

hhmi bulletin

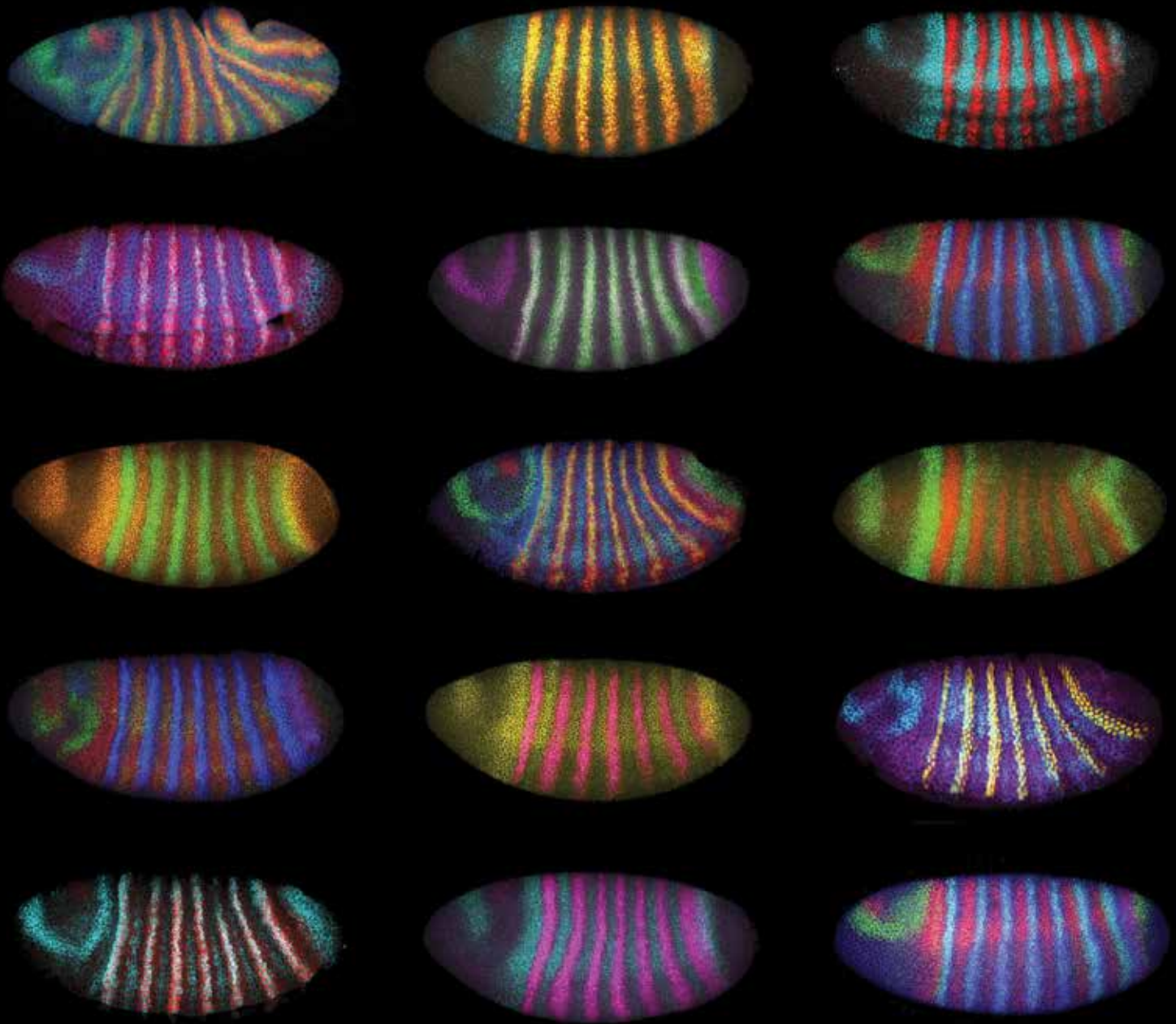
IN THIS ISSUE

Neural Theory at Janelia
Marta Zlatic in the Footlights
Bacterial Barcodes

Small Forces, Big Impact

Mechanical stress plays a
vital role in the development
of both animals and plants





12

Like the creases in a sheet of origami paper, the stripes that pattern these fruit fly embryos guide forces that will govern their shape. As an embryo becomes longer and thinner, lines of protein are laid down along the growing fly's head-to-tail axis. Each vibrant band consists of one of three members of the Toll receptor family. The receptors allow the cells to differentiate themselves from their neighbors and perform the directional movements necessary to remodel the growing embryonic tissue.

Contents

Spring '15 Vol. 28 No. 02

Departments

PRESIDENT'S LETTER

03 A Generation at Risk

CENTRIFUGE

04 Making It Real

05 Craftsman

BENCH REPORT

06 Digging Deeper Beyond Itch

08 Autism's Genetic Roots

10 Open and Shut Case

PERSPECTIVES & OPINIONS

30 The Theory Connection

32 Q&A – What book have you read that influenced your work?

CHRONICLE

34 *Science Education*
From Insight to Action

36 *Toolbox*
Barcoding Bacteria

38 *Lab Book*
T Cell Burnout
Got Thirst? Here's Why
Salvaging SNAREs

OBSERVATIONS

With Theory Comes Order

Features

12 Show of Force

Scientists are learning myriad ways that small forces add up to a big impact on the development of organisms, from plants to animals.

18 All the World's a Stage

Marta Zlatic is as capable of portraying the Greek heroine Hecuba as she is of pulling back the curtain on the neural circuitry of fruit fly larvae. At both pursuits, she's won wide acclaim.

24 Navigating the Torrent

Experimental neuroscientists and theorists at Janelia are joining forces to make sense of – and improve – the deluge of data coming out of labs.



All the World's a Stage, page 18

Web-Only Content

▶ Watch a zebrafish's brain light up as it takes a virtual swim.

▶ Witness tiny pores called stomata develop on a leaf.

▶ See a mouse drink with gusto after a prompt from a light-triggered protein.

▶ Travel inside the body to experience an allergy-induced immune cell reaction.

▶ Observe how mechanical forces can shape cells and tissues.

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Martin Nicolausson

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Illustrator **Graham Roumieu** (“Digging Deeper Beyond Itch,” page 6) is the creator of three faux autobiographies of Bigfoot. His work also has appeared in *The Atlantic*, *The New York Times*, *Harper’s*, an animated commercial for corn dogs, and elsewhere.



Bryn Nelson (“Autism’s Genetic Roots,” page 8) is a former microbiologist who decided he would much rather write about microbes than mutate them. Now an overcaffeinated science writer based in Seattle, he has published articles in *The New York Times*, *Nature*, *Discover*, *Mosaic*, and *Scientific American*.



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A photographer and filmmaker, **David Friedman** (“The Theory Connection,” page 30) explores stories of science, technology, and human nature through portraiture and storytelling. His current projects include an ongoing look at the lives of inventors from all walks of life.

