purify them.

The risk, in retrospect, was huge. I wasn't even 30 years old, but I had the chutzpah of youth. It seemed very challenging, but I didn't perceive the risk. It never occurred to me that the whole thing could fail, even though I went down a number of blind alleys. Hughes support allowed me to keep going.

You clearly did a good job mentoring Brian Kobilka. What, to you, makes a good mentor in science?

There's no single right way to mentor. I love interacting with my trainees, getting to know them, and assessing what makes them tick. What I do is a lot like coaching—you play to people's strengths. Let's say I've got a guy who's five-foot-seven and a terrific shooter. I don't make him a center. And I don't make my seven-foot-tall player a point guard. My goal for every trainee—and I've had more than 200 of them—is the same. I want them to experience what it's like to work at their

2012
NOBEL PRIZE IN
CHEMISTRY

Paul Fetters

potential, to be running as fast as they can run. For some people, like Kobilka, that's a four-minute mile. For others it's an 11-minute mile.

If my career was limited to just the experimental results I've reached with my own hands, nobody would've heard of me. Going from an idea to the right experiment is not a trivial thing. It requires great players and good coaching.

Your lab still produces a slew of research papers. How do you maintain enthusiasm for the work after so many years?

A lot of being successful is leveraging your gifts to the maximum extent possible and protecting yourself from inevitable failures. Gifts cannot be explained or fundamentally changed—though you can tinker around the edges.

For example, throughout my life I was a long-distance jogger. I'm totally devoid of athletic talent, but with hard training I could get to seven minutes a mile. I have a friend, Alton Steiner, who was once a champion swimmer and runner, a real athlete. Then he had some back problems, and he didn't run a speck for two years. One day he called me up, and I mentioned that I was running a 15K race the next day. He decided to join me. It was a miserable, humid day. The first mile we do at about a seven-and-a-half-minute pace. In the second mile I slow down a bit, and by the third mile I'm down to 8:30. He looks fine. Eventually he looks over sheepishly and says, "Do you mind if I go up ahead?"

I finished the race, but it was not pretty. And he's there sipping a lemonade, having finished at an average pace better than my fastest pace. You can tinker around the edges. But at my best, I can't eat his dust. That's what a gift is. After all these years, I haven't a clue how I still have this exuberance for science. I guess it's a gift.

What's the next frontier for your lab?

Lately we've been focused on beta arrestins, a class of molecules we discovered about 20 years ago that we still don't fully understand. They interact with receptors when they're stimulated, thereby turning off signaling, hence the name. But in the last few years we've discovered that beta arrestins can act as signaling molecules in their own right. That suggested the possibility of developing a new class of therapeutic that stimulates the biological pathways with desirable results and arrests the pathways with less desirable results.

Still, my job is to look at the big picture. Every experiment's results suggest five different things you could do next. They're all interesting. That's the problem. But we want to go from here to there, and my job is to keep us on the trail.

I often tell people there are four keys to success in science. The first is focus, the second is focus, the third is focus, and you can figure out the fourth. If anybody learned that lesson from me it was Brian Kobilka. After he left my lab, he became obsessed with getting a crystal structure of the receptor. It took him about 15 years. In 2007, he solved the structure of the beta-adrenergic receptor [the G-protein-coupled receptor that responds to adrenaline] and later the structure of the receptor interacting with its G protein. That's a remarkable accomplishment.

One of the things I learned by running races is you don't look up. You don't look ahead. You look down. You put one foot in front of the other, and you just look at the road in front of you. You don't worry about what's out there. Before you know it, you get to the end. ■

— SARAH GOFORTH

WEB EXTRA: For more about Robert Lefkowitz and his work, see the multimedia slideshow at www.hhmi.org/bulletin/winter2013.

Clare Mallison

Tethered to the Wind

Because the night was windy, biologist Craig Mello had set his alarm for 5:00 a.m. He woke, put on a wetsuit, and crossed his Rhode Island backyard to the sandy shore of Narragansett Bay. The September morning was still dark when he sped away from the beach, his feet on a surfboard, his body tethered to a crescent-shaped kite above him. He zipped across the water at 20 miles per hour, propelled by the wind.

By 8:45 a.m., Mello was back on terra firma, meeting with scientists up the road at Brown University.

"Kiteboarding with the moon trail on the water is just beautiful," says Mello, an HHMI investigator at the University of Massachusetts Medical School, an hour's drive to the north of his home. From early spring until late fall, Mello kiteboards whenever the wind is right, often at dawn or moonrise. "With the sun you get a golden path. With the moon you get a silver path."

It's a high-intensity pursuit that reconnects Mello to the bay he explored by sailboat as a teenager during summer visits to his grandparents. The first time he steered a Sunfish, he recalls, "I got totally hooked." In sailing, Mello found a doorway to "just being—being out there and experiencing nature, being in the moment and not trying to accomplish anything." He felt the same when he later learned to windsurf.

Kiteboarding, he's found, amplifies his communion with sea and sky. "When you are sailing, you're controlling the boat. When you're windsurfing, you're holding up the mast like a sidestay—you're part of the boat. And when you are kiteboarding, your body is the connection between the kite, which is floating in the sky, and the board floating in the water. You are like the fulcrum," says Mello. "You are the connecting point." It took him a whole summer to get upright on the board, but according to the six-foot-three, 52-year-old Mello, "It's pretty effortless once you know what you're doing."

Just as some people obsessively check their phones for email, Mello checks his to track the wind speed and direction at Conimicut Lighthouse, across Narragansett Bay from his living room window. He needs to know if the wind is right for the mile-long tack toward the green islands on the horizon.

Oddly, Mello might not have tried kiteboarding if he hadn't won the Nobel Prize—the 2006 prize for physiology or medicine that he shared with Andrew Fire for their discovery of a type of gene silencing called RNA interference. When an old windsurfing buddy wrote with congratulations, he mentioned that he hoped Mello had tried kiteboarding. He had not. The friend met Mello on the beach to show him the ropes.

His zeal for the sport parallels his motivation to do research. "I am basically the same way about the lab as I am about kiteboarding," says Mello. "I love it."

Managing the demands of kiteboarding is similar to repeating a mantra, says Mello's wife, yoga instructor Edit Mello. "His mind is able to be so busy that it's actually restful for him."

Even when he's far from the water, Mello says, "It's like the kite is always flying there in your mind. It's a refuge you can go to without going anywhere." *—Cathy Shufro*

FOR MORE INFORMATION: To learn about Mello's latest research, see "Cellular Search Engine," page 10.

Something's Brewing

Like any self-respecting Dutchman, Jasper Akerboom owns a pair of wooden shoes. If they seem out of place in his garage in Ashburn, Virginia, so does everything else: honey, barley, herbs, and raisins; a row of 15-gallon drums; an old microscope; a funny smell.

Akerboom, 35, is a scientist at the Janelia Farm Research Campus, which explains the microscope. The rest is thanks to his all-consuming hobby, homebrewing.

Making beer is half art and half science, says Akerboom, so it's the perfect diversion for a restless researcher. In the lab of biochemist Loren Looger, Akerboom's day job entails creating and testing molecular probes that help visualize how neurons work in the brain.

Akerboom, who goes by "Jaapie" to friends and readers of his beer-focused blog, is serious about his homebrewing. In a three-ring binder, he lists the chemical features of water in every public aquifer in Virginia (water quality can significantly affect the flavor profile of beer). He uses local ingredients when possible: hops grown in Loudoun County, water from the tap, and organic spelt grain. Most homebrewers, however, order well-characterized strains of brewer's yeast from online catalogues.

Not Akerboom. The environment is rife with yeast; more than 1,600 species have been described to date, and the brewer's yeast *Saccharomyces* has hundreds of variants. He knew how to isolate yeast, so why not put a finger to the wind and see what sticks? He began in his backyard, leaving jars of broth open to the air. "The trick is to create a medium that bacteria find disgusting, so you have to do less purifying," he explains.

Akerboom dabs the broth onto Petri dishes and watches for the yeast's trademark shiny white colonies. Then he cultures the colonies to make sure they are yeast (hence the microscope) and watches to see if it actively ferments. He followed this protocol with yeast collected from upstate New York, where his wife's family lives, and from Janelia's grassy hillside roof, with dreams of dispensing a "homegrown brew" in the onsite pub.

Unfortunately, the beer made with Janelia yeast tasted medicinal, "sort of like an old Band-Aid," Akerboom recalls. The New York strain was "highly undrinkable." But the two varieties of yeast from his backyard produced delicious brews. He named his favorite Wild Farmwell Wheat. On a visit to the Lost Rhino Brewing Company, a few miles from Akerboom's home, fellow Janelian Peter Lee introduced Akerboom to the brewmaster Favio Garcia. Taken with the idea of using local yeast for a beer, Garcia worked with Akerboom to adapt the Wild Farmwell Wheat recipe for a large scale and sold it as a summer wheat beer. Akerboom donated the yeast in exchange for a chance to learn more about the commercial brewing process.

Someday Akerboom would like to run his own brewery. And he's already thinking about the marketing side of the business. "Half of a person's decision to pick a beer to order is based on what the tap looks like," he acknowledges with a smile. He keeps a shoebox of handcrafted taps next to the clogs in his garage. He carved his favorite from the wood of an old Dutch ice skate, the kind that straps to a shoe. He found the skate at a flea market in Maryland, but the hand-painted emblem bears the name of the small Netherlands town where his mother grew up. "I'm a scientist, but I'm an artist too," he says. "The details are important."

—Sarah Goforth

Adam Auel

WEB EXTRA: For a slideshow of Akerboom's garage brewery, go to www.hhmi.org/bulletin/winter2013.
FOR MORE INFORMATION: To read Akerboom's beer blog, go to Jaapie.org.

Clare Madison

Musical Magnet

Andrey Shaw doesn't know why students with backgrounds in music gravitate to his lab, but they do. A few years ago, he had so many that he decided to put on a recital. "People who hadn't played instruments since junior high were dusting off their skills," he says. "It was totally amazing."

"We had a rule that nobody could play solo, so people would have to practice together," he laughs. Shaw performed as well, playing a four-handed piano piece, Dvorak's *Slavonic Dances*, with a student.

He'd love to repeat the performance, but it's hard to carve out the time from his studies of the immune system. "Science has been so all-absorbing," says the HHMI investigator at Washington University in St. Louis.

Finding time to play music used to be easier for Shaw. As a child in Seattle, he spent four or five hours a day polishing sonatas, waltzes, and nocturnes. Through college, he was on track to become a concert pianist. After earning a degree in music from Columbia College, he began giving piano recitals in New York City. But crippling performance anxiety made him unsure about his career choice. "So I had this brilliant idea that I would go to medical school. That could be my 'straight job,' and I could play music on the side."

He enrolled in medical school at Columbia University but found himself wandering over to the Manhattan School of Music to put his hands on the piano keys instead of going to class. "I flunked my first anatomy exam," he says. With that wake-up call, he started paying more attention in school and playing piano a lot less. He earned his medical degree but realized that a career as a physician would not allow him time to pursue music as well.

He started visiting labs, casting about for something that grabbed his interest. He found it, unexpectedly, in the lab of virologist Jack Rose. "I walked into the lab and I could immediately tell that was what I wanted to be doing," he remembers. "I don't think I realized until that point how creative science is. It truly spoke to me." He was struck by how "craft-like" conducting an experiment can be. He was also excited about the creative freedom to pursue his ideas wherever they took him. "Good science is about trying to think outside the box, about what no one else has thought of before," he says. "As scientists, we have an incredible level of freedom."

Shaw sees many parallels between playing music and conducting scientific research. "Much of what you do is tedious and repetitive. It requires a Zen-like state—you have to sit down, focus, and be in the moment," he says. "It's not that different from sitting at the piano to master a piece by practicing it over and over."

He finds his greatest satisfaction working with young scientists, supporting and mentoring them, as he did when they practiced together for the lab recital. "I love finding what it is that makes people tick and getting them excited about it," he says.

—Lauren Ware

upfront

WEB ONLY CONTENT

RICKETY FOR A REASON
Researchers investigate a fragile but powerful enzyme in the cell membrane. Read the story at *www.hhmi.org/bulletin/winter2013*.

How many mutations does it take to study an enzyme's architecture? One hundred and fifty one, in the case of the mysterious rhomboid protease. By changing the amino acid sequence of the enzyme in 151 ways, HHMI scientists found the building blocks most important to maintaining the enzyme's framework, which turns out to be rather rickety—and for good reasons. Read about that, and how the water-dependent enzyme does its job in a water-repellant environment, in the *Bulletin*'s online edition.

Cellular Search Engine

Caenorhabditis elegans *germ cells constantly scan their contents looking for unfamiliar genetic material.*

FOR A CELL, THE PAST INFORMS THE PRESENT. WE HUMANS HAVE search engines like Google and Yahoo to sift through the Internet's gobs of historical information and learn from others' mistakes and successes. In some cells of the worm *Caenorhabditis elegans*, it turns out, a type of RNA, called Piwi-interacting RNA (piRNA), and its partner, an Argonaute protein called Piwi, run a similar search. The piRNA and protein continuously peruse the cell's library of data and detect how it previously dealt with a particular molecule—whether an invading virus or a cell's own genetic material.

That historical review determines the cell's next step, according to research by HHMI investigator Craig Mello of the University of Massachusetts Medical School. piRNA—short stretches of 21 to 36 nucleotides—exists in tens of thousands of different sequences in germ cells (those that become eggs and sperm) of many animals including humans, but scientists have struggled to understand its purpose. Mello's lab has now uncovered the reason the molecules are so ubiquitous and exist in so many forms in *C. elegans*: so they can pair with essentially any genetic sequence they encounter during their endless scanning.

"The Internet is full of information, yet we can navigate it pretty efficiently because we have search engines," says Mello. "Argonaute proteins are like cellular search engines, and their small RNA cofactors are like the short search queries we type into Google." The piRNAs have a huge capacity for scanning, explains Mello, because they allow imperfect pairing—like when you misspell a search query, but still find what you're after. In short, Mello has discovered, the piRNA-based search engine can find everything out there, like running many millions of two- or three-word searches in Google to assemble every Web page on the Internet.

Mello's findings suggest that to make sense of this massive search process, the piRNA system interfaces with two other pathways that serve as cellular memories of "self" and "non-self" RNA. Sequences that were seen before in a previous generation ("self" RNAs) are thought to be protected from piRNA silencing by a pathway involving an Argonaute protein called CSR-1. Sequences not seen before lack this pathway's protection and so they are recognized by the Piwi/piRNA complex. In this case, the complex recruits a different Argonaute, dubbed WAGO, to create a permanent memory of the "non-self" RNA sequence.

The Mello lab stumbled upon this system while attempting to introduce foreign genes into *C. elegans*. When scientists insert a new gene into the worm's germline—the genetic material passed down to future generations via germ cells—the gene is sometimes expressed and sometimes silent. Mello's lab group wanted to know why expression is so unpredictable, even when genes are inserted into exactly the same corresponding spot in the genome.

To follow expression, the team attached coding sequences for jellyfish green fluorescent protein (GFP) to the foreign genes. By identifying worms whose germlines glowed green, they could easily see whether the associated gene was silenced. Then, they began crossing worms—those that had silenced the gene and those that hadn't—with each other. In the resulting offspring, they expected to see half the brightness of the green fluorescence—the gene inherited from one parent would be on, the second gene off. But the germlines of cross progeny were dark.

"You get this transfer of silencing from one copy of the gene to another," says Mello. "And this was permanent, very stable silencing." Even in future generations, they found, GFP was always turned off.

However, this is only half of the story, Mello says. Equally remarkable was the observation that, in another line of worms, active versions of the engineered genes had become resistant to the transfer of silencing over time and instead activated the silent genes. These observations suggest that in some cell lines, silencing trumps, while in other cases, the on-switch prevails. Importantly, the researchers found, once an on-or-off decision was made, it held true for every descendant of that animal for generations. "The animal is actually remembering which genes are supposed to be on and which genes it wants off," says Mello.

The connection with piRNA came when Mello's team repeated the experiments on cells that lacked Piwi, the protein that binds to piRNA molecules, and found that genes that had been silenced in other iterations of the tests were now always turned on. Through a series of experiments, the scientists provided evidence that piRNAs are forever scanning every bit of free RNA in germ cells. They also showed that molecular memories were maintained through two groups of molecules: one that signals activation, another silencing. Both rely on different Argonaute proteins to establish the memories.

Nicholas Blechman

When a piRNA successfully binds to RNA, the attached Piwi protein kicks into action: If the RNA sequence has not been seen before, the Piwi turns on a molecular "non-self" pathway that enforces silencing. If the RNA sequence has been seen before, it is recognized by the "self" Argonaute pathway, and Piwi allows the cell to express the gene. The findings were published in two papers in the July 6, 2012, issue of *Cell*.

While an animal's immune system is built to recognize foreign particles or cells, the piRNA system offers a second level of protection at the genetic level. If a virus, for example, gets past a worm's immune system, it can insert its genes into the worm's genome. The piRNA system can ensure that those viral genes remain turned off—it's a second line of defense. "Instead of recognizing a structural feature as foreign, the animal is looking at the sequence information itself," says Mello.

But the system—which Mello has dubbed RNAe, for RNA-induced epigenetic silencing—could also be a way that organisms generate heritable diversity that can be acted on by natural selection. Epigenetic silencing is any form of inherited genetic regulation that allows two organisms with the same set of genes to express those genes differently. Most previously known epigenetic mechanisms are based on protein modifications or chemical tags on chromatin or DNA, but RNAe is based on an inherited RNA signal and is the first epigenetic mechanism discovered to scan new genes by comparing them with a memory of self RNA expression.

"The bottom line," Mello says, "is that cells appear to have a previously unappreciated level of information technology sophistication, including both an actual memory of every gene that's been expressed and a constant surveying of information to keep track of what's new."

■ – SARAH C.P. WILLIAMS

WEB EXTRA: Read Nobel laureate Craig Mello's friendly advice to newly-minted Nobelist Robert Lefkowitz at www.hhmi.org/bulletin/winter2013.

Sister Act

Peering inside an intact brain shows that related neurons work together.

As a systems neuroscientist, Yang Dan integrates functional studies in animals with computer programs, computational tools, and statistics.

Leah Fasten

DURING ONE OF HER REGULAR VISITS TO THE INSTITUTE of Neuroscience in Shanghai, China, Yang Dan had an Ah-ha! moment. As she listened to a science seminar, the HHMI investigator at the University of California, Berkeley, realized that she could test, once and for all, a key hypothesis of how mammalian brains are organized. "Right after the seminar, I went to talk to Song-Hai Shi, the speaker," she says.

Shi, from Memorial Sloan-Kettering Cancer Center in New York, had just told the audience that neurons in the developing cortex that come from the same parent cell—called sister neurons—are more likely to be connected than two neighboring non-sister neurons. "I immediately wondered if the sister neurons had similar functions," Dan says, her voice still rising with excitement about that 2010 conversation.

Whereas Shi's team had been working with slices of fresh brain tissue from a rodent, Dan's question required studies in live animals. She wanted to peer inside the brain of a mouse and see if individual pairs of sister neurons are functionally connected, meaning they respond to the same stimuli. As a systems neuroscientist, Dan pairs natural context—in this case, intact brains—with computer programs, computational tools, and statistics to tease apart neural responses in live animals.

To examine pairs of sister neurons, Dan's team injected a small amount of a viral vector encoding a fluorescent protein into the developing brain of mice. With such a low concentration of the virus, only a limited number of the neural progenitor cells were infected. As those cells divided, the daughter cell neurons inherited the gene encoding the fluorescent protein. Whereas Shi's team used the labeling approach to study sister pairs in slices removed from the animal, Dan's team used two-photon microscopes to study the neurons in the intact brain. They saw that the sister neurons, identified by their fluorescent label, were more likely than randomly selected neighboring neurons to respond to similar stimuli. As predicted, the sister neurons not only shared a parent cell but were functionally related as well.

Of course, seeing that the sisters share a function is very different from understanding how that happens. One hypothesis is that the sister cells remain physically connected via a shared radial glial cell, which guides their movement during development. To find out whether each neuron retains some physical linkage to other sister cells from the same parent cell, the team injected a drug (or in separate experiments, a mutant protein) to disrupt gap junctions, which are channels that connect the cytoplasm of one cell to another. Without the gap junctions, the sister neurons no longer shared a function, showing that the functional relationship depends on a physical one.

"It was a pretty simple idea, but important," Dan says about the work, published June 7, 2012, in *Nature*. The experiments support a long-standing model of how the mammalian cortex is organized, she explains. In the 1950s, scientists discovered that the neurons in the cortex are organized in columns, somewhat like the ribs of a fan, with each column responding to a particular type of stimulus. When Pasko Rakic at Yale University found that sister neurons also line up in columns that parallel the functional columns, he proposed that the developmentally related columns form the basis of the functional ones and dubbed it "the radial unit hypothesis."

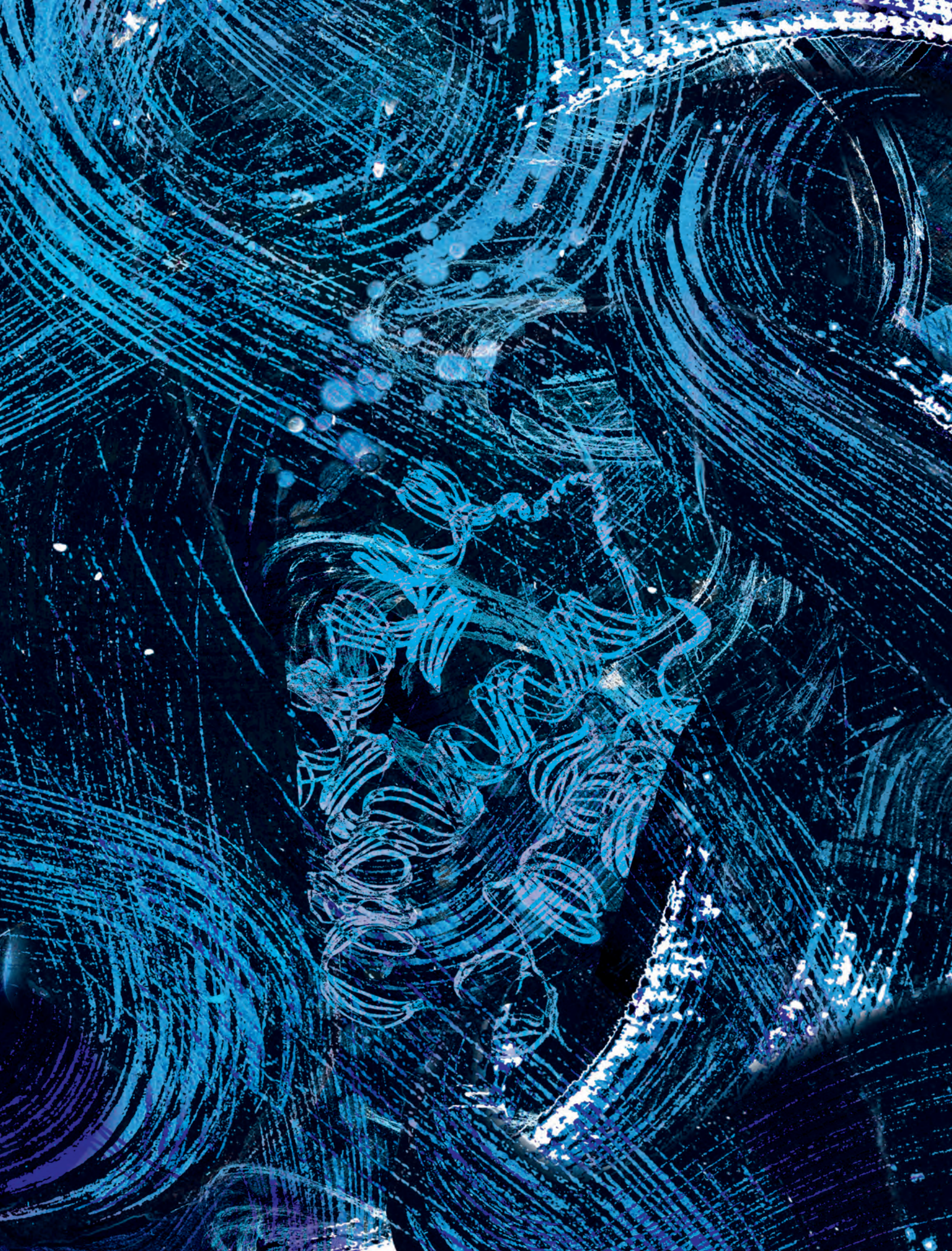
The work from Dan's team, done in collaboration with Shi's group in New York, is the first hard evidence supporting that model. "There were some hints, but this was proof in the intact system," Dan says. "Columnar organization really talks about the function, and that is something you can look at only in live animals."

Dan continues to explore how neurons work together in the mammalian brain. Her team recently turned its sights on inhibitory neurons, which modulate how other neurons respond to a stimulus. They reported in the August 16, 2012, issue of *Nature* that of three molecularly identifiable types of inhibitory neurons in the visual cortex, one type helps fine-tune the range of stimuli that its partner neurons respond to.

Dan also has begun probing the function of the prefrontal cortex, the region of the brain responsible for more complex reasoning. The fine-scale circuitry of the prefrontal cortex has been largely unknown territory, but with her cell-labeling technique—adapted from Shi's work—she is poised to explore at the level of individual neurons.

> "If I had missed that seminar, we would not have thought about the sister-neuron project at all."
>
> YANG DAN

When Dan thinks about Shi's seminar now, she pauses and her voice drops just a bit. "It was a pretty fateful moment. If I had missed that seminar, we would not have thought about the sister-neuron project at all. It really opened up a big line of research for us." ■ –RABIYA TUMA



A STRUCTURAL REVOLUTION

A quarter century ago, structural biology was stalling. HHMI created a way to energize it.

By Nicole Kresge
Illustration by Simon Pemberton

Part 1 of 2. In the next issue, our series on HHMI's structural biology program continues with a look at some of the research that is coming out of the program.

IMAGINE

there is a way to peer deep inside a cell, past the cytoskeleton and the organelles, beyond the large molecular complexes. A technology that reveals intimate details about a single protein's structure, down to the location of its tiny carbon atoms. Now imagine that this method with the potential to unlock the secrets of biology is so obscure, expensive, and elaborate that only a handful of people can take advantage of it. This is, in essence, what structural biologists were up against in the mid-1980s.

"At that time, barely anybody could do structural biology because there wasn't enough money to get all the necessary equipment," says Thomas Steitz, an HHMI investigator at Yale University.

Steitz and his colleagues needed help, and assistance arrived in the form of an HHMI initiative. In 1986, the Institute created a program to fund structural biology research around the country. Over the next quarter century, the initiative produced three Nobel laureates (see Web Extra, "A Trio of Accolades"), five high-powered x-ray beamlines, scores of innovations in microscopy, hundreds of protein structures, and answers to long-standing questions in biology.

"From the very beginning it was a very popular program with the Trustees and it was absolutely welcomed with great delight by the structural biology community," says Purnell Choppin, who was then chief scientific officer of HHMI and became president in 1987. "Many people have told me that the Hughes program really transformed structural biology, not only in the United States but abroad as well."

The Dawn of Structural Biology

Architects like to say that form follows function—a building's shape should be based on its intended purpose. The same concept applies to the structure of biological molecules: their forms reflect their functions. Learning what a molecule such as a protein looks like can lead to ways to encourage or hinder its activity, which might be especially helpful if that protein lowers blood cholesterol levels, for example, or is part of a virus.

Unfortunately, protein molecules are much too small to be seen by light microscopes and even most electron microscopes. Structural biologists have developed technical workarounds, however. One of the earliest and most powerful techniques is x-ray crystallography, which involves the often arduous process of coaxing millions of copies of a molecule to organize themselves into a repeating three-dimensional pattern—a crystal. After working for weeks, even months, to grow a protein crystal, scientists then pelt it with intense beams of x-rays, thereby destroying their hard work but also obtaining valuable data.

Each atom in the crystal scatters the x-rays, producing what's called a diffraction pattern. By rotating the crystal in the beam, scientists can gather diffraction data from many angles. With help from a high-powered computer, the data are translated into a three-dimensional map of the coordinates of each of the molecule's atoms.

Linus Pauling and Robert Corey at the California Institute of Technology were the first scientists to use x-rays to probe the structures of amino acids—the building blocks of proteins. Combined with information from other groups, what they found was simple, yet profound: an elegant spiral of amino acids called an alpha-helix—one of the fundamental structures found in almost all proteins. They published their results in 1951.

Less than a decade after Pauling and Corey's remarkable discovery, Max Perutz and John Kendrew of Cambridge University went bigger. They solved the structures of the proteins hemoglobin and myoglobin with x-ray crystallography, a feat for which they were awarded the 1962 Nobel Prize in Chemistry.