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President's Letter

A Generation at Risk

EARLIER THIS YEAR, I had the good fortune to attend a day of events at the White House that celebrated science students and called on philanthropies like HHMI to continue helping to drive science forward. Reflecting on successful STEM scholars, President Obama said this:

“It’s not enough for us to just lift up young people and say, ‘great job, way to go.’ You’ve also got to have labs to go to, and you’ve got to be able to support yourself while you’re doing this amazing research. That involves us as a society making the kinds of investments that are going to be necessary for us to continue to innovate for many years to come.”

In recognizing commitments to emerging scientists, the president called out a new program, “Faculty Scholars,” that HHMI has created with the Bill & Melinda Gates Foundation and the Simons Foundation. Through this program, we will together award up to 70 grants, every two to three years, to early career basic researchers and physician scientists who have the potential to make unique contributions to their fields. The first round of the Faculty Scholars competition launched in March, and we plan to make the first grant awards in the fall of 2016. Our three organizations will invest a total of \$148 million in research support over the program’s first five years.

“We need to expand our reach, nurturing greater numbers of early career scientists with the funding, mentoring, collaboration, and training they need to successfully establish careers today.”

—ROBERT TJIAN



We plan to identify and support not only accomplished researchers who have already demonstrated their capacity for important scientific contributions, but also promising scientists who have not yet reached their potential.

Today’s emerging scientists face increasingly tough odds. The percent of all NIH research grant funding awarded to scientists under the age of 36 has dropped from 5.6 percent to 1.3 percent over roughly the past decade. The average scientist is now 42 years old before obtaining his or her first R01 NIH research grant. With this reality, a growing number of promising researchers are leaving the U.S. for opportunities overseas or, worse, abandoning science altogether, prompting some to label them “a generation at risk.”

Scientists at the beginning of their careers need adequate funding to be aggressive with their research programs. In that early phase, generally between years 4 and 10, start-up funds from a university become exhausted, just when it’s time to start ramping up. For

these scientists to persist, they must have help to clear the pathway.

HHMI already supports some excellent early career scientists in the United States and abroad. But there is much more to do to help launch the next generation of scientists. We need to expand our reach, nurturing greater numbers of early career scientists with the funding, mentoring, collaboration, and training they need to successfully establish careers today. Doing this well, at scale, will require significant, sustained leadership and collaboration – the kind of support best provided by big, strategic organizations coming together to create change.

I personally look forward to meeting the newest members of HHMI’s community when our first crop of Faculty Scholars arrives at HHMI for meetings. Wait and see what they will do.

Making It Real

WHEN SHE LECTURES about sex determination to her Life Sciences 1b students, evolutionary geneticist Hopi Hoekstra gets personal. Really personal. She starts by scoping out the undergraduates in Harvard's Science Center auditorium and picking a male student who says he's not easily embarrassed. She stands beside him and asks the other 450-some students to watch him while counting to five in unison. After "... five," Hoekstra asks for estimates of how many sperm the young man produced during the preceding few seconds. Then she delivers the answer: about 7,500.

"I try to get the students excited about the material before the lecture even starts," says Hoekstra, an HHMI investigator at Harvard University. Hoekstra calls this "motivating the material."

"Starting lectures with a story – from personal stories, to mysteries of the field, to historical facts – helps get the students' imaginations going." Without the motivation, Hoekstra says, "it's just a bunch of facts."

To set the stage for her lecture on epigenetics, she uses a family story. She projects a photo of her grandmother, who survived the 1944 *Hongerwinter* in Nazi-occupied Holland, and explains that her grandmother subsisted on tulip bulbs while pregnant with Hoekstra's mother. When in utero, Hoekstra's mother was forming the ovum from which Hoekstra herself would eventually develop. Through

that egg, Hoekstra may have been directly affected by her grandmother's privation. In other words, environmental signals might have triggered epigenetic changes in her mother's ova – heritable changes, but ones that alter how genes are expressed rather than the DNA itself.

"I'm part of this lineage," Hoekstra tells the class. Then she shows a snapshot of her son, three-year-old Henry. "He's the real test case," she explains – a test case for the *indirect* effects of what his great-grandmother endured. Research has shown that epigenetic changes in people who suffered from malnutrition can predispose their descendants to diabetes and obesity.

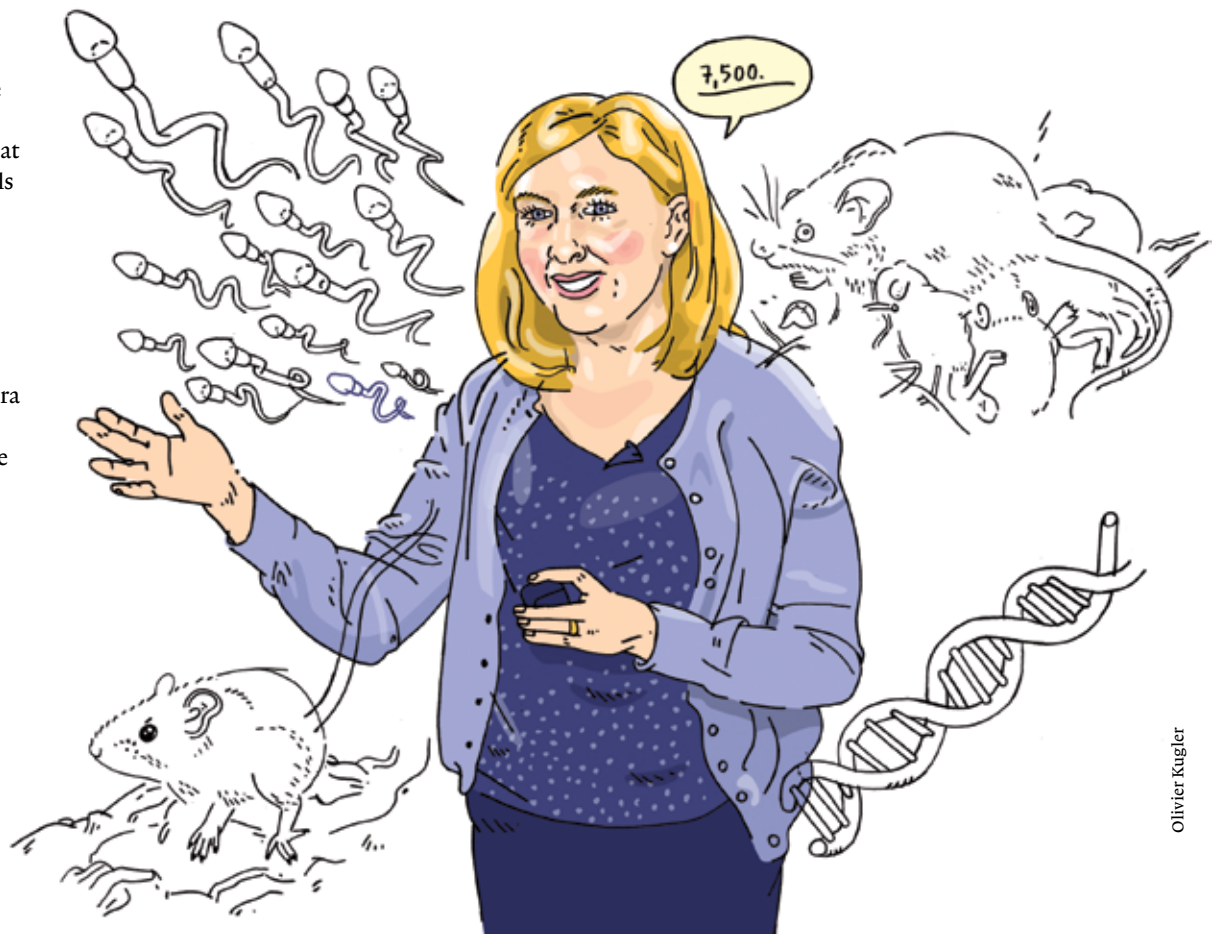
It's only then that Hoekstra says, "Let's see how this works at the molecular level." She's pretty sure that if she'd skipped the story

and simply announced, "Today's lecture is about methylation" – one form of epigenetic alteration – her students would have been far less engaged.