"There were a number of rods in the original myoglobin structure and everyone believed those rods were alpha-helices," recalls David Davies, a structural biologist at the National Institutes of Health who was at that time a visiting scientist in Kendrew's lab. "Pauling had proposed the alpha-helix in 1951 but no one had actually seen one. So, in 1959 John and I [analyzed] a section through one of these rods in a higher resolution model of myoglobin and there was an alpha-helix. It was fantastic."

In addition to publishing his work in the *Proceedings of the Royal Society of London*, Kendrew described the myoglobin structure in a 1961 *Scientific American* article. To help nonscientists understand this groundbreaking discovery, he enlisted the talents of scientific illustrator Irving Geis to create the first molecular illustration meant for a general audience (see Web Extra, "Illustrating the Invisible").

The '80s Tech Boom

Those first few discoveries made clear that x-ray crystallography would be a huge player in deciphering the nature and function of molecules. Although it took Kendrew more than 10 years to deduce the structure of myoglobin, subsequent technological advances sped the pace of discovery. "When I first started as a postdoc, if you could determine a structure in three to five years you were doing well," recalls Brian Matthews, a biophysicist and HHMI alumnus at the University of Oregon. "By the time I came to Eugene in 1970 to start my own lab, the first structure we worked on took three of us a year. That was considered extraordinarily quick."

"The 1980s were a time when a lot of the technologies that are now the backbone of structural biology and crystallography were

introduced," says Johann Deisenhofer, an HHMI alumnus at the University of Texas Southwestern Medical Center. By the middle of the decade, three developments had pushed crystallography into its heyday. The first was the recombinant DNA revolution. Genetic research had finally made it possible to clone DNA and make ample amounts of any protein. "It was a wonderful moment because we recognized that we were going to be liberated from the constraint of working on proteins that happened to be very abundant," says Stephen Harrison, an HHMI investigator at Harvard Medical School.

The second advance was the availability of computers that could handle the complex algorithms that turned a diffraction pattern into a molecular map. It became possible to do scientific computations that were unthinkable in Kendrew's day.

Third, and perhaps most significant, was the availability of a powerful new source of x-rays: the synchrotron. These massive machines fling subatomic particles faster and faster around a huge ring—about the size of a football field—until they approach the speed of light. The powerful radiation emitted by these flying bits of matter can produce x-rays about a thousand times stronger than the ones created in the average laboratory, allowing scientists to speed up their data collection by as much as 100-fold. "This was very important because it turned out in the long run that a lot of our laboratory-based x-ray facilities were not good enough for the job," says Deisenhofer.

Paul Fetters

Purnell Choppin, HHMI president emeritus, recalls the enthusiasm among the Trustees and the scientific community for the Institute's commitment to structural biology.

Breaking the Barrier

By 1985, nearly 200 protein structures had been solved, almost all of them by using crystallography. Despite this incredible progress, the field was stalling. The technology was there, but it was elaborate, expensive, hard to use, and often inaccessible.

In a 1985 report to the Board of Trustees, HHMI President Donald Fredrickson wrote, "Soon the access to [the technologies] and the paucity of persons trained to use them will be the critical barrier to continued progress in cell biology."

The situation prompted Fredrickson to assemble a committee to determine what the Institute could do to break through this barrier. Davies and seven other structural biology experts met in Boston on a Saturday in early March. They spent the day evaluating the state of structural biology and deliberating about how HHMI could support its development. The final verdict: The Institute should create several structural biology laboratories at research hospitals and medical schools around the United States, each associated with an existing HHMI "unit." Each of the new laboratories would have 1 or 2 principal investigators and a team of 6 to 10 associates, all funded by HHMI. The cost of purchasing and maintaining all the necessary equipment—computers, microscopes, x-ray generators—would be covered. The intention was to make the resources available to HHMI investigators and other scientists at the universities as a way to bolster the field as a whole.

The Trustees supported the scientific leadership's decision, allocating about $25 million initially and promising $60 million over the next five years. Structural biology became the fifth major area of research for HHMI, joining cell biology and regulation, genetics, immunology, and neuroscience. Despite the prevalence of x-ray crystallography, the new program also committed to supporting emerging technologies such as electron and optical microscopy, magnetic resonance imaging, and nuclear magnetic resonance (NMR).

"Crystallography wasn't the only tool in the world, but it was the dominant tool," says Purdue University's Michael Rossmann, who was then a member of HHMI's Scientific Review Board. "The labs that were funded were fairly solid crystallographic labs, but many of them have blossomed out to using other tools as they became available."

Eight scientists at six institutions were selected for the program: David Agard and John Sedat at the University of California, San Francisco (UCSF); Stephen Harrison

and the late Don Wiley at Harvard University; Wayne Hendrickson at Columbia University; Florante Quiocho at Baylor College of Medicine; Stephen Sprang at the University of Texas Southwestern Medical Center; and Thomas Steitz at Yale University.

HHMI also agreed to support the creation of a protein crystallography facility at the National Synchrotron Light Source at Brookhaven National Laboratory. More scientists would now have access to a high-intensity x-ray source (see Web Extra, "Accelerating Discovery").

A Torrent of Findings

The initiative worked and had a cumulative effect.

"Yale already had a center for structural biology that included five senior investigators studying diverse problems," says Steitz. "HHMI provided technical support, technicians, equipment, and soon there were about a hundred postdocs and students who were using the Yale facility."

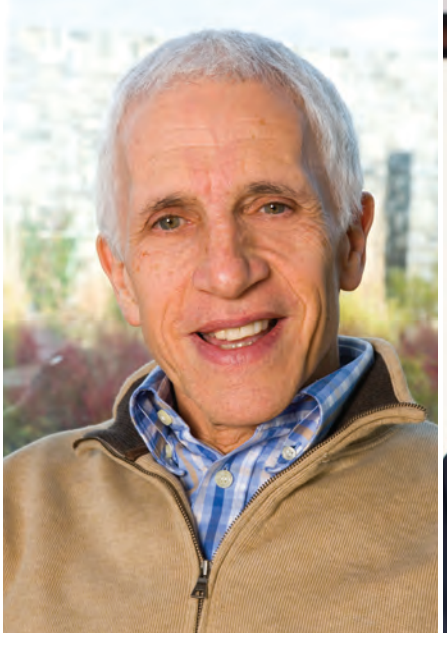
Collaborations, and even new facilities with new tools, took hold. "Because I had one foot in the medical faculty, I started interacting collaboratively with a variety of scientists around the medical school," says Harrison. "The dean asked me to help lead a small center for structural biology in the medical school to try to enhance its presence. I decided that one of the things this new center should do was spearhead a modest initiative in cryoelectron microscopy."

It wasn't long before, as Davies recalls, "the findings started coming out in torrents."

In 1987, just one year into the initiative, Don Wiley at Harvard University answered a central question in immunology. For decades, scientists had wondered how the immune system could tell the difference between normal healthy cells and infected cells. They knew that a molecule called the major histocompatibility complex (MHC) played a role in tagging the unhealthy cells. But how did MHC flag down the passing T cells that would trigger an immune response? Using x-ray crystallography, Wiley and his student Pamela Björkman, who later became an HHMI investigator, showed that MHC contains a deep groove—perfect for cradling a short piece of protein, much like a hotdog in a bun. He surmised that MHC uses the groove to present foreign peptides to the T cells, which recognize the non-native bits of protein as a signal to act.

X-ray crystallography also proved to be an extremely powerful tool for obtaining information that could be used in fighting viruses. "There was an intense focus on HIV at the time," says Harrison. "This was only shortly after the discovery of the virus that causes AIDS; there were no adequate drugs, and there was still a scramble to understand as much as possible about the virus and its properties to assist in thinking about therapeutics." Wiley and Harrison decided to use the flexibility provided by HHMI funding to devote some of their joint effort to work on HIV.

Viruses usurp normal cellular processes to slip inside a cell and hijack its molecular machinery. One of the weapons that allows HIV to gain entry into a host cell is a molecular complex called gp120/gp41. The virus uses the complex to clamp onto a cell surface molecule called CD4. Wiley and Harrison focused their efforts on this entry process. Their work revealed some of the radical changes in shape that gp120 and gp41 undergo when the complex binds to CD4. Conformational changes in gp120 alert gp41 that it is time to go through its own transformation, which, in turn, launches a series of events that lead to the membrane fusion necessary for the virus to enter the host cell. Other HHMI investigators contributed crucial components of this picture, notably Wayne Hendrickson and his student Peter Kwong. According to Harrison, these early HHMI-based structural studies have become one of the foundations underlying current work on HIV vaccines.



Stephen Harrison's program at Harvard revealed how HIV changes shape to enter a host cell and Tom Steitz at Yale studied the virus's enzyme reverse transcriptase.

Another of HIV's armaments is reverse transcriptase. This enzyme allows the virus to merge its genome with that of its host, tricking the unsuspecting cell into producing new virus particles to invade neighboring cells. Fortunately, reverse transcriptase has a fatal flaw—it isn't normally found in human cells, making it an ideal target for drugs. But the enzyme mutates very rapidly—another strategy that results in a very elusive quarry.

In the late 1980s, Steitz began work on solving the structure of reverse

Harrison: Paul Fetters Steitz: Brian Ach / AP, ©HHMI

"RIGHT NOW, WE ARE AT THE POINT WHERE THE TECHNIQUES HAVE BECOME ALMOST PERFECT." – JOHANN DEISENHOFER

transcriptase bound to the experimental drug nevirapine. When nevirapine was discovered, it fit snugly into a pocket on reverse transcriptase where it easily blocked the enzyme's activity. It wasn't long, however, before reverse transcriptase mutated, making the drug useless. Steitz's crystal structure uncovered the reason why. Several parts of the pocket had changed shape, and the drug could no longer bind and inhibit the enzyme's activity. Luckily, several other areas of the pocket were unaltered, and they were used to create a new version of nevirapine.

But How Does It Fold?

A phenomenon that puzzled structural biologists for decades is how a string of amino acids can fold into an orderly three-dimensional shape like Pauling and Corey's alpha-helix. Brian Matthews, who became an HHMI investigator at the University of Oregon three years after the program started, set about answering this question. Using a protein called phage T4 lysozyme, he methodically substituted each of its amino acids with a different amino acid and then compared the resulting three-dimensional structures using x-ray crystallography.

His conclusion? Proteins are very tolerant. "Many people had the idea that a protein structure is very complicated—that there's very fine balance between the unfolded and the folded protein," says Matthews. "People believed that if you just randomly made substitutions here and there you would probably prevent folding or at least seriously compromise the protein." Matthews found the contrary: He made multiple substitutions at many sites and the three-dimensional structure was not changed at all. The amino acids on the surface of the protein were especially forgiving—Matthews changed many of them and the protein remained active.

David Agard, an HHMI investigator at UCSF, tackled the protein-folding problem with a different technique—NMR spectroscopy. This powerful method is useful for looking at smaller proteins in buffer solutions. The molecules are exposed to a giant magnet that causes their nuclei to absorb and re-emit electromagnetic radiation. This emitted energy gives clues about each atom's orientation and location in the protein.

Agard chose a small bacterial enzyme, called alpha-lytic protease, to look at the different shapes a protein assumes as it folds into its final active form. Normally, these intermediate structures are difficult to examine because they're so unstable and transient. But Agard discovered that if he cut off a piece of the enzyme called the proregion, the protease would be frozen in an intermediate form, which could easily be visualized with NMR spectroscopy. His experiments revealed a surprise: The proregion helps alpha-lytic protease fold into a very unstable, high-energy form before assuming its final active shape.

Not ones to shy away from new technology or big projects, Agard and his UCSF colleague and then HHMI investigator John Sedat also developed several microscopy techniques to help them look at protein machinery at work inside cells.

"Our HHMI support allowed us to make leaps in technology that wouldn't have been possible any other way," says Agard. "At the time, the electron microscopists generally weren't doing big complex cellular things. So we had to combine methodologies and formulate new strategies for collecting and processing data in three dimensions."

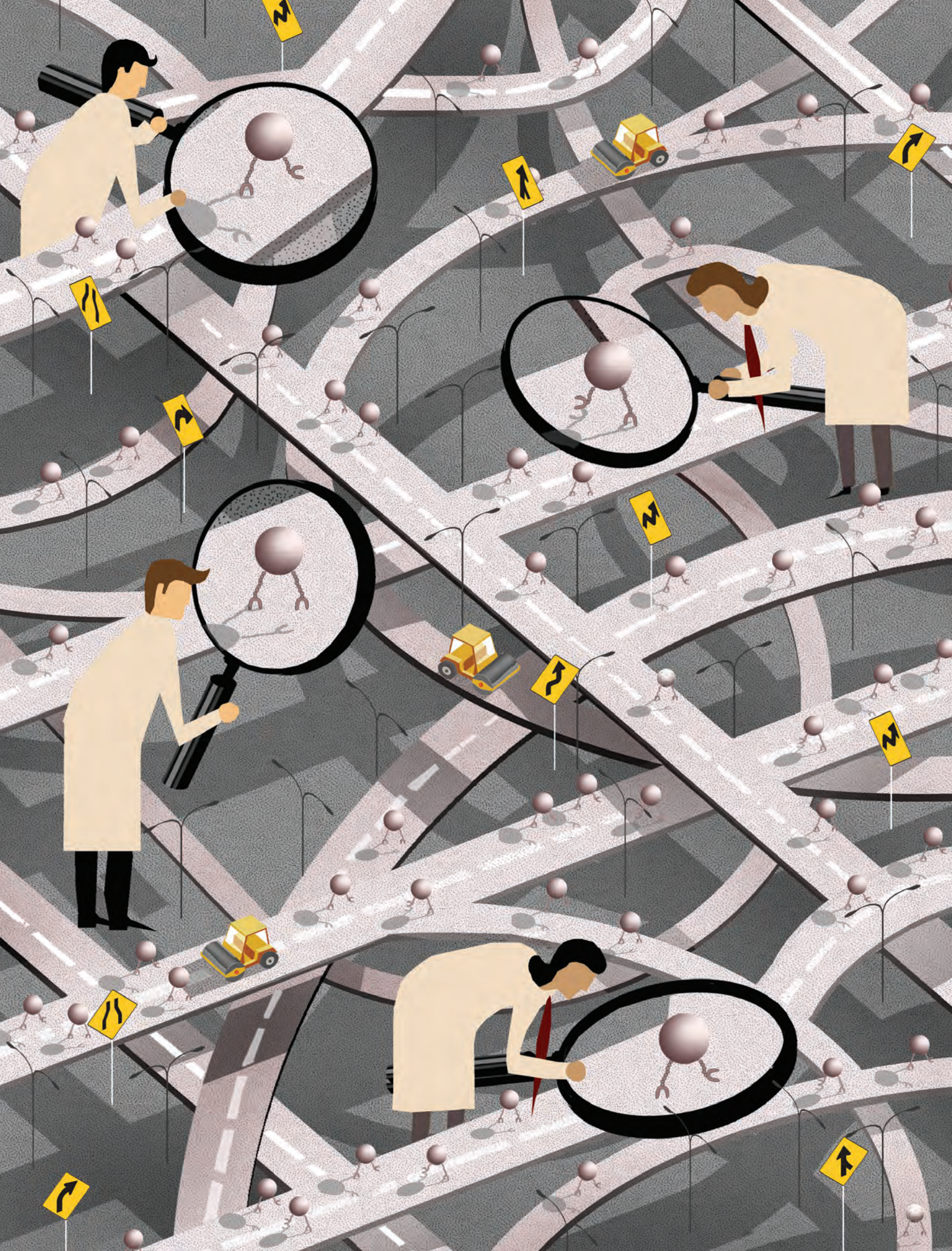
One of the techniques they pioneered was cryoelectron tomography, which involves flash-freezing a cell, photographing it from different angles, and combining the photos to create a three-dimensional model of the cell's contents. Agard and Sedat used this technique to examine a molecular complex called the centrosome, which is responsible for ensuring that equal numbers of chromosomes are distributed to the mother and daughter cells during cell division. Their images revealed just how the centrosome goes about organizing microtubule fibers in the cell and how the chromosomes then line up on the fibers and move to their respective ends of the dividing cells.

The Shape of Things to Come

Today, there are 36 investigators at 24 institutions in the HHMI structural biology program. More than 86,000 protein structures have been solved and submitted to the Protein Data Bank, an international repository for structural data. "Right now, we are at the point where the techniques have become almost perfect," says Deisenhofer. "As soon as you have a crystal you can almost certainly determine the structure in a relatively short amount of time. It's become a standard technique in many laboratories."

In the coming years, emerging technologies are likely to increase the number and type of molecules that can be studied. For example, a team of scientists led by HHMI investigator Axel Brunger at Stanford University is using a free-electron laser that shoots x-rays at very small crystals of proteins that are hard to crystallize (membrane proteins, for example), opening up a world of structures that had been off-limits for x-ray analysis.

(Continued on page 40)



ONE FOOT IN FRONT OF THE OTHER

INSIDE CELLS, SPECIALIZED PROTEINS MARCH DOWN A NETWORK OF HIGHWAYS CARRYING MACHINERY AND MESSAGES. RESEARCHERS ARE LEARNING REMARKABLE DETAILS OF HOW THEY MANAGE IT.

BY SARAH C. P. WILLIAMS
ILLUSTRATION BY ADAM SIMPSON

To get from Boston to San Francisco, a person has a few choices: drive a car, hop on a bus, fly in an airplane, hitchhike from city to city, maybe even ride a bike. Circumstances will dictate which way works best. If the traveler is in a hurry, the bus might take too long. Carrying a large suitcase makes a bike impractical. And if the person is short on cash, a plane ticket may be too expensive.

In every type of living cell, materials jet around in a similar variety of manners. The way cellular cargo travels depends on its size, where it's headed, how quickly it must arrive, and how much energy is available. Some chemicals circulate passively through a cell, with no need for energy or a road, but others—like building materials needed at the end of a growing cell or chemical messengers that must reach the nucleus—need to move quickly toward a set destination.

Beyond that, some of the goods must first be packaged and then picked up by vehicles that follow an ever-changing highway to the right destination.

In the past half-century, scientists have revealed how cells build these thoroughfares, and they've uncovered specialized proteins that walk along the roads' lengths carrying freight. But plenty of questions remain: How does the cell control the transportation? How do the walking proteins coordinate their steps to keep grounded on their tracks? How can materials hitch a ride on cellular freeways if there isn't energy to spare?

"It's become clear that there is an enormous platter of movements that have to be executed by the cell," says HHMI investigator Ronald Vale of the University of California, San Francisco. "Chromosomes have to be separated, a cell has to pinch in two, materials made in one place have to be delivered to another place in the cell. All of those features of life are dependent on physical motion."

That physical motion is generated by three types of molecular motors that can walk down tracks inside cells: myosin, which walks on actin filaments, and two microtubule motors—kinesin, which carries cargo from the center of a cell outward, and dynein (the largest and least understood), which carries cargo from the periphery toward the cell's center. Most of the motors in the cell have two "feet," which alternate steps as they move. But each protein also has distinct quirks in its movement, a unique form of regulation, and a different role in keeping cells alive.

For devising ways to study molecular motors in detail, Ronald Vale, along with colleagues Michael Sheetz and James Spudich, received the 2012 Lasker Basic Medical Research Award.

Ryan Anson / AP, ©HHMI

Research by HHMI investigators and others has revealed that when any of the molecular motors fails, it causes not only traffic jams and lost messages but also faulty construction and demolition of the cells' roads, and that can lead to disease. Understanding the process better, scientists think, can help them learn how to rev up the engines of the motors, keep their steps on track, and rebuild the transport systems that are needed to keep a cell alive.

THE RIGHT MOTOR FOR THE JOB

Since the dawn of microscopy, scientists peering into the innards of cells have seen many moving parts. The earliest experiments on mobile proteins studied muscle cells, an obvious place to look for molecular movement. More than 50 years ago, scientists isolated two proteins—myosin and actin—from muscle cells. Andrew Huxley and Hugh Huxley (no relation) independently proposed that actin thin filaments slide across myosin thick filaments in the presence of the cellular energy molecule ATP. As this idea gained traction, it also became clear that isolated molecules of myosin could walk along actin filaments, suggesting a way that materials in the cell could

"THERE IS AN ENORMOUS PLATTER OF MOVEMENTS THAT HAVE TO BE EXECUTED BY THE CELL." – RON VALE

be transported as well as providing a way to study myosin motors (see Web Extra sidebar, "Stepping Back in Time").

By the mid-1980s, scientists at Stanford University were using a microscope to watch myosin carry plastic beads along actin filaments in non-muscle cells. Vale, a graduate student at Stanford at the time, got caught up in the excitement of seeing cellular movement and wanted to try the same experiments on proteins from nerve cells, where materials could be seen moving through the cells' long axons. He expected to turn up myosin as the vehicle responsible for this transportation. Instead, Vale, together with Mike Sheetz and Tom Reese, isolated another molecular motor—kinesin—and they began focusing their attention on it.

Within 10 years, Vale and colleague Robert Fletterick had solved the structure of kinesin, helping to explain the molecular underpinnings of how the molecule walks along microtubules, dynamic tubes that run throughout cells. And he showed that the three-dimensional arrangement of atoms that make up kinesin was highly comparable to that found in myosin. "By using what was known about myosin," says Vale, "we could bootstrap experiments and apply prior knowledge on myosin to understand the workings of kinesin, the newer kid in town."

Kinesin and myosin have globular heads that attach to their tracks and flexible tails that extend outward and carry cargo. The proteins' heads break down ATP to convert energy into work. For each ATP consumed, the motor protein takes a step forward (see Web Extra animation).

Shortly after Vale's discovery of kinesin, he and his colleagues found evidence for another motor that moved along microtubules in the opposite direction. Two years later, Richard Vallee discovered that this second motor protein was dynein, which had been identified decades earlier for its role in flagella—whiplike tails that can propel entire cells, such as sperm. This newly discovered dynein—called cytoplasmic dynein—transports cargo along microtubules, serving a different function from the dynein that propels cells. The key difference between cytoplasmic dynein and most kinesins is the direction of transport: dynein molecules move along microtubules pointed at the cell's center; kinesin walks outward. Having a distinct molecule for each task lets cells fine-tune traffic control.

Cytoplasmic dynein, though, looks different from myosin and kinesin. It has a very large wheel-like motor domain that binds multiple ATPs and a more complex tail region to connect to cargo. Because of its large size and complex structure, it has been harder to study than myosin and kinesin. For Vale, the challenge is enticing—since 2002, his lab has primarily focused on studying dynein.

"One of the really intriguing parts of dynein is that the key nucleotide binding site is a very large distance from the microtubule binding site," says Vale. The distance between dynein's ATP-binding site and its feet is four times the size of the whole kinesin molecule, he says. So how does the energy-generating portion of the protein communicate with the walking feet?

In 2011, Vale's team solved the crystal structure of dynein, more than a decade after the structure of kinesin, and got some hints as to how the long-distance communication could work. They discovered a buttress—a section that supports the lanky top of the protein—that may be involved in transferring information from the ATP-binding head to the protruding feet that bind to the microtubule track. But questions remain about how dynein takes steps with a structure that's so different from the other two motors.

"At this point, we can't articulate a complete model of how dynein produces motion," says Vale.

This much is known: when dynein or kinesin stops carrying goods across the cell, the cell stops functioning. Mutations in kinesin have been linked to kidney disease and an inherited neuropathy. Mutations in dynein are involved in motor neuron degeneration and can cause chronic respiratory infections (because the movement of mucus through the respiratory tract is inhibited). In 1997, Vale along with three colleagues launched a drug-development company, Cytokinetics, based on their research on molecular motor proteins. Their first drug, omecamtiv mecarbil—an activator of cardiac myosin—is in phase 2 clinical trials for the treatment of heart failure. The company also is conducting early tests of a treatment for amyotrophic lateral sclerosis, or Lou Gehrig's disease.

At Children's Hospital Boston, HHMI investigator Elizabeth Engle has stumbled upon another class of diseases linked to

"MICROTUBULES ARE OFTEN CONSIDERED THE PASSIVE TRACK FOR MOVEMENTS TO OCCUR ON. BUT IN THE CELL THEY ARE EXTREMELY DYNAMIC." – EVA NOGALES

mutations in molecular motors and their roadways. She studies inherited eye disorders in which the muscles of the eye don't develop properly. The root of the problem, her lab has discovered, is that dynein and kinesin don't properly carry messages up and down the microtubules of growing neurons. The consequence: the neurons don't connect to the correct muscle tissues. In genetic screens designed to pinpoint the cause of this problem, Engle's lab has revealed mutations in the proteins that make up microtubules as well as in a specific type of kinesin. Now, they're probing how the kinesin mutation changes the motor protein's function.

"These human genetic studies are highlighting amino acid residues vital to specific functions of both kinesins and microtubules," says Engle. "Thus, we can translate the human findings backward to enlighten more basic studies of these proteins and their interactions."

TALES FROM THE ROAD

What about the other side of the cell cargo story, the roads themselves? Within the cell, road construction—and destruction—is an around-the-clock, nonstop job. To control the flow of goods between its neighborhoods, a cell is as likely to shut down or open new roads as it is to impose changes on vehicles.

"Microtubules are often considered the passive track for movement to occur on," says Eva Nogales, an HHMI investigator at the University of California, Berkeley. "But in the cell they are extremely dynamic."

As a model for understanding how microtubules shrink and grow, Nogales studies mitosis, the process by which a cell copies and divides its genetic material to separate into two cells. Once the cell divvies up its chromosomes—the structures that contain the genetic material—microtubules pull the chromosomes to opposite sides of the cell. Kinetochore proteins enable this movement by linking microtubules to a special region in each chromosome and then remaining attached as the microtubules shrink or grow. When the microtubules shorten at the proper time, the two copies of each chromosome—each attached to one set of tubules—separate. Nogales wants to know how the cell regulates this process and how the many other steps in mitosis are coordinated with microtubule arrangement and movement. Using cryoelectron microscopy, her lab group has shown how kinetochore proteins bind to microtubules and allow movement of chromosomes.

"Somehow there has to be a feedback between the checkpoint that allows mitosis to proceed and the microtubules," says Nogales. That feedback is mediated by phosphorylation of the protein complexes that tether microtubules to the chromosomes. To visualize the effect of this phosphorylation on the microtubule-chromosome attachments, Nogales has turned again to cryoelectron microscopy.

"Our studies are ultimately just snapshots in a movie that is very dynamic," says Nogales. As technology evolves, she says, the process will become even clearer (see Web Extra movie).

Like research on kinesin and dynein, understanding the role of microtubules in mitosis has applications in human health. For example, stopping the rearrangement of microtubules during cell division, or the separation of chromosomes, is one way to halt the out-of-control growth seen in cancer cells. Already, the drug Taxol (paclitaxel) is being used to treat some types of cancer, where it stops microtubules from rearranging, thus blocking mitosis and cell division. The company Cytokinetics is investigating additional drugs that target kinesin motors for treating cancer.

And as Nogales has probed deeper into the biological details of microtubules, she's realized that their processes for shrinking and growing provide an interesting means of intracellular transportation of cargo. Her goal is to understand these processes at the molecular level.

"In addition to microtubules enabling the movement of these motor proteins, they're also growing and shrinking themselves at the same time," explains Nogales. "So the cell can actually couple this growing and shrinking with the movement of materials. They do this by a process that we are barely starting to understand."

Scientists have shown, she says, that as microtubules grow, some proteins hop on and off the tips of the developing roads. It's like getting a ride across the country by grabbing onto the back of a cement roller as it builds a new road, rather than paying for a bus. Many of the proteins that hitch a ride in this way are ultimately involved in contact with the outer membranes of cells. By riding the tip of a growing microtubule, a protein is assured a prime spot at the membrane when the microtubule reaches it. The process, Nogales thinks, could allow proteins to move to a distinct location without using energy.

CELLULAR GPS

Proteins at the growing ends of microtubules also likely help the microtubules find their targets. At the University of California,

San Francisco, HHMI investigators Lily Jan and Yuh Nung Jan have discovered one apt example. The Jans study ion channel proteins located in neurons' plasma membranes, which selectively allow potassium in and out of the cells. They knew that, after the channel proteins were produced inside the cell, the proteins were delivered to the membrane by hitching a ride on kinesins that stepped along microtubules to the cell's edge. But they were puzzled to see that the channel proteins were always delivered to clusters of the proteins specifically located in the axons of neurons. How did the microtubules know in which direction to head?

"In central neurons like the motor neurons, across the board evolutionarily, the channel goes down to the same spot," says Lily Jan. "In giant squid, in humans, in mice. So we wanted to know how this destination is reached."