Hoekstra's research involves evolutionary changes in wild populations, such as deer mice. But she thinks a lot not just about evolution, but also about how to teach it. After every lecture, she jots comments on the blue folder holding her lecture notes – suggestions to herself such as, "Can move faster through first

part" and "Leave more time for rat-licking examples." In June 2014, Hoekstra was awarded a Harvard College Professorship, a five-year appointment that recognizes excellence in teaching.

She believes she'll attract more students to science by demonstrating that biology research is very much a work in progress. For instance, last fall she informed her students that, as recently as two years ago, their textbook didn't even have a chapter on epigenetics. "They think of everything as solved, but the doors to this have just opened. They can think, 'Wow, there's so much still to be discovered!'" –Cathy Shufro



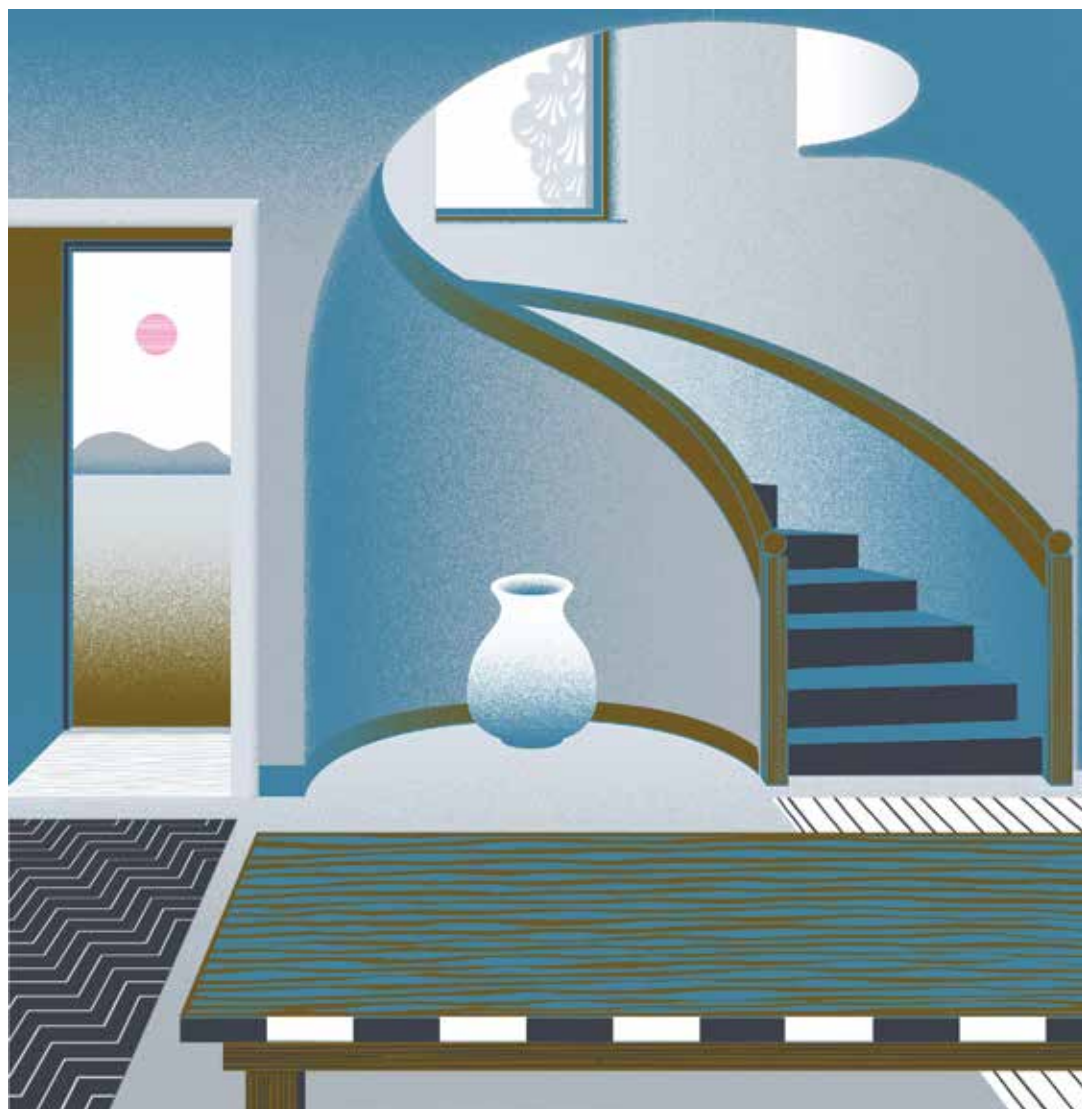
Craftsman

WHEN GREG HANNON was a graduate student at Case Western Reserve University, he liked to hang around the civil engineering department. Friends there would let the young molecular biologist use the department's wood and metal shops.

He learned to run a milling machine and operate a lathe. He built a wine rack, bookshelves, and gel boxes for his lab. Then one day, in a dusty back corner of a wood store, Hannon found what he believes was some of the last Cuban mahogany imported into the United States. "I spent more than a month's grad student salary on it," recalls Hannon, who looked at the raw mahogany and saw a dining room table. "I'd never built such a substantial piece of furniture, but I just jumped right in."

Hannon had the foresight to choose a trestle design: the table's structure was supported by four wedges that could easily be knocked out, allowing it to be disassembled and transported. When he left Case Western in 1992 and headed east for postdoctoral studies at New York's Cold Spring Harbor Laboratory, Hannon brought the table.

At Cold Spring Harbor, Hannon would pioneer the study of RNA interference: harnessing for research or therapeutic purposes the nucleic acids used by cells to regulate gene expression and protein synthesis. He also



built benches, more tables, cabinets, a dresser. He added a room to his house.

There's some overlap between his avocational and vocational pursuits. Woodwork and applied molecular biology are both exercises in problem solving – handling inevitable confrontations with the unexpected.

A skilled builder "has a thought process geared toward that and can think ahead any number of steps," says Hannon. "Thinking about how you build things on a molecular level, the process is the same."

In other ways, though, he finds building a release from the lab. It requires total, measure-twice-cut-once focus. "I'm not a meditation kind of guy, but I imagine it's something like that," Hannon says of pottery-throwing, another of his

manual pursuits. "The clay knows what your mood is."