The Jans discovered that a protein called EB1 is key—without it, the microtubules guiding the channels' paths don't reach the right spot. Their lab group has gone on to show that EB1 is also important in guiding channel proteins to the right spot in heart cells.

Not all neuronal proteins, of course, are delivered to the channel cluster that the Jans study. In fact, most neurons are heavily dotted with another common delivery site: synapses—the structures between cells that neurons use to communicate. So microtubules and motor proteins must find an astonishing array of locations.

"You can imagine kinesins as cars driving the length of microtubule roads," says HHMI investigator Kang Shen of Stanford University. "But not every car will drive to the end of the road; they'll get off at different exits." Shen has discovered that kinesins, not surprisingly, are critical to placing the synapses at different exits along the roads that traverse a neuron's axon. Kinesins carry synaptic vesicle precursors—sacs containing the components of synapses—along microtubules and then distribute them at different points on the axon.

"We've found that when kinesins have some mutations, they appear to drop off their cargo too early," says Shen. "Other mutations do the opposite, traveling the whole length of the road without depositing the cargo." In his latest work, Shen has shown that kinesins also carry microtubules to ensure they are oriented correctly in the dendrite, where neurons receive chemical messages. This finding, slated to be published soon, illustrates the diverse jobs that molecular motors have, he says, and the complexity of their functions. He plans to use synapse placement as a system to study how kinesins are regulated in the cell to carry cargo to precise locations.

Throughout mammalian cells there are 45 types of kinesins, 40 versions of myosin, and at least 14 different dyneins. Each carries goods—ranging from entire organelles, such as mitochondria and large chromosomes, to signaling chemicals—to a distinct destination. Yet the various motor proteins display small differences in how they process ATP, how they step along actin or microtubules, what cargo they can carry, and how their function is regulated. Now that the basic structure of each has been elucidated, and the complexity of their pathways revealed, scientists are primed to delve into questions about these differences and the regulation of each.

"We have these 45 kinesins that are involved in an enormous range of biological activities," says Vale. "And for the vast majority of them, we don't understand how they're deployed or targeted within cells."

But as the implication of gaining knowledge about these motor proteins becomes clearer, the interest in them is growing, says Vale. "This field has broadened as many more scientists have become involved," he says. "People studying cancer or signaling or developmental biology often encounter some kind of molecular motor that is relevant to their research problem. I think that will continue to be true." The road to understanding molecular motors, he says, is far from over. ■

Eva Nogales studies how proteins hitch a ride on microtubules as they shrink and grow. Kang Shen studies the way kinesins deliver cargo essential to nerve signaling.

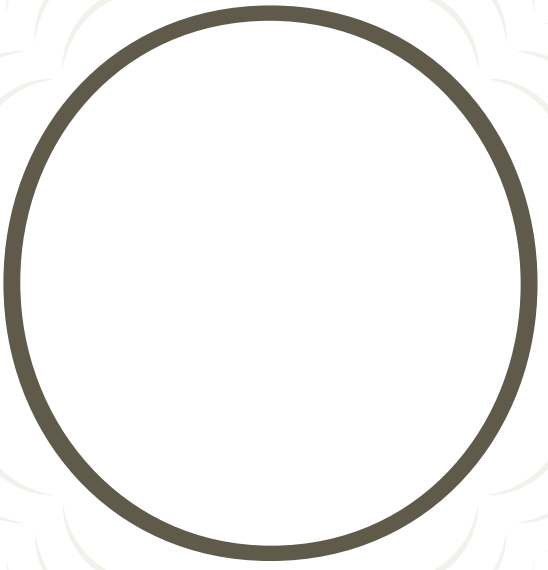
Nogales: Ryan Anson / AP, ©HHMI Shen: George Nikitin / AP, ©HHMI

WEB EXTRA: To read more about the history of molecular motors research, and to see a movie and animation of motors in action, go to www.hhmi.org/bulletin/winter2013.

A HAPPY OASIS

Once Sangeeta Bhatia created her lab the way she wanted—as a supportive, intellectually challenging environment—the creative juices began to flow.

by Jennifer Michalowski • photography by Jason Grow



On Fridays, the usually tranquil space of HHMI investigator Sangeeta Bhatia's office at the Massachusetts Institute of Technology (MIT) gets a little crowded. The room fills with energy as her team gathers in two groups—liver researchers first, then the cancer team—and clusters, about 10 at a time, around a table better suited for 6. Her students and postdoctoral fellows have her full attention as they share new data and devise future experiments, jumping up occasionally to sketch out an idea on the room's white board. "I love Fridays," Bhatia says. "They're nothing but science."

Bhatia's lab group works at the intersection of technology and medicine. Her research program has two main goals: engineering a lab-grown liver that can one day be implanted into patients, and using nanomaterials to design better ways to detect tumors and deliver therapeutics to cancer cells. Her group is attacking these problems—plus a few others—from several angles, integrating the tools of tissue engineering, materials science, and microfabrication.

How can members of a group that is simultaneously studying liver biology, tissue regeneration, and cancer, not to mention stem cells and infectious disease, find common ground? "Everyone here is working on something very, very different," points out one member. But there is a constant exchange of ideas among the biologists, chemists, computer scientists, and engineers who gather in Bhatia's office. All are eager to learn from one another, and they often make unexpected connections and come to creative solutions.

That's because a collaborative nature is a prerequisite for joining Bhatia's lab. It's not enough for a job candidate to have the right scientific knowledge or technical skills: All members of the lab must weigh in on each potential member, and they look for people who will contribute to the amiable environment for which the lab is known. "We've turned away smart, ambitious people because we didn't think they'd be good citizens," Bhatia says.

To keep her team motivated as they work to create better solutions for patients, Bhatia is intent on crafting a supportive and sustaining environment for herself and the members of her lab. Curiosity, innovation, and a drive to improve human health are prized. So too are time and energy to spend outside the lab. "I want this to be a happy little oasis, a place where everyone wants to come," she says of the scientific community she oversees.

As a student at MIT and Harvard Medical School, studying both bioengineering and medicine, Bhatia didn't exactly dream about running an academic lab of her own. Her professors seemed harried, her labmates worked through the night, and the intensity and competitive atmosphere did not mirror the lifestyle she wanted. "When I looked up the pipeline," she recalls, "I wanted to know whether there were people who were married, had kids … were normal." And as an aspiring female engineer in the 1990s, Bhatia found few role models in academia.

Her parents, immigrants from India who placed a high value on education, had actively encouraged Bhatia's curiosity and aptitude for science and math. "As a child of Indian immigrants, there's sort of a limited menu of career choices," she observes. "My dad used to ask me, 'What are you going to be, a doctor, an engineer, or an entrepreneur?'" She was determined to become part of the new field of bioengineering by the time she was in high school, when her father brought her to a friend's lab at MIT, where researchers were investigating ultrasound therapies for cancer. "I was really captivated by the idea that engineers could use instruments to impact human health," Bhatia says. She wanted to do that, too. She just assumed she'd do it within the biotechnology industry.

Bhatia is quick to acknowledge that her career might have taken a different path. "But then someone reminded me that, as an academic, you can build the group in your image: You can make the culture one you want to live in." Her graduate work devising a system to grow liver cells in the lab had fueled her curiosity and sparked countless ideas for new experiments. So straight out of medical school, she took a faculty position in the bioengineering department at the University of California, San Diego (UCSD). She set herself up to explore her lingering questions about the liver,

"I WAS REALLY CAPTIVATED BY THE IDEA THAT ENGINEERS COULD USE INSTRUMENTS TO IMPACT HUMAN HEALTH."

added a new cancer focus to her research, and took care to surround herself with people who shared her values. "And I loved it," she says.

She returned to MIT in 2005, moving with her family back to the Boston suburb where she grew up. Her Laboratory for Multiscale Regenerative Therapies is located on the sunny fourth floor of MIT's new Koch Institute for Integrative Cancer Research. From her office, she can gesture toward many of the area labs her team is collaborating with to explore liver biology, cancer therapy, stem cells, and infectious disease. "Multiscale" means the group is working with both nanotechnologies and microtechnologies. Because of their small size, nanoparticles, so tiny that about 1,000 of them could fit across a human hair, behave differently than larger particles, and Bhatia's team is exploiting their unique electromagnetic properties in its cancer research. The microtechnologies, such as the tools they use to produce their artificial livers, are still tiny but about 1,000 times larger than nanoparticles.

PEOPLE-FOCUSED CHOICES

Bhatia has a strong instinct for matching her group's interdisciplinary strengths to some of the most troublesome clinical problems. As a graduate student, she was so fascinated with the medical courses required for her Health Sciences and Technology program that she kept taking "just one more course," until finally she decided to complete a medical degree. Now, as an "accidental doc," she speaks fluently with clinicians about the challenges their patients face and the limitations of current technology.

In deciding what research to pursue and how to go about it, Bhatia is guided by a clear objective. "Sangeeta has always been someone who wanted to make an impact, to leave the world better than she found it," says Christopher Chen, a graduate school classmate of Bhatia's who is now a bioengineer at the University of Pennsylvania. They have remained close friends, and Bhatia calls him her "science buddy," scheduling regular phone calls to consult about the rewards, frustrations, and nitty-gritty details of running a lab.

Chen recalls that during the clinical portion of their graduate training, he and Bhatia had a shared goal: to record each day in their notebooks "one really good idea" inspired by their interactions with patients. Although Bhatia was deeply into liver research by that time, "her ideas weren't just about the liver," Chen says. "They were about infectious disease, how to improve a surgical technique … all kinds of things. I think she would have made an impact no matter where she landed."

A SUPPORTIVE ENVIRONMENT—FOR THE LIVER

Bhatia's enthusiasm for her work is obvious as she speaks animatedly about the wonder of the liver, the organ she learned to love as a graduate student in the lab of tissue engineer Mehmet Toner at Massachusetts General Hospital. The pinkish-brown triangle carries out more than 500 functions in the human body, including

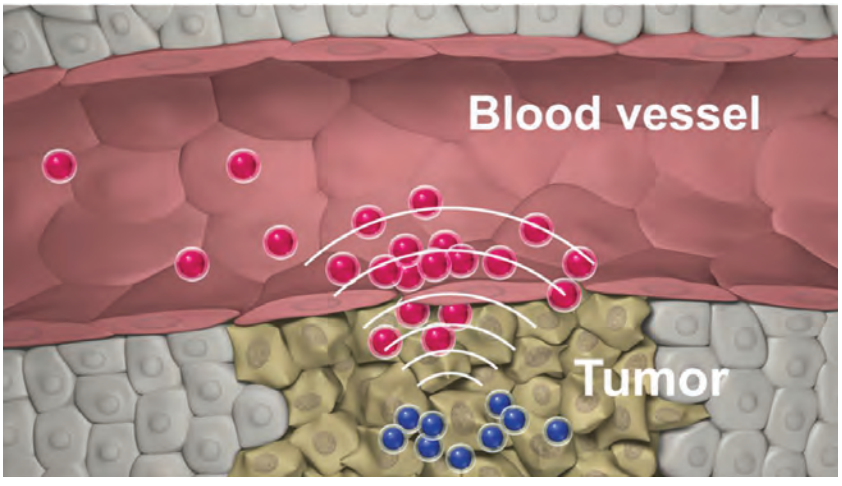
Bill Gallery

Bhatia and former grad student Alice Chen built 3-D microlivers and then implanted them in mice, where they functioned like livers.

removing toxins, generating energy, storing vitamins and minerals, and helping to regulate fats and sugars in the bloodstream. Before her graduate work, researchers faced challenges growing hepatocytes, the cells responsible for most of these functions, in the laboratory. Removed from a complete liver, the cells promptly died. In Toner's lab, Bhatia found a way to stabilize them by mimicking the liver's architecture.

In the body, hepatocytes are sandwiched between two layers of extracellular matrix and receive support from neighboring cells called stroma. Hepatocytes and stroma do not establish this structure on their own when placed in a dish, so Bhatia used microfabrication techniques to pattern tiny circular spots of collagen, a component of the extracellular matrix, onto the surface of a culture dish. She let hepatocytes establish themselves on the collagen dots and then added stromal cells. The cells thrived.

Grown in this way, the micropatterned co-cultures live for several weeks. Bhatia has refined the model since she and Toner first reported it in the *Journal of Biomedical Materials Research* in 1997, and today she and her collaborators are using it to explore what happens to liver cells as conditions such as drug exposure and viral infection progress. Rockefeller University virologist Charles Rice says that his lab used to rely on a cell line developed from cancerous hepatocytes, which poorly reflected the behavior of normal cells, to study the hepatitis C virus (HCV). When they infected the Bhatia lab's co-cultures with HCV in 2010, they had a much more reliable model of the virus, which affects about 150 million people worldwide. The two labs have continued to work together to design a fluorescent indicator that lets them identify which hepatocytes have been infected with HCV, so they can trace early cellular events in viral infection and test the effects of potential therapies.



Bhatia's cancer team designed nanoparticles that quickly zero in on a tumor, then call a swarm of drug-delivering nanoparticles to the site.

HIGH EXPECTATIONS

One challenge of the liver, Bhatia says, is that its extraordinary multitasking makes it virtually irreplaceable with anything but new liver cells. "When the liver fails, you support the person medically and put them on a transplant list," she says. "Sadly, lots of patients die on that list." Bhatia fully expects to change that—and her high expectations are infectious.

Alice Chen, who completed her Ph.D. in Bhatia's lab in 2011, was taken aback when Bhatia asked her during their first meeting what she would do if she cured liver failure by the time she graduated. But suddenly, she recalls, it seemed possible. "She just always expected excellence," Chen says. "Of course, we didn't cure liver failure, but I'm really proud of what I was able to accomplish under her guidance." Chen transformed the surface-bound liver cultures into three-dimensional "microlivers" by supporting the cells in a hydrogel matrix. The structures, which are about the size and shape of a contact lens, carry out normal liver functions when implanted in mice, according to their paper published in the *Proceedings of the National Academy of Sciences* in 2011. Chen hopes they will improve researchers' ability to test potential drugs. Now, as a lead scientist at the California biotechnology company Auxogyn, Chen says when she is presented with a difficult or discouraging problem, she often finds herself thinking "What would Sangeeta do with this information?"

Scaling up the microlivers to take over function in the human body could take decades, Bhatia acknowledges. A lab-grown liver for transplant would probably need to be 10 to 30 percent the size of an adult liver, and there are a variety of hurdles to overcome. "We're trying to work on all the major bottlenecks," she says.