Both woodworking and pottery also offer more immediate gratification than his work usually provides. "You make progress every day," says Hannon. "That's not always the case with science, which is punctuated by periods of insight followed by the long, hard slog of making things work."

Last fall, after 23 years at Cold Spring Harbor, Hannon began to relocate his laboratory to the University of Cambridge in the United Kingdom, with a full transition expected in June of this year. It was a daunting shift. He went from postdoc to full professor and HHMI investigator at almost literally the same bench.

But as difficult as it was to move, Hannon is finding in

Cambridge a needed change of pace: a chance to do more applied research, exposure to new ideas, a jolt out of intellectual complacency.

It also offers a wealth of home projects. He and his wife are renovating a 110-year-old country house. They're putting in new floors, redoing the bathrooms, and building chicken runs and goat pens for what may well become their own home dairy.

Hannon is also rebuilding the house's main staircase. He approaches this new challenge with some trepidation; completing the restoration could take years. But he knows the results will be enduring. In the dining room of the house stands his 25-year-old mahogany table. —*Brandon Keim*

Bench Report

figure out what the proteins do. And it's no easy task – there are 31 different versions of the receptor in mice and 8 in humans. So far, the HHMI early career scientist has learned that some of the receptors sense itch and others sense pain. His lab team's most recent discovery is somewhat of an anomaly: they've found an Mrg receptor on immune cells that plays a role in drug allergies.

Allergic reactions occur when immunoglobulin E (IgE) antibodies perceive a threat to the body – often an otherwise harmless substance like cat dander or pollen. The IgE antibodies bind to these substances and dock onto specific receptors on mast cells. These immune cells are packed with granules of histamine and other proinflammatory molecules that lead to the hives, irritation, or pain associated with allergies. When triggered by IgE antibodies, mast cells release their payload, producing an allergic reaction.

Though mast cells are typically activated by an allergy-specific IgE antibody, they can also be activated by a wide range of other unrelated things, explains Benjamin McNeil, a postdoc in Dong's lab at Johns Hopkins University. "People who display severe allergic reactions to environmental chemicals, FDA-approved drugs, and parasites, for example, often don't have any IgE antibodies against these things," he says. "Their reactions are mast cell-mediated, but via a different pathway." The majority of compounds that trigger such antibody-independent reactions are collectively known as "basic secretagogues," because of their positive, or basic, charge and their ability to cause mast cells to secrete their granules.

When McNeil joined the lab, in 2011, he picked what he thought was a fairly simple project: figure out the function of a human Mrg receptor called MRGX2. "It was supposed to be a straightforward project," McNeil says. "I was a neuroscientist and wanted to study sensory biology. We thought that MRGX2 was on neurons, too, so this was a way for me to learn the techniques in the lab while characterizing the receptor." But, as is often

the case in scientific research, it wasn't that simple. The very first thing McNeil discovered was that MRGX2 is not expressed in neurons.

In 2006, a group of Japanese scientists published a paper suggesting that MRGX2 might be involved in non-IgE mast cell responses. Following up on this lead, McNeil discovered that ample amounts of the protein were expressed in human and mouse mast cells. In fact, he says, "It's the most mast cell-specific gene in the entire genome. The only way to really define a mast cell is by this." In other words, if a cell expresses the MRGX2 gene, it's definitely a mast cell.

The next step was confirming the receptor's function. After McNeil discovered that a similar Mrg protein – Mrgb2 – exists on mouse mast cells, he created mice that didn't express the gene to test the protein's affinity for different compounds. Chemicals that triggered the receptor would cause an allergic reaction in normal mice but not in the knockout mice. McNeil went on to test every basic secretagogue he could get his hands on. Strikingly, everything he tried seemed to activate the receptor. "After that, I realized, 'Okay, this is a really bizarre receptor,' and started expanding the list of candidates," he recalls.

Digging Deeper Beyond Itch

Scientists identify an immune cell receptor that may be at the root of some drug allergies.

XINZHONG DONG HAS been scratching an itch for the past 14 years. Like a case of hives that won't go away, the Mrg family of proteins has been nagging at Dong ever since he discovered the receptors on sensory neurons in 2001. He's been consumed with trying to



“It’s really remarkable that there’s just one single receptor for all of these substances.”

—BENJAMIN MCNEIL

In the end, the list of compounds that activated the Mrg mouse analog receptor was huge. It included insect venoms, cancer and HIV medications, neuromuscular-blocking drugs commonly used for anesthesia during surgery, and several molecules in a class of antibiotics called fluoroquinolones. All of the compounds had two characteristics in common – they were small and positively charged. “They are otherwise completely unrelated, structurally speaking,” McNeil says. “They can differ by a factor of ten in their size and have totally different compositions. It’s really remarkable that there’s just one single receptor for all of these substances.”

The study, published in *Nature* on March 12, 2015, has opened the floodgates for dozens of new experiments and collaborations in

the Dong laboratory. McNeil is screening other suspected allergy-causing compounds for binding to MRGX2. Dong has started working with scientists at GlaxoSmithKline to find small molecules that can block MRGX2 but still allow IgE-mediated activity in mast cells. He’s also developing a drug-binding assay for MRGX2 that will allow pharmaceutical companies to test whether their new compounds elicit allergic reactions.

And there’s also more work to be done on other Mrg family members. “We still have many other genes to study,” says Dong. “We don’t know exactly where they are located, where they’re expressed in tissues, and what their functions are.” Clearly, it’s an itch he’s not ready to stop scratching any time soon. —Nicole Kresge

Bench Report

Autism's Genetic Roots

Using a fresh approach, researchers are making progress in unraveling the genetic complexities of the disorder.

AUTISM IS NOTORIOUS for the broad range and variable intensity of symptoms that can impair a child's behavior, communication, and social interactions. Scientists have long known that developmental disability is strongly influenced by genetics, but pinpointing a cause for autism's onset has proven frustrating. Even identical twins can have vastly different forms of the condition.

A promising "genotype-first" strategy pioneered by HHMI Investigator Evan Eichler of the University of Washington is now giving the search for answers a much-needed boost. Instead of beginning with a detailed characterization of an individual's symptoms and then hunting for the responsible gene or mutation, Eichler sequences large amounts of DNA from multiple patients and then seeks out shared genetic variants that may point toward common paths to the disorder.

The approach has yielded a growing list of autism subtypes in which children with the same mutation have a highly similar suite of symptoms. "I feel like we're kind of where cancer was as a field 20 years ago," Eichler says. Cancer, once widely considered a single disease, has since become an umbrella of more than 200 types, each with its own genetic and environmental determinants. "I like to think that this is what we're doing in the case of autism: we're helping to unravel this complexity. But we're doing it with genetics first," he says.

A decade ago, autism researchers began finding genetic clues in often sporadic

mutations known as copy number variations, or CNVs, in which big chunks of chromosomal DNA are missing or duplicated. Eichler and his collaborators discovered that some of these CNVs occurred almost exclusively in children with autism or developmental delays.

Soon thereafter, a series of technological breakthroughs began allowing "next-generation" DNA sequencing methods to more quickly and cheaply spell out the genetic code of autism patients. With the new tools, Eichler and colleagues began sequencing the exome – the 1.5 percent of the genome that encodes proteins – of affected individuals and their parents. This time, the researchers focused on single base-pair mutations and small insertions or deletions to zero in on specific genes. Even with only 20 families in that study, "we were thrilled with the candidate genes that we were seeing," Eichler says. "There was already handwriting on the wall that this was going to be productive."

When he and other researchers applied the same strategy to a much larger collection of DNA from patients, parents, and unaffected siblings, they found more evidence for an excess of "gene-killing" mutations in autism patients. After pooling their results, however, the lab teams found that the vast majority of mutations mapped to separate genes. "Just as there are many roads to Rome, there are many ways to get to an autistic state," Eichler explains. Despite the excitement of seeing a strong genetic signal, the researchers realized they would need many more DNA samples to prove the involvement of any single gene.

To scale up the effort, Eichler organized a large data-sharing consortium of labs, and a December 2012 paper in *Science* targeting 44 candidate genes demonstrated the group's newfound power. By sequencing the genes in nearly 2,500 individuals with autism spectrum disorders, the consortium found disruptive mutations in six genes that accounted for an estimated 1 percent of all sporadic cases.

As the sequencing effort accelerated, distinct genetic subtypes of autism began to emerge. Tellingly, individuals with the same disrupted gene tended to share similar physical, clinical, and behavioral traits. Based on early evidence implicating a gene called *CHD8*, for example, Eichler and colleagues sequenced the

gene again in 3,700 individuals with autism or developmental delay. As they reported in a July 2014 *Cell* study, their analysis yielded 15 independent mutations – and the tally has since surpassed 20.

By having the children re-evaluated by pediatricians, psychiatrists, and other clinicians, the researchers discovered that although only half of the individuals are intellectually disabled, most have autism and macrocephaly, or overly large heads. Most also have experienced severe gastrointestinal problems, and Eichler suspects that the same genetic subtype might contribute to a sleep dysfunction that keeps the children awake for several days at a time. In zebrafish, his lab found that knocking out the equivalent gene resulted in fish with somewhat larger heads and impaired digestion.

Among eight children with a sporadic mutation in a separate gene called *DYRK1A*, by contrast, Eichler's lab and collaborators in Australia and Europe found that all have autism, intellectual disabilities, and unusually small heads, or microcephaly. As described in a February 2015 *Molecular Psychiatry* study, many also have pointed chins resulting from a skull deformity that often requires surgery to remove overcrowded teeth. In fruit flies, researchers discovered the same gene mutation 20 years ago and named it "minibrain"; *DYRK1A* is also one of two genes linked to cognitive deficits in Down syndrome.

Eichler and colleagues are now screening thousands of families for mutations in 250 candidate genes associated with a half-dozen protein networks tentatively linked to autism. When clinicians eventually develop potential interventions for the disorder, he says, knowing which network is impaired in which patient may help determine the right course of therapy.

In the interim, he notes, a genetic diagnosis may help parents understand the basis of autism, connect with other families for support, and realize that they're not to blame for their child's condition. "I think it adds a certain human aspect to all of this research," Eichler says. "After years of being in this business, I find this part to be sometimes better than the *Nature* and *Science* papers: the fact that for these individual genes, we're really making an impact on families' lives in what feels like very substantial ways." –Bryn Nelson

"Just as there are many roads to Rome, there are many ways to get to an autistic state."

—EVAN EICHLER

Evan Eichler uses a genetics-first approach to unravel autism's complexities.



Bench Report



To see an animation of 24 hours of stomata development, go to hhmi.org/bulletin/spring-2015.

Open and Shut Case

Researchers are uncovering details of the molecules that control the development of stomatal pores on a leaf's surface.

IT'S NO WONDER that Dominique Bergmann talks about her *Arabidopsis* cells acting like toddlers and adolescents. Just the way a parent marks her kids' heights on a doorframe, she's spent her career charting plant cell development.

"How do you go from a naïve stem cell with all sorts of possibilities to being

committed to a fate as a differentiated cell?" wonders Bergmann, an HHMI-GBMF investigator at Stanford University.

"We don't have anywhere a whole record of how an organ is built from the beginning to the end."

But Bergmann has now made huge strides in tracking from start to finish the development of discrete multicellular structures on a leaf and in plotting the decisions their cells were making along the way. In an enterprising new study published April 6, 2015, in *Developmental Cell*, her laboratory tracked the shifting genetic programs of developing leaf cells as stem cells arise, differentiate, and form a complete structure.

The ambitious investigation was possible, in part, because the structure she studies is made up of just two specialized guard cells that form a pore on the leaf's surface. The so-called stoma, Greek for "mouth," allows plants to breathe in carbon dioxide and expel oxygen. Stomata formation unfolds simply and elegantly over two days on the leaf surface, where it can be watched in real time. That gave Bergmann an edge: in 2006 and 2007, her lab team identified the three transcription factors that orchestrate, in sequence, a leaf cell's entry into the stomatal lineage, its initial commitment to become a guard cell precursor, and its terminal differentiation into a guard cell.

When that sequence is disrupted by mutations in the transcription factors, the appearance of stomata on leaves is altered. SPEECHLESS (SPCH) and MUTE mutants sport no "mouths," while FAMA mutants, named for the Roman goddess of spreading rumors, produce an overabundance of dysfunctional mouths. These three molecules provided Bergmann with a handy way of isolating cells at each developmental stage, so that her team could document how changes

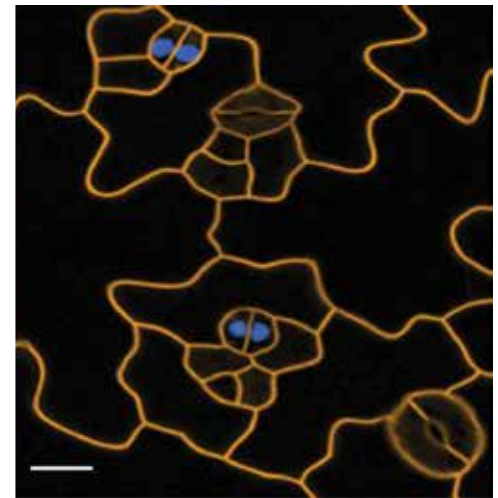
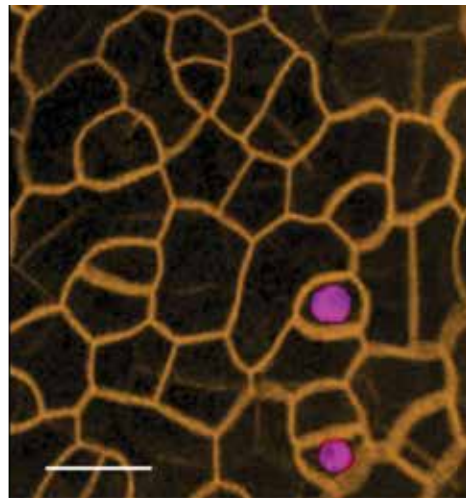
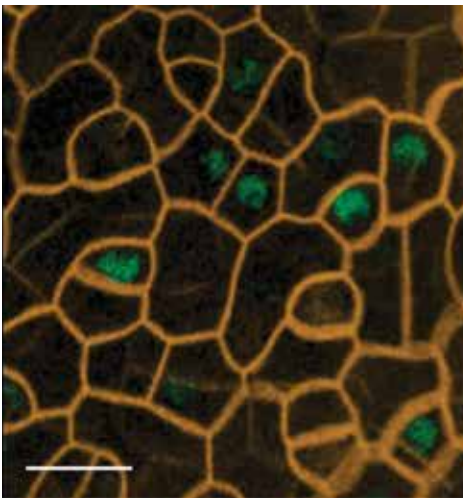
in the cells' genetic programs might direct developmental decisions.