One of the biggest obstacles is obtaining enough cells, which do not multiply in the lab and must come from patients. Several researchers in her lab are exploring ways to accelerate the cells' growth or direct the development of expandable progenitor cells. They're also examining the role of the cells' interactions with one another and with components of the extracellular matrix. In the meantime, Bhatia says, "We're trying to mine our inventions for near-term applications."

One of the most satisfying applications came last year when Hepregen, the company Bhatia cofounded with former student Salman Khetani in 2007, used the liver cultures to compare the effects of several compounds, including one that had been a candidate therapy for hepatitis C infection until it caused unexpected toxicity in a

"I LOVE MY SCIENCE, BUT I DON'T THINK ABOUT IT 24/7!"

clinical trial. The drug had not caused liver toxicity when tested in animals before the trial's launch, but when Hepregen tested it on human liver cells grown with Bhatia's system, toxic effects were evident. Researchers found a closely related compound that could be administered to the liver cells without toxicity and began a new clinical trial. "That was really gratifying," Bhatia says.

In her relentless quest for new opportunities, Bhatia used a sabbatical in 2008 to consult with public health experts and infectious disease specialists about how her lab might best make a difference in global health issues. She learned that the malaria pathogen *Plasmodium vivax*—less studied than its more virulent cousin *Plasmodium falciparum*—was particularly difficult to diagnose and treat because of its ability to lie dormant in the liver. After talking with malaria researcher Stephen Hoffman at the Maryland-based biotechnology company Sanaria, the researchers showed that *P. vivax* can grow inside the Bhatia lab's liver cultures. Now Bhatia's and Hoffman's groups are working to recreate the pathogen's elusive hypnozoite stage with hopes that they can use the model to screen potential antimalarial drugs.

CANCER

Alongside the liver studies, about half of Bhatia's lab is devoted to developing nanotechnologies that improve cancer diagnosis and therapy. "The program has evolved in an opportunistic way," Bhatia says. A conversation in 2000 with Sanford-Burnham Medical Research Institute biologist Erkki Ruoslahti triggered Bhatia's first thinking about cancer technologies. Ruoslahti had been screening for molecules that would adhere to the lining of blood vessels that surround tumors and healthy tissues. Bhatia and Ruoslahti realized the peptides he identified could be used to target nanoparticles to those sites within the body, and the two teamed up to begin experiments with nanosized semiconductors known as quantum dots. They soon recruited UCSD materials scientist Michael Sailor to the project and the three began introducing new properties, such as a porous or biodegradable structure, into their nanoparticles.

Today, Bhatia's cancer team is taking cues from biology to design more efficient nanoparticle systems. They are designing two-particle systems in which one particle finds the tumor and then acts as an antenna to attract a second particle designed for diagnostic detection or therapy delivery—not unlike how different cell types work together to signal and treat a microbial infection, Bhatia says. They also would like to engineer a system in which simple components can achieve complex behaviors when they come together in large numbers and are working on mimicking the kind of swarming behavior seen when birds flock and ants forage for food.

THE MYTH OF THE SCHOLAR

Despite her focus and drive, she is not interested in perpetuating "the myth of the scholar," Bhatia says. "You don't have to think about science 24/7. I love my science, but I don't think about it 24/7!"

Her students aren't entirely convinced. "She's very devoted to her iPhone," they say. "If you email her at 1 a.m., you might hear back right away." But they know those emails come late because Bhatia stays off the computer in the evenings until after her daughters' 9:00 bedtime.

"When they're awake, it's all about them," she says. The girls' soccer games and dance recitals (Bollywood is the current favorite) sometimes trump discussions of experimental design. And on Wednesdays, Bhatia works from home so she can be waiting at the school when the afternoon bell rings. "Earlier in my career, I used to tell people I was working 'off-campus,'" she says. "Now everyone knows Wednesdays are Mommy Day."

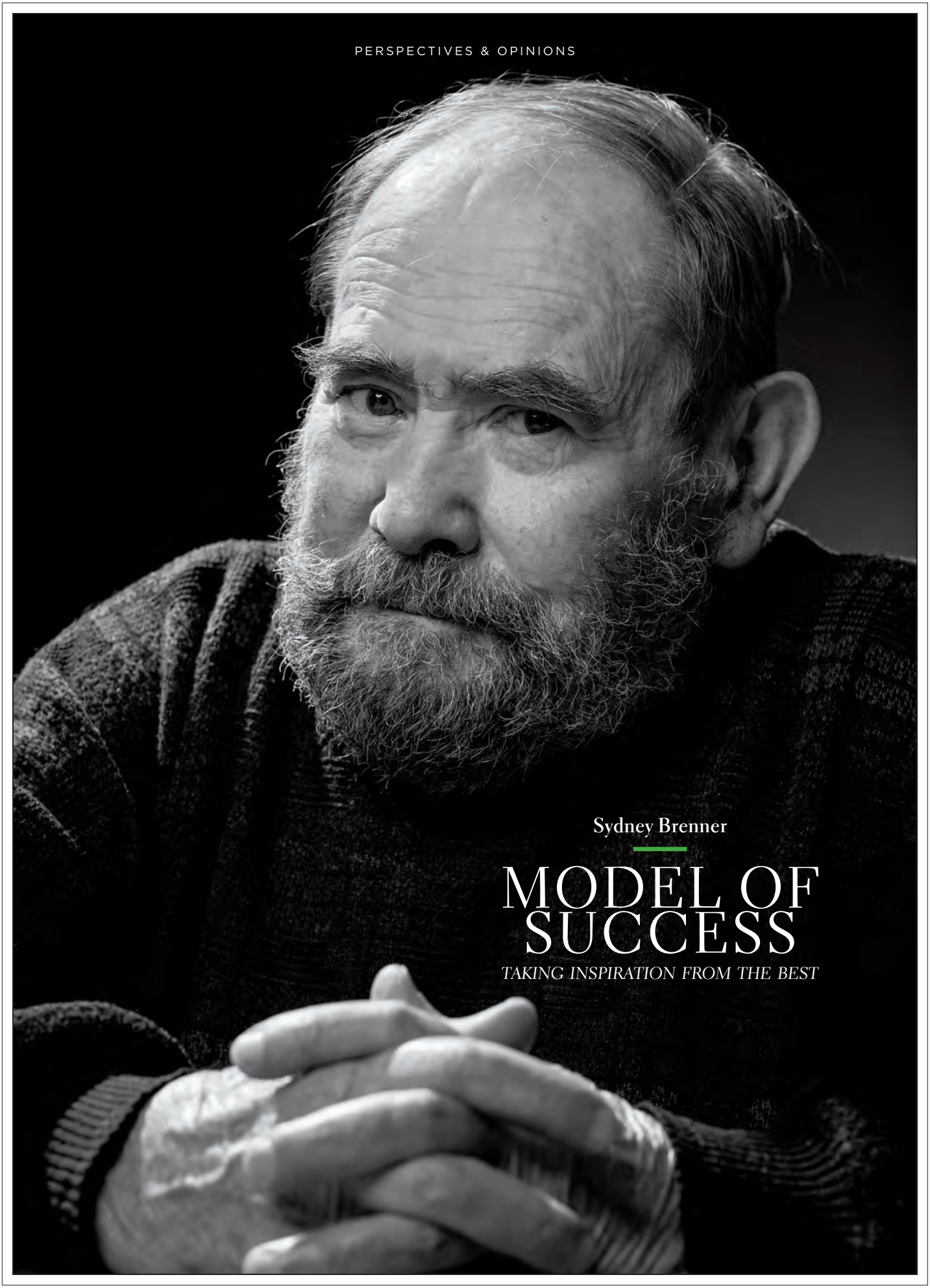
She and her husband Jagesh Shah, a systems biologist at Harvard Medical School, are instilling in their daughters the same curiosity about the world that shapes their lives. "They love science," she says delightedly, perhaps in part due to the home experiments the four conduct together. The girls are also regular guests at the annual outreach event for middle school girls organized by MIT's Society of Women Engineers. Bhatia and a handful of classmates launched the program, which they called Keys to Empowering Youth (KEYs), when she was a graduate student.

"This age group had been identified as the pinch-point in the pipeline, where girls begin to lose interest and drop out of math and science," she says. "So we wanted to bring them in so they could experience the 'gee-whiz' aspects of a high-tech lab that they'd never be exposed to in the classroom."

Now she is the KEYs faculty advisor and hosts an event in her lab each year. This year's session had a glitzy Lady Gaga theme, and each girl went home with a sparkly hydrogel they'd made by entrapping glitter in a prepolymer solution. Bhatia's hopeful that the exposure to female engineers will help keep the girls inspired and engaged as they advance in their education. "Plus," she says, "I just love that my five-year-old knows the word 'hydrogel.'"

She's similarly pleased with her trainees, who say her mentorship offers rigorous and well-rounded preparation for their future endeavors. Gabe Kwong, a postdoctoral researcher who

(Continued on page 40)



Sydney Brenner

MODEL OF SUCCESS

TAKING INSPIRATION FROM THE BEST

Andrew Cutraro

At the famously innovative Medical Research Council's Laboratory of Molecular Biology (LMB) in Cambridge, England, Sydney Brenner made his mark. When his former grad student Gerry Rubin created HHMI's Janelia Farm Research Campus, he modeled it after both the LMB and Bell Labs, hoping to replicate their vibrant, visionary cultures. Today, Brenner spends part of his year at Janelia, as a senior resident fellow.

You've been involved with Janelia since the beginning. Is it working?

Janelia Farm was, and still is, a very good idea. The problems being undertaken here are very important. It is the last frontier for biological sciences—how the brain gets built and how it works. Along with developing new imaging technologies, Janelia is meant to discover the principles of the construction of neural networks. It's taking a very extended view, starting with model organisms.

Isn't similar research going on elsewhere?

No. There are two ways of approaching complex biological systems. You can do systems biology and try to deduce what happens inside from measuring behavior. But it actually won't work: you cannot make the measurements accurately enough.

Or you can construct a wiring diagram of neurons and ask if you can use it to explain behavior. That's what's going on at Janelia. Many things have to be solved simultaneously, and a big effort has gone into techniques. We've continued the development of all forms of microscopy, with tremendous progress.

But one of the important ideas here is that you shouldn't just develop techniques and wait for someone to use them. The tools should be ripped out of the hands of the engineers and used, and that's exactly what's happened here.

Can you give a good example?

There is a recent paper by Scott Sternson on certain neurons in the mouse hypothalamus. He uses optogenetics and techniques he's developed, such as new ways of stimulating neurons, for an analysis of the eating circuit. It actually tells you why animals want to eat and how you can control that.

How important is the fact that Janelia researchers don't need to apply for grant money?

If you go, as I do, to the Salk or Scripps Institute, the talk isn't about science; it is about how am I going to get enough money to maintain my research. That is the talk all over America.

In that sense, this is a wonderful haven, and we really need centers like this to provide opportunities for young people. That is going to be very important for science generally and specifically for the future of American science.

Have HHMI and director Gerry Rubin succeeded in creating a culture of innovation like that at LMB?

In the LMB, we were more tightly packed—this place is very grand. It may be just a romantic sentiment, but I feel that when you are living in more of a warren, you may be more productive. We're getting closer to that ideal at Janelia, as we fill the last third of the building and people begin knocking into each other in the corridors. What's terribly important in a lab is keeping it open and keeping the conversation going all the time.

In addition, at the LMB we never dragooned anybody into a project. So I did suggest, and we've implemented, having postdocs at large at Janelia who do multiple things—we call them junior fellows. I think it is these people who will make the bridges, and I think we need more of them.

What other challenges does Janelia face?

One challenge is the absence of an intellectual community beyond the labs. Cambridge was fantastic because we could be with people in different subjects. It can be hard to explain science to a professor of English literature, but if you succeed, it teaches you. Gerry is bringing endless people here for meetings and outside lectures, but it's hard to fix this.

How much do you get involved in the science at Janelia?

Because I live on site, I go a lot to the pub and talk to everybody. It's important to encourage them and tell them how difficult it was in the old days. I also have my own little project here. I'd like to know, how is it specified in the genome to build the very complex things we have in our bodies? You could call it the final code.

INTERVIEW BY JOHN CAREY. *Sydney Brenner is a Nobel laureate and senior fellow of the Crick–Jacobs Center at the Salk Institute in La Jolla, California.*

Q&A

How do you encourage innovation in your lab?

Great scientists are also great innovators. New ideas, techniques, and devices form the bedrock of scientific discovery. Here, four researchers share how they inspire innovation in the lab.

–EDITED BY NICOLE KRESGE

Carolyn R. Bertozzi

HHMI INVESTIGATOR
UNIVERSITY OF CALIFORNIA, BERKELEY

"I put forth 'grand challenges' during group meetings and one-off hallway encounters. Usually these incite eye rolling, but once in a while a coworker takes a serious interest in a 'moon-shot' project. I also aim to give my students and postdocs a great degree of independence in crafting their research projects. For postdocs headed for academic careers, I encourage exploration of new territory that could form the basis of their future research program. Finally, I send trainees to conferences and encourage them to attend sessions outside their immediate research area."

Eric Betzig

JFRC GROUP LEADER
JANELIA FARM RESEARCH CAMPUS

"I try to encourage my group to:
a) Not follow the academic herd.
b) Keep an eye out for the unexpected.
c) Focus on what's not working—understanding the problem is half the battle.
d) Work harder than the other guy.
e) Not get trapped by their success."

Craig S. Pikaard

HHMI-GBMF INVESTIGATOR
INDIANA UNIVERSITY BLOOMINGTON

"There is no simple formula. Innovation requires having a sense of what questions to pursue, possessing a broad enough perspective to piece together clues and synthesize hypotheses, maintaining a healthy skepticism of existing models, grasping the limitations of current technologies, knowing how and when to seek out collaborators, and being willing to go for it when a promising idea comes to mind. Imagination, creativity, and exploration are what make science fun, and in my lab, we revel in these aspects of our work. I look for creative junior colleagues like those described in an ad for IBM fellows decades ago: dreamers, heretics, gadflies, mavericks, and geniuses."