"If you think about the transition of the adolescent years into adulthood, a lot of things happen during that time," says Bergmann. "We wanted to capture this sort of growing up over developmental time. What genetic switch is flipping in these cells to say, 'I'm done with being a stem cell; I'm going to commit myself to something?'"

The team set out to use known cell-sorting techniques with fluorescently tagged versions of SPCH, MUTE, and FAMA, among other markers, to identify and separate the different developmental stages. However, those techniques were intended for free-floating mammalian immune cells, not plant cells locked together by cell walls.

Adapting techniques pioneered by HHMI-GBMF Investigator Philip Benfey, Bergmann's group was able to dissolve the cell walls, but they still had to amass enough of the fleeting intermediate stem cells to get an accurate readout of the cells' entire array of actively expressed genes. The sorting, combined with gene sequencing, revealed which genes were active at each transition in stomata development.

Images from a time series in stomata formation. Cells at different developmental stages are marked by nuclei labeled green (SPCH, stem-cell like), pink (MUTE, committed), or blue (FAMA, differentiating). The first two panels show the same group of cells at an early time point; in the third panel, taken a day later, cells are maturing. Scale bar in each is 5 microns.



But, Bergmann admits, “It was a miserable experiment to do,” requiring tens of thousands of seedlings to get at least 2,000 cells of each type.

Once her team had a list of the active genes present in each developmental stage of a stomatal cell’s life, they compared their genetic profiles to those of plant cell types that had been characterized in other labs. They found that the earliest stem cells, tagged and sorted by SPCH, were highly variable and didn’t quite match the profiles of any other plant cells out there.

“It was like a bunch of toddlers running around. There was great promise there, but you couldn’t predict anything,” says Bergmann. “We interpreted that as reflecting their pluripotency.” (This observation complemented another recent finding by her lab team – that SPCH oscillations reset some leaf cells’ identity to the stem-cell state, so the number of stomata on the leaf can adjust to environmental changes, such as in temperature or humidity.)

To study the next developmental stage, in which cells become the precursor guard mother cells, the team tagged and sorted cells using MUTE as a marker. At this stage,

they found, cells had activated genes related to chromatin or DNA modification, which seemed to signal commitment. “They settled down – they are not turning on a bunch of functional proteins yet, but they are no longer pluripotent,” Bergmann explains. “They’ve locked into that developmental path.”

In the final, differentiated guard cell stage, the team saw some of those functional proteins come online, including active genes that encode sensory systems and ion channels that regulate the opening and closing of stomata.

Bergmann now feels she has an overarching view of the stoma’s development – from a messy infancy full of potential, through an adolescence focused on choosing a path, to an adult phase with its fate locked down. “We see the cell go from a chaotic state, where it could do anything, to becoming more and more controlled,” she says.

The keys to that process, it turns out, are the transcription factors that her lab identified: it’s SPCH that opens the genetic doors to various nascent possibilities and FAMA that shuts those doors to lock in the cell’s mature fate. –Kendall Powell

“How do you go from a naïve stem cell with all sorts of possibilities to being committed to a fate as a differentiated cell?”

–DOMINIQUE BERGMANN

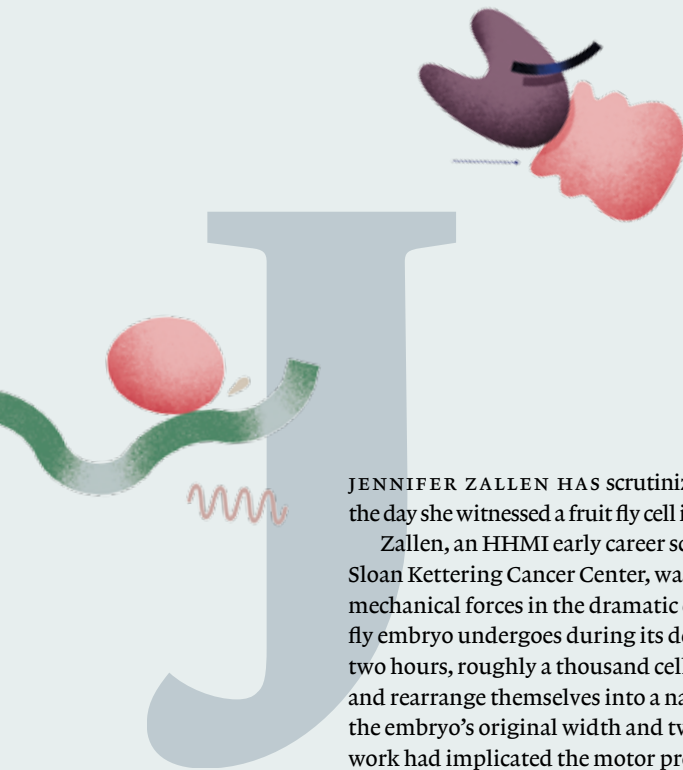
The background is a dark blue field filled with various abstract, colorful shapes and patterns. There are wavy lines in shades of pink, light blue, and white. Some shapes resemble biological structures, such as a pink bean-like shape with a small brown sphere on top, a purple curved shape with a white arrow pointing to it, a green cloud-like shape with a yellow sphere inside, and a brown sphere with a light blue ring around it. There are also several blue rings and a black jagged shape at the bottom right. The overall aesthetic is scientific and artistic.

Show of Force Scientists are learning
myriad ways that small forces
add up to a big impact on the
development of organisms,
from plants to animals.

BY RACHEL EHRENBERG



ILLUSTRATION BY MARTIN NICOLAUSON



JENNIFER ZALLEN HAS scrutinized millions of cells, but the day she witnessed a fruit fly cell in a tug-of-war stands out.

Zallen, an HHMI early career scientist at Memorial Sloan Kettering Cancer Center, was exploring the role of mechanical forces in the dramatic elongation that a fruit fly embryo undergoes during its development. Over a mere two hours, roughly a thousand cells mobilize en masse and rearrange themselves into a nascent fly that's half the embryo's original width and twice its length. Previous work had implicated the motor protein myosin in this mass movement, so Zallen's team labeled myosin with a fluorescent tag and rigged a video camera to the microscope so they could watch the protein in action. Then, her postdoc Rodrigo Fernandez-Gonzalez delicately poked the tip of a glass needle into the embryo and sucked in the tiniest bit of fluid, yanking on a nearby cell.