Martin D. Burke

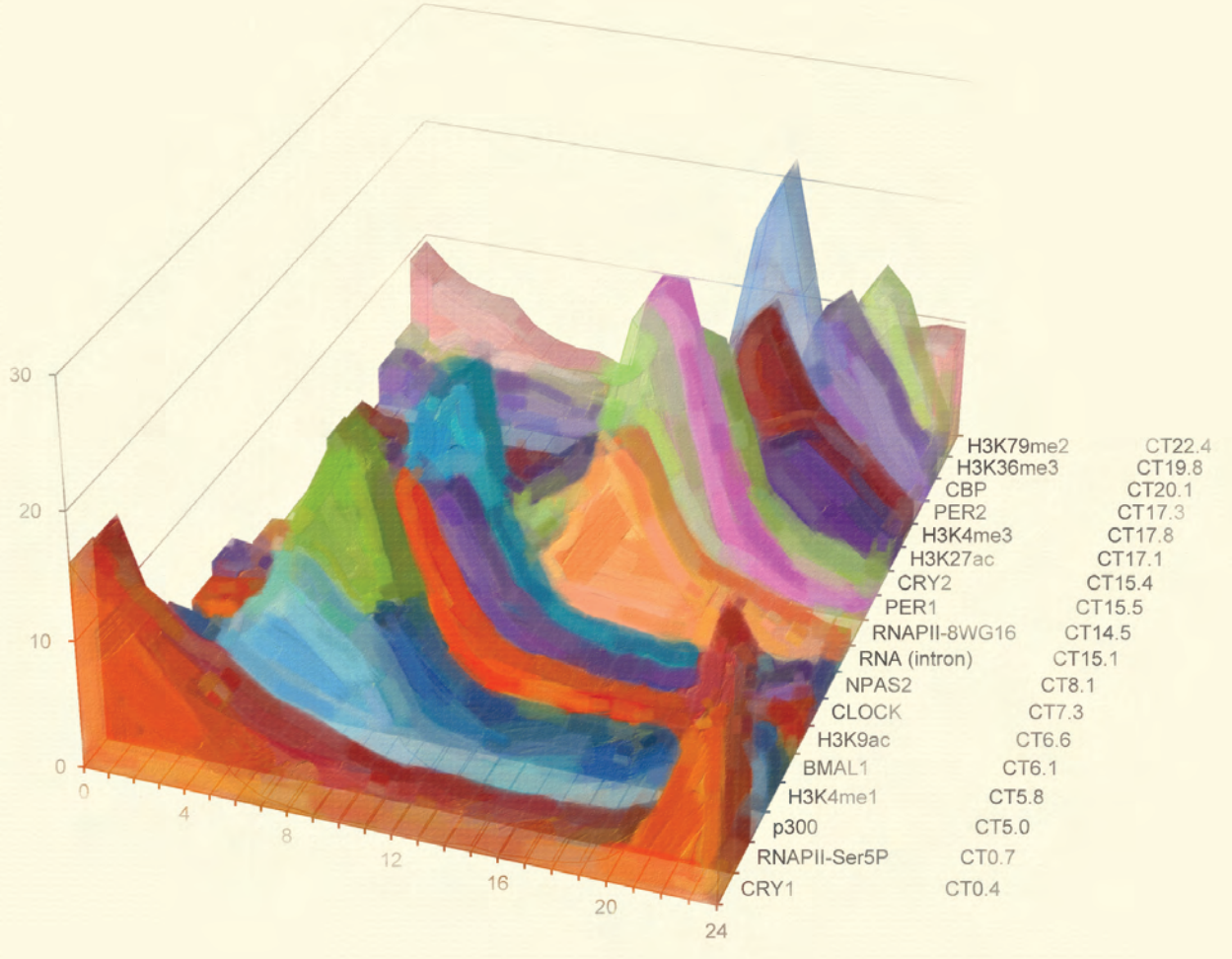
HHMI EARLY CAREER SCIENTIST
UNIVERSITY OF ILLINOIS AT URBANA–CHAMPAIGN

"I try to recruit highly innovative students ... and then stay out of their way!"

Bertozzi: Courtesy of Carolyn Bertozzi Betzig: Paul Fetters Pikaard: A.J. Mast / AP, ©HHMI Burke: L. Brian Stauffer

chronicle

The circadian clock is the body's master choreographer, synchronizing a range of bodily functions to the day-night cycle. The clock's cogs consist of a few core proteins that act as transcription factors to maintain the body's 24-hour rhythms. To get a global view of how these cogs work, HHMI investigator Joseph Takahashi undertook an in-depth study of where and when the transcription factors bind to genes in the liver cells of mice. As he depicts in this painting, Takahashi discovered that regular fluctuations in the amount of transcription factors cause a daily wave of genome-wide transcription. To read more about this research, visit www.hhmi.org/bulletin/winter2013.



Joseph Takahashi / University of Texas Southwestern Medical Center

Trash Is Treasure

LANDFILL-BOUND MATERIALS TAKE A DETOUR TO ENLIVEN CLASSROOM LEARNING.

ON A HOT MONDAY MORNING IN JULY, A WAREHOUSE IN GARDENA, California, buzzed with activity. The 6,500-square-foot space looked like a child's fantasy playground with green walls covered in giant spools of thread and floors lined with row after row of blue plastic barrels full of yarn, shiny Mylar, glass, and other sundry items.

Everything in the warehouse was originally destined for a California landfill. But Steve and Kathy Stanton are repurposing all of it for Trash for Teaching, or T4T, an innovative program that reuses industrial cast-off materials to create discovery-based science learning experiences for students.

The Stantons run a local company that makes boxes for commercial candy manufacturers. In 2004, Steve, who was trained as an architect, drove by a huge trash bin full of yellow foam "fingers" behind a paint roller manufacturer. He stopped to help himself to a bag of foam. The business owner, who couldn't understand why Stanton wanted the trash, was not pleased. Today, after eight years of successful programs in area schools, Stanton gets a different reaction—close to 100 businesses donate their weird and wonderful trash to support T4T, anywhere from monthly to once per year.

T4T originally focused on arts but turned its rubbish-repurposing energy to science in 2011. The organization is working with 150 California schools to create science-based afterschool programs fed by rolling carts stuffed with supplies. The group also helps schools integrate discovery-based learning programs into STEM (science, technology, engineering, and math) classes from elementary to high school. T4T is part of a larger movement to return discovery to classrooms.

Steve Stanton says they try to move 1,000 pounds of materials out of the warehouse every month, all aimed at encouraging students to think creatively and critically. For example, one open-ended challenge asks kids to design the ultimate recycling machine, able to transport a can into a blue bin 15 feet away. According to

Jason Ford

Shiva Mandell, T4T's director of creative programming, hands-on learning and experimentation often create an excitement for science that the participants didn't know they had.

"Collaboration and trial-and-error testing are more important than the underlying specific science concepts, so it doesn't look or feel anything like traditional textbook learning," he says. "From our work so far, it seems to be much more important to provide students with challenging experiences so they can strengthen their own critical thinking skills and their notions of tenacity instead of just learning particular content."

Hands-on learning can be one of the first things to be axed in cash-strapped school districts, leaving students in the dark about the scientific process. James Maloney, who runs the Caltech Classroom Connection, one of several HHMI-funded programs that use T4T's preassembled science kits in Pasadena schools, says that students often struggle to imagine what scientists actually do. "They're curious, but they don't know what real science looks like and what the scientific process is actually like."

He says that T4T's kits give kids the opportunity to ask a question and then do a controlled investigation to try to find the answer. One kit, for example, has students investigate reflection, refraction, and other properties of light using metallic film, wax paper, a Petri dish, and a pen clip. "Alongside that," says Maloney, "there is an environmental message about using waste-stream materials that would otherwise end up in the garbage."

Each material has a story, and as Steve and Kathy walk around the warehouse, they share a bit about each one. The donated Petri dishes are new but past their expiration date (yes, medical-grade plastic has a date of expiry); they're unusable for hospitals but fine for classroom work. The eyeglasses are from club membership optometry shops—T4T receives around 100 pounds per week. Clothing manufacturers donate the yarn, Mylar, and string. Slot-machine parts come from nearby Los Angeles—in fact, Kathy estimates that 99 percent of the materials come from businesses within 25 miles of their warehouse. And, since 2004, the program has also diverted more than 50,000 pounds of waste from landfills.

Pallets of materials are piled behind a vast floor-to-ceiling denim curtain that Mandell and his wife spent their honeymoon sewing. T4T is a labor of love for the handful of people who devote their time to the effort. Creating and tweaking the discovery concepts can take months, says Mandell. By using recycled supplies, though, the kits can be assembled much more cheaply than if the organizers had to purchase the materials.

T4T is continually developing more ways to use the materials in classrooms and afterschool programs. One kit asks high school students to create a tin can-style telephone out of medical specimen cups (the kind used to collect urine samples) and string. Dave Zobel, a science writer who helped create the lesson plans, says that he is always surprised by the unexpected ways the students use the materials to invent phones. For example, one group of teens used pieces of laser-cut Styrofoam as the phone and found that the material carries sound even better than plastic.

Getting students excited about science and technology is the first step to filling the high-tech jobs of the future, says Steve Stanton. In California alone, STEM-related jobs will number nearly 1.2 million by 2018, and Stanton says the school system is not doing its job if kids are not prepared to excel in that job market. He hopes that putting together supplies from a dream-like warehouse may help ignite a spark in students—from Styrofoam telephones to the next great innovations to improve the world. ■ –KATHARINE GAMMON

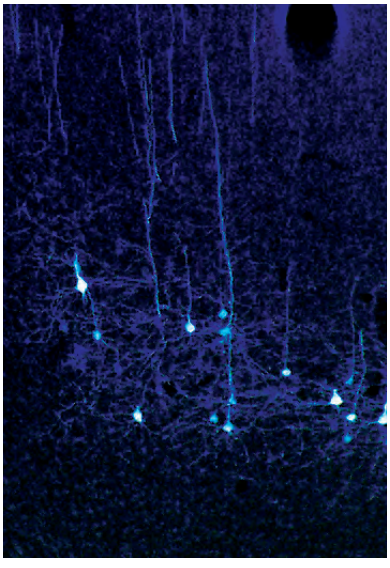
> "From our work so far, it seems to be much more important to provide students with challenging experiences so they can strengthen their own critical thinking skills and their notions of tenacity instead of just learning particular content."
>
> –SHIVA MANDELL

WEB EXTRA: To see a YouTube video of T4T in action in the classroom, go to www.hhmi.org/bulletin/winter2013.

Melody-Modifying Mice

AN UNEXPECTED BRAIN CONNECTION REVEALS
THAT MICE CAN LEARN NEW FEATURES OF THEIR SONGS.

Many a man believes the way to a woman's heart is by wooing her with a song. Apparently, mice think so too. New research by HHMI investigator Erich Jarvis shows that a mouse can alter the pitch and sequence of its ultrasonic serenade to match those of its rivals, which may increase its chances of winning the affection of nearby females.



Neurons (white) connecting the motor cortex to the larynx allow mice to copy their rivals' ultrasonic serenades.

In addition to the squeaks we would recognize, a male mouse can belt out tunes too high-pitched for humans to hear. But by recording the sounds and lowering their pitch on playback, we can also appreciate the melodies. These songs, thought to be part of courtship, have long been considered innate rather than learned. However, researchers noticed that the vocalizations sound similar to those of songbirds, which can modify melodies on the basis of what they hear. "That led me to question whether or not mice have any type of vocal learning behavior," says Jarvis.

Vocal learners such as humans and songbirds have a strong direct connection between their motor cortex, which manages fine motor control, and the part of their brainstem that tells the larynx how to move. Jarvis and his graduate student Gustavo Arriaga discovered that mice do too. "It's not exactly what we see in humans," says Jarvis, whose lab is at Duke University Medical Center. "In mice there are a few connections, but in songbirds and humans there are a lot."

The researchers also found that when two male mice are placed in a cage with a female, the smaller, presumably less dominant, male shifts the pitch of his song to match that of the other male. Their findings, published October 10, 2012, in *PLoS ONE*, contradict a long-held assumption that mice are not vocal learners.

"We think that mice are vocal learners but to a limited degree," says Jarvis. "This is not an all-or-none type of behavior. We propose it is a continuous spectrum of traits." Mice fall somewhere in the middle. Next, Jarvis plans to figure out whether other animals, such as nonhuman primates, have overlooked learned vocalizations. ■ –NICOLE KRESGE

IN BRIEF

GETTING TO THE ROOT OF THINGS

Flowers and seeds are a plant's most visible assets, but the real action happens below the earth's surface. A vast world of microbes in the soil do everything from providing nutrients to helping plants survive extreme drought. In the first large-scale analysis of this microbial microcosm, scientists led by HHMI Gordon and Betty Moore Foundation investigator Jeff Dangl looked at hundreds of types of bacteria that mingle with the roots of the mustard plant *Arabidopsis thaliana*.

To catalog the diversity of the root bacteria, Dangl and his team at the University of North Carolina at Chapel Hill, in collaboration with the Department of Energy's Joint Genome Institute, planted more than 600 microbe-free seedlings in local soil samples. As the plants grew, the researchers analyzed the bacterial content of the roots and surrounding soil.

The resulting microbiome, published August 2, 2012, in *Nature*, showed that the soil close to the roots contains only a subset of all soil bacteria. Within the roots, even fewer types of bacteria settle in. This finding suggests that something is selecting which bacteria associate with the roots. "We don't know if it's the host's ability to attract the bug or the bug's ability to inhabit the niche, or both … but now we can do the analysis because we have defined conditions for reproducible experiments in this complex ecosystem," says Dangl.

A TOXIC ENVIRONMENT FOR KIDNEYS

Chronic kidney disease has been on the rise for two decades and no one knows why. According to new research from HHMI investigator Friedhelm Hildebrandt, the problem could be faulty repair of DNA that's been damaged by environmental toxins.

To find the causes of kidney disease, Hildebrandt and his team at the University of Michigan Medical School looked at the exomes—DNA regions that code for proteins—of 50 families with childhood kidney disorders. They discovered mutations in four genes associated with chronic kidney disease: *FAN1, MRE11, ZNF423,* and *CEP164*. Surprisingly, all four genes control DNA repair. The group reported its findings in the August 3, 2012, issue of *Cell* and the August 2012 issue of *Nature Genetics*.

The DNA repair system fixes errors in the genetic code caused by slipups during DNA replication or by genotoxins—environmental factors that damage genetic material. When the system fails, mutations remain and the cells cannot function properly.

While the connection between DNA repair and kidney failure is not clear, the kidneys may be particularly susceptible to DNA degeneration caused by genotoxins because their job is to eliminate many toxins from the body. Hildebrandt believes that the discovery of this disease mechanism will help in diagnosing kidney failure and offers clues for developing treatments.