Myosin flooded to the site, enabling the pinned cell to contract and then escape, essentially pulling itself away from the needle. "It was fast," says Zallen, "too fast to involve changes in gene expression in the nucleus." There was no change in the cell's genetic makeup and no chemical signal recruiting myosin to the needle's tip. Yet the cell

and its neighbors had exhibited an immediate and collective response to the tug of the suction. Just experiencing mechanical tension appeared to be enough to kick myosin into gear.

Those experiments, published in *Developmental Cell* in 2009, are part of a growing effort by scientists to elucidate the role of mechanical forces in shaping biological tissues and, ultimately, entire organisms. The research is not only yielding new insights into the stunning aesthetics of animal and plant morphology,

but it may also lead to new tricks for controlling plant architecture or for halting cancer's spread.

Scientists have long appreciated the idea that mechanical forces are integral to creating shape and form. Nearly a century ago, in the introduction to his treatise *On Growth and Form*, Scottish scientist D'Arcy Thompson wrote, "Cell and tissue, shell and bone, leaf and flower, are so many portions of matter, and it is in obedience to the laws of physics that their particles have been moved, molded and conformed."

The importance of those physical laws to the formation of healthy tissues and organs has also long been acknowledged: weight-bearing exercise is crucial for maintaining strong bones, and turgor pressure on cell walls of plants keeps leaves and flowers from wilting. But during the molecular revolution of the 1990s, the role of genes, signaling molecules, and proteins in development took center stage, partially obscuring the important role of physical forces.

A revival is now underway. Armed with knowledge gleaned during the molecular era, along with new tools for manipulating and imaging cells and unprecedented computing power, scientists are reexamining the profound impact of force. By zeroing in on cells as they are squeezed and stretched in real time, researchers can untangle the developmental consequences of force generation, propagation, and detection. Efforts to quantify these actions, as well as the cellular players involved, are leading to testable models that may eventually reveal how tissues and fully fledged organisms take shape.

Push and Pull

Some striking examples of the importance of mechanical forces are coming from studies of the developing embryo of that workhorse of the lab, the fruit fly *Drosophila melanogaster*. Unlike plant cells, which for the most part don't move (though there are exceptions), the cells of developing flies and other animals often travel as they realize their ultimate fate.

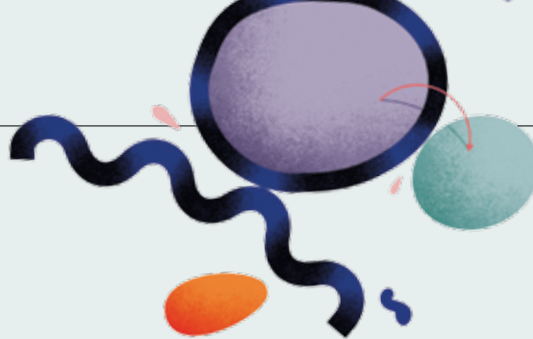
"During development, cells often move a great distance from where they are born to where they need to end up," says Zallen. "These cells have to navigate through complex mechanical environments, and they are constantly pushing and pulling on each other as they move."

The motor protein myosin II, best known for its role in muscle contraction, has emerged as the primary mediator of this pushing and pulling. Some of myosin II's jobs include helping cells divide and travel as they contribute to the development of tissue-level structures such as grooves and tubes.

When Zallen was a postdoc in the Princeton University lab of HHMI Investigator and Nobel Laureate Eric Wieschaus in the early 2000s, she was investigating how turning on certain genes in a particular spatial pattern could orient cells as the *Drosophila* embryo elongated. Here, myosin II came into play; during elongation, the force-generating protein accumulated at cell borders along the fly's head-to-tail axis. Continuing this work in her own lab, Zallen found that the accumulation and contraction of myosin was driving a

Jennifer Zallen thinks that cells might use force as a compass, to help them move in the right direction.





“During development, cells often move a great distance from where they are born to where they need to end up.”

—JENNIFER ZALLEN

coordinated, multicellular movement that led to a dramatic change in the embryo’s shape.

Zallen began to wonder if cells might detect and make use of the forces generated by their neighbors, inspiring the tug-of-war experiments and cementing the role of mechanical tension in regulating myosin’s activity. Her team showed that once a single cell starts to constrict, that force spreads: pulling on neighboring cells causes them to constrict as well, producing a contractile cable that extends across cellular neighbors like a long rubber band.

“We’re very interested in the possibility that cells could use these forces as a compass to help them move in the right direction,” says Zallen. “There is an increasing appreciation that forces can act as signals that influence the shape, fate, and behavior of cells to enable them to assemble into tissues during development.”

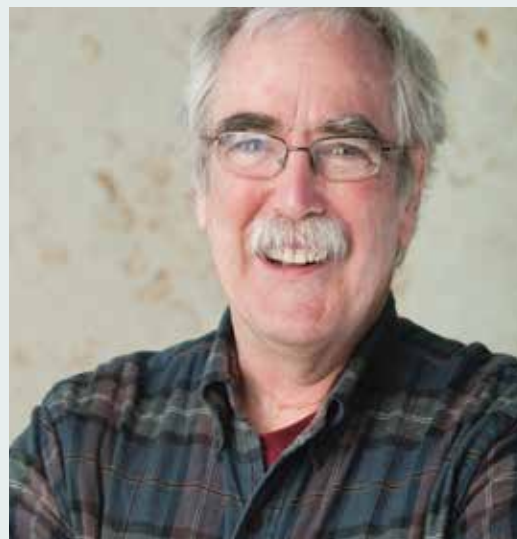
Zallen’s lab group has since shown that as the *Drosophila* embryo elongates, the contraction of these long cables of myosin – and myosin’s partner in crime, the cytoskeletal protein actin – draws cells into unexpected intermediate structures, little flower-like conglomerates called rosettes. These rosettes form when cells align into columns and shrink their connected edges together, thanks to the contracting myosin cable that pulls several cells into contact at a single point. The rosettes then disassemble in a direction perpendicular to the way they formed. The process transforms a cluster of cells that started out as tall and thin into one that is now short and wide, promoting elongation of the fly embryo.

Now the researchers are starting to pinpoint various cellular players that guide and respond to the jostling involved in embryo elongation. In 2012, Zallen and her team reported that rosettes don’t form properly in embryos that aren’t able to make the signaling molecule Abl, an enzyme that enables cells to adhere to each other under tension and execute group cell movements. Her team also discovered the cell-surface proteins that are laid down in bold stripes along the embryo’s head-to-tail axis and

that guide the direction of cell movements to help make the embryo longer and thinner. This patterning directs myosin’s contractile machinery, orchestrating the mass movement of elongation, reported Zallen and colleagues in *Nature* in November 2014.

Wieschaus compares the patterning that precedes force generation in *Drosophila* to imaginary dotted lines on a sheet of origami paper showing where creases need to go to fold it into a bird. “Once you have a pattern, it gives you a way to localize forces; then you can get form,” he says. The idea that tissue remodeling might result from the concerted action of assemblies of cells rather than from individual cells is a view of development that’s gaining traction, he adds.

“We began by thinking of the problem as a whole bunch of bricks. If you could understand how each brick behaved, you could put that all together and say how it leads to a whole organism,” Wieschaus explains. “But over the past couple of years, we’ve become aware that maybe it’s easier – or more useful or more correct – to realize that changes in morphology are bigger than single cells.”