Gustavo Arriaga and Erich Jarvis / Duke University

Tunneling Out

BULKY RNA–PROTEIN PARTICLES TAKE A SURPRISING ROUTE THROUGH THE NUCLEAR MEMBRANE.

Molecules in a cell's nucleus have only one way out. They must travel through channels, called nuclear pores, which bridge the otherwise impassable double membrane surrounding the nucleus. At least, that's what scientists thought. Now, a research team including HHMI investigator Melissa Moore at the University of Massachusetts Medical School (UMass) has upended that notion with the discovery of an alternative passageway used by RNA–protein complexes to exit the nucleus.

While studying how motor neurons communicate with muscle cells in fruit flies, UMass geneticist Vivian Budnik noticed something unusual in the cells: a build-up of large, round particles in the space between the two layers of nuclear envelope. A staining procedure showed that the membrane-bound particles did not contain DNA, so Budnik consulted Moore, an RNA expert. They discovered the particles were chock full of messenger RNA destined for the cytoplasmic soup. But how did those large marbles of protein-studded RNA leave the nucleus?

The team saw a tantalizing parallel in virology: herpes virus, which assembles in the nucleus, is also too large to exit via a nuclear pore. Instead, it tunnels through the nuclear envelope, wrapping the envelope's inner membrane around itself and forming a bubble-like vesicle that fuses with the outer membrane, which allows the virus to escape into the cytoplasm.

"This really made my jaw drop," says Moore. Though scientists had known about the viral export mechanism for years, no one had ever looked to see whether cells used a similar tunneling mechanism.

The UMass team confirmed that the RNA–protein particles use the same exit strategy as herpes virus and identified other proteins involved that have been linked to human neuromuscular diseases. The findings, published May 11, 2012, in *Cell*, suggest that disruption of this nuclear export pathway hinders connections between motor neurons and muscle cells. "It's unbelievable how many previously unexplained things make sense now," says Moore.

Large RNA–protein particles exit the nucleus by fusing with and budding through the nuclear envelope.

Vivian Budnik / University of Massachusetts Medical School

Evidence in the literature indicates this alternative form of nuclear export may occur in organisms ranging from yeast to mammals, says Moore. The team is now investigating these other possibilities. ■ –MEGAN SCUDELLARI

IN BRIEF

AN IMMUNOLOGICAL ARMS RACE

Protein kinase R is one of the most potent weapons in a cell's protective arsenal. The enzyme lies dormant until triggered by a replicating virus. Then, it springs into action, shutting down protein production inside the cell, effectively halting viral replication. HHMI early career scientist Harmit Malik recently discovered how one virus outwits this defense mechanism by bulking up its genome.

The vaccinia virus uses two proteins—E3L and K3L—to fend off protein kinase R. E3L prevents protein kinase R activation, while K3L interferes with the enzyme's ability to stop growth and division. Malik's team at the Fred Hutchinson Cancer Research Center infected cells with a strain of virus lacking E3L and watched the virus evolve. After just a few rounds of replication, the virus learned how to outfox protein kinase R.

Investigating the evolved virus's genome, the researchers saw increased numbers of *K3L* genes. By increasing *K3L* gene copies, the virus not only produces more K3L proteins to combat protein kinase R, it also enhances the probability that K3L will acquire a mutation that makes it a better weapon. Once the virus has the desirable amino acid substitution, the number of *K3L* genes declines. The group published their findings August 17, 2012, in *Cell*.

Malik plans to look for other adaptation-driven gene expansions that enable viruses to keep pace in such arms races.

THE ORIGINS OF DOUBT

What goes on in the brain when we experience doubt? Alla Karpova, a group leader at Janelia Farm Research Campus, is helping to zero in on the answer.

Karpova studies the brain's medial prefrontal cortex, which helps guide decisions by weighing past choices. To determine what happens when the brain switches from certainty to uncertainty, she created a treat-dispensing machine for rats. Over time, the rats learned that the machine's left lever dispensed treats more often than the right. After a rat learned to favor the left lever, Karpova recorded neuronal activity in its medial prefrontal cortex and saw a stable pattern of nerve pulses.

When she changed which lever dispensed treats, however, things got chaotic in the brain. Cortical cells started to fire in unpredictable patterns. Soon, hundreds of cells were firing at random. The cells had crossed a threshold, resetting the network and allowing the brain to let go of certainty, says Karpova. The reset coincided with the moment when the rats started doubting the left lever and exploring the right one. The study was published October 5, 2012, in *Science*.

Karpova aims to identify the trigger that sparks these activity changes. Her team suspects the neurotransmitter norepinephrine. If correct, they can use it to manipulate medial prefrontal cortex activity to determine cause and effect.

CONTINUED FROM PAGE 19
(A STRUCTURAL REVOLUTION)

Then there's the new trend of combining techniques to look at larger assemblies, like Agard and Sedat did. For example, Steitz, who shared the 2009 Nobel Prize in Chemistry for solving the structure of the ribosome—a huge complex of RNA and protein—is blending cryoelectron microscopy and crystallography to capture snapshots of the ribosome as it goes about its job transcribing proteins.

"Between cryoelectron microscopy and the free-electron laser, I imagine we won't need crystals at all," says Agard. "The combination of these tools will make it so that we understand the structure of all the molecular complexes at high resolution in isolation and at moderate resolution in the context of the cell. The ability to look at how these complexes are interacting, how they interchange, and what their dynamics are, that's where I imagine things going." ■

WEB EXTRA: Visit www.hhmi.org/bulletin/winter2013 to read multiple sidebars and see a slideshow on the evolution of molecular illustration.

CONTINUED FROM PAGE 31
(A HAPPY OASIS)

developing a diagnostic urine test for tumor-derived proteins, points out that with no two people working on the same project, "you're very quickly expected to become the leader in your own subfield."

But apart from discussions of data and experiments, lab members find Bhatia always makes time for counseling about careers and personal decisions as well. "I keep expecting that level of involvement to diminish, but it never does," says fifth-year graduate student Meghan Shan.

"I'm really proud of my students," Bhatia says. "They've gone on to do really varied things." Many are running their own academic labs, she says. Others are entrepreneurs, launching companies to develop stem cell therapies, nutrition technologies, or reproductive technologies. One student is working to help introduce a biotechnology sector in his native Portugal. Another has become one of the only women on the faculty of her department at the Indian Institutes of Technology.

Bhatia's unfaltering support for women in engineering is motivated both by her own experience and by hard data. Early in her career, Bhatia says, she was hyperaware that she was usually the only woman in the groups of engineers she encountered. "In the beginning, I felt really fragmented," she says. "I used to overthink everything." As one of only two female students in her graduate program, she says, she used to puzzle over whether she should wear skirts to class, or if she'd be better off downplaying her femininity.

Confident and self-assured now, Bhatia laughs at the memory. But studies have shown that until women in academia make up about 30 percent of their field, they continue to face a "chilly climate," she says. Female engineers have not yet reached that critical mass. "The key is to not get complacent," Bhatia says. "The data show that unless you keep actively working on diversity, you can rapidly lose the gains made by the hard work of so many."

Bhatia, who in 2006 founded the first diversity committee for the Society of Biomedical Engineers, still often finds herself the only woman in the room—or the only M.D., the only non-Caucasian, or the only mom. "But I don't think about that anymore," she says. "Once you have a set of accomplishments that you're proud of, the rest of it kind of falls away. Now I have a very coherent sense of my identity. I have a vision that I'm excited about and an amazing team to drive it." ■

Mikey Burton

REALLY INTO MUSCLES

Whether in a leaping frog, a charging elephant, or an Olympic sprinter, what happens inside a contracting muscle was pure mystery until the mid-20th century. Thanks to the advent of x-ray crystallography and other tools that revealed muscle filaments and associated proteins, scientists began to get an inkling of what moves muscles. The then revolutionary "sliding filament model" developed independently by researchers Hugh Huxley and Andrew Huxley (no relation) set the stage for later discoveries of cytoskeletal motor proteins and helped jumpstart the field of structural biology.

My first involvement in muscle work was in 1949 as a research student in a small group supported by the Medical Research Council, at the Cavendish Laboratory in Cambridge. This was the group that eventually grew into the MRC Laboratory of Molecular Biology, but at that stage it consisted of Max Perutz, John Kendrew (my PhD supervisor), Francis Crick, and myself. I was supposed to be working on the X-ray analysis of crystalline proteins, but I had grown restive at the lack of concrete results in that field (this was several years before Perutz showed that the heavy atom technique could work on proteins), and as a sideline, I was exploring the use of a microcamera that Kendrew had suggested to me, a device employing a narrow glass capillary to collimate down an X-ray beam to allow patterns to be recorded from very small selected areas of biological specimens.

Reading through Perutz's reprint collection during long night vigils over water-cooled X-ray generators, I became intrigued by the problem of muscle structure and the contraction mechanism. I previously had no biological training, as I will explain presently, and was amazed to find out that the structural changes involved in contraction were still completely unknown. At first I planned to obtain X-ray patterns from individual A-bands, to identify the additional material present there. I hoped to do this using some arthropod or insect muscles that have particularly long A-bands, or even using the organism *Anoploductylus lentus* Wilson, which my literature search revealed had A-bands up to 50 Qm in length! However, getting the microcamera built was a lengthy process, and in the meantime I also became very interested by Schmitt et al's early work on muscle ultrastructure in the mid-1940s, and the X-ray diffraction patterns that Bear had obtained from air-dried specimens. He had used such material because of the very long exposure times necessary, so as to get more protein into the X-ray beam than a fully hydrated muscle would allow.

But I had learned from Perutz that the whole secret of getting good high-resolution X-ray diagrams from protein crystals was to maintain them in their native fully hydrated state, in their mother-liquor. So I wondered whether a whole host of new details might not spring to light if one could obtain a low-angle X-ray diffraction pattern from a live, fully hydrated muscle. I knew from the earlier work on dried material that the size of the structural units present was likely to be in the hundreds of Angstroms range, and so I set about constructing a slit camera with the necessary high resolution.

Excerpted from "A Personal View of Muscle and Motility Mechanisms," by Hugh E. Huxley, published in *Annual Review of Physiology* (1996) 58:1-19. Reproduced with permission of Annual Reviews in the format Republish in a journal/magazine via Copyright Clearance Center.

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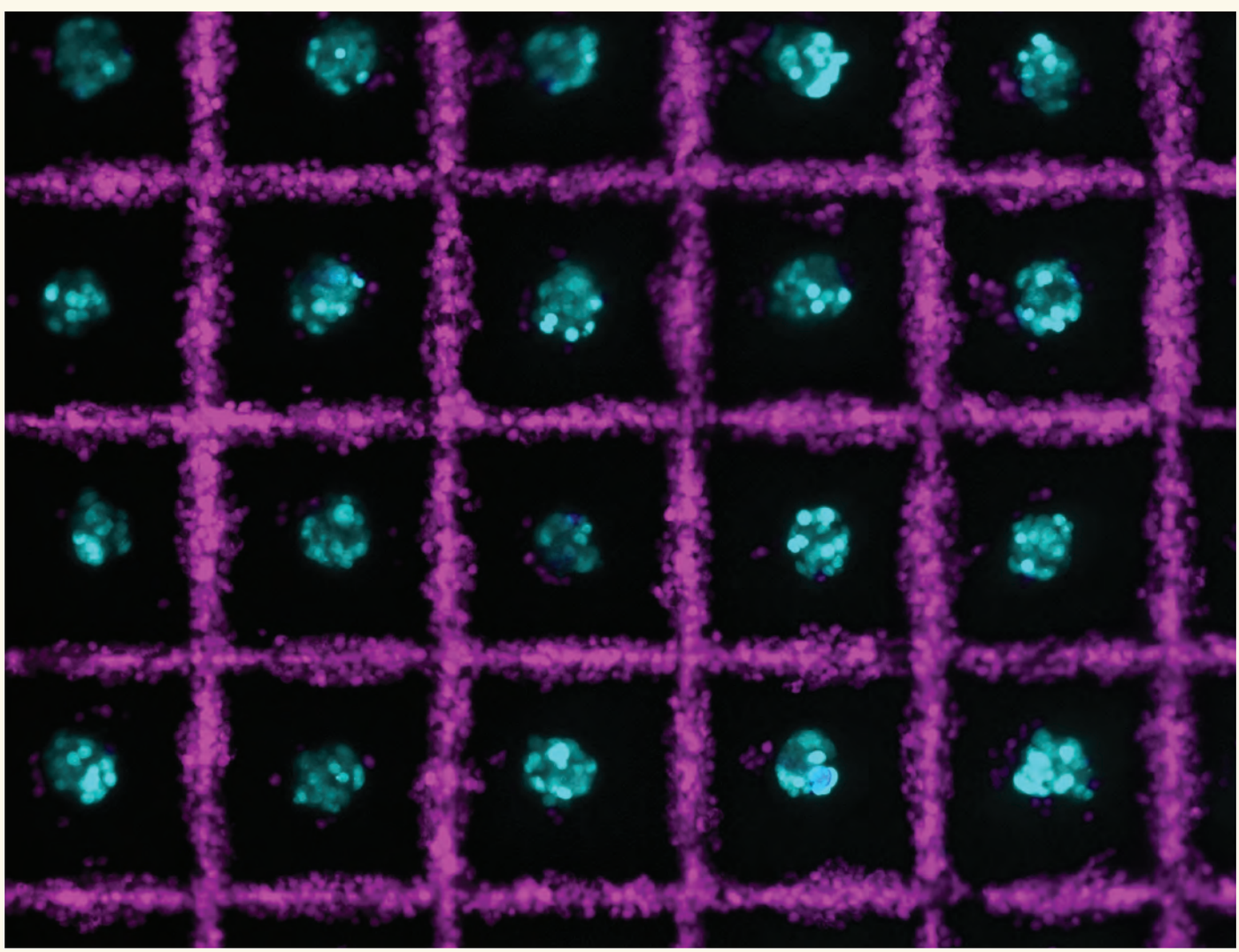
Grid Locked

Our cells often work in near lockstep with each other. During development, a variety of cells come together in a specific arrangement to create complex organs such as the liver. By fabricating an encapsulated, 3-dimensional matrix of live endothelial (purple) and hepatocyte (teal) cells, as seen in this magnified snapshot, Sangeeta Bhatia can study how spatial relationships and organization impact cell behavior and, ultimately, liver function. In the long term, Bhatia hopes to build engineered tissues useful for organ repair or replacement. Read about Bhatia and her lab team's work in "A Happy Oasis," on page 26.



Bhatia Lab