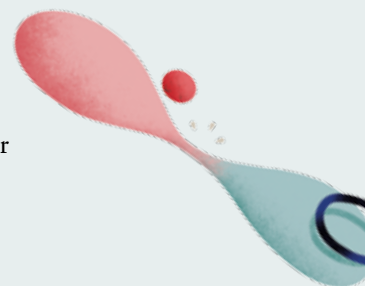


Eric Wieschaus’s lab team studies how force influences the flow of groups of cells.

Mass Movement

There’s growing evidence that networks of contractile myosin cables are a force to be reckoned with during embryonic elongation in organisms other than the fly. In 2012, for example, Gerd Walz of the University Hospital Freiburg in Germany and HHMI Early Career Scientist John Wallingford of the University of Texas at Austin and colleagues showed that myosin cables play a crucial role in the elongation of kidney tubules in two model vertebrates, the frog *Xenopus* and mice. Other research, by Masatoshi Takeichi and colleagues at the RIKEN Center for Developmental Biology in Japan and Ann Sutherland and colleagues at the University of Virginia, revealed a similar role for myosin cables during the development of the chick and mouse neural tube.

Recent work also implicates physical forces at work in metastatic cancer. Research from Valerie Weaver’s lab at the University of California, San Francisco, as well as in



➤ To see forces at work in plants and animals, go to hhmi.org/bulletin/spring-2015.

labs elsewhere, has found that the physical stiffness of the extracellular matrix, a web of fibers outside the cells, plays a prominent role in the aggressiveness of breast cancer. Enhancing the mechanical stiffness of this matrix activates a protein that aids the tumor's ability to spread, Weaver and colleagues reported in *Cancer Research* last year. The stiffness, which puts physical tension on epithelial cells, also drives malignancy by downregulating the cells' production of an important tumor suppressor protein, Weaver and her colleagues recently reported in *Nature Medicine*.

A view of cells as social constructs, which respond to mechanical cues en masse, might lead to ways to interfere

with those cues when they're implicated in disease, says Zallen. "It may be useful to think about the metastasis of certain cancers as a group activity," she says.

Wieschhaus has taken this holistic approach to the extreme. His lab had been investigating the development of the ventral furrow, an inward folding of cells that characterizes the transition of the *Drosophila* embryo from a single sheet of cells into three germ layers that ultimately differentiate into adult organs and tissues. This stage occurs right after the cell membranes form, when the fly-to-be transforms from one giant cell

with many nuclei to an embryo of 6,000 cells.

Working with his Princeton colleagues Oleg Polyakov, Konstantin Doubrovinski, and Bing He, Wieschhaus developed an approach that uses two-photon imaging and fluorescent beads to track the flow of cells as the ventral furrow forms. The researchers discovered that the infolding results from mechanical forces in the form of pulsing contractions driven by myosin on the cell surface. Mathematical modeling revealed that the cells were flowing much like a viscous fluid – behavior captured in the tidy Stokes equations of physics, which describe the flow of such fluids as paint and lava. This raised the question of how ventral furrow development would proceed if the embryo weren't partitioned into the individual units we call cells.

To investigate the forces in the absence of cells, Wieschhaus's team knocked out two genes – *slam* and *dnk* – that direct the development of the cell membranes. Remarkably, they found that, during ventral furrow formation, a fruit fly embryo without cell membranes behaved very similarly to one with cell membranes. As the team reported in *Nature* in April 2014, the process proceeded in a messier and slower fashion than it did in an embryo with cell membranes, but

the flow patterns were essentially the same, upending the cell-focused view.

"As cell biologists, we believe that cell membranes are really important for everything," Wieschhaus says. "But we found that we could eliminate all the partitioning, have this big goo of cytoplasm flowing like a fluid, and yet the embryo goes on and does its stuff."

A simple model of mechanical forces via myosin constriction could account for the changes in shape that lead to ventral furrow formation, Wieschhaus says. Similar squeezing at a cell's apical end occurs during folding in other places and other embryos, including during the development of the *Drosophila* respiratory system and the closure of the *Xenopus* neural tube. Such commonalities suggest that transmitting force via viscous flow might be another fundamental mechanism for establishing form.

Pattern and Form

Mechanical forces may also explain patterns observable with the naked eye, including an enduring mystery of plant architecture. While many animals undergo a massive rearrangement of cells to form an embryo that then, in essence, simply enlarges, plants' growth is often indeterminate – that is, they can add new leaves, branches, and flowers until death. This new growth isn't haphazard; it follows a conspicuously regular pattern that's observable by looking, for example, head-on at the tip of a new shoot. It turns out that mechanical forces generated in the hotbed of plant embryonic growth – the shoot apical meristem, a concentrated region of dividing cells – are crucial in driving this predictable geometric morphology.

Ever since the ancient Greek scholar Theophrastus noted this striking regularity in the arrangement of leaves around a plant stem, botanists, physicists, and mathematicians have been trying to explain how such systematic placement, called phyllotaxis, arises. (More modern-day scholars who have studied the phenomenon include D'Arcy Thompson, who noted the crisscrossing patterns that form spirals in pinecones and sunflower heads.) It was phyllotaxis that turned the attention of Elliot Meyerowitz, an HHMI investigator at the California Institute of Technology (Caltech), toward the role of mechanical forces in generating form.

Using live imaging techniques, Meyerowitz and his colleagues had been investigating how differing concentrations of the plant hormone auxin related to patterns of plant growth. It had long been known that auxin was crucial in determining where each new flower or leaf appeared on a plant; experiments dating back to the 1930s demonstrated that daubing a paste of auxin onto the meristem prompted the growth of new plant organs. So Meyerowitz – with a team that included his then Caltech colleagues Bruce Shapiro and Marcus Heisler, plus Henrik Jönsson, then of Lund University in Sweden, and Eric Mjolsness of the University of California, Irvine – began tracking the concentration of auxin in meristem cells of the model plant *Arabidopsis*.

Previous work in several laboratories had suggested that the membrane protein known as PIN1 was probably an auxin pump, controlling the direction of the hormone's flow out of



Elliot Meyerowitz investigates the impact of mechanical stress on growth in plants.





A view of cells as social constructs, which respond to mechanical cues en masse, might lead to ways to interfere with those cues when they're implicated in disease.

cells in the plant shoot's meristem. But PIN1's location isn't fixed in cell membranes: the pumping protein can move around, and something was directing it to membrane regions that were adjacent to cells already high in auxin. Meyerowitz and his colleagues suspected that auxin's influence on the location of the PIN1 pump could lead to a particular spatial pattern of high auxin concentration, and that this might account for the regularity of phyllotactic spirals and whorls. If cells somehow sensed their own high auxin and recruited PIN1 to the nearest membrane regions in adjacent cells, the auxin concentration would increase even more in the original cell. This would induce growth of a new leaf or flower. But as auxin concentrations increased locally, neighboring cells would become depleted in auxin, leaving a spot with no leaf or flower. Cells farther away from this auxin-depleted site would, by comparison, have more auxin and thus would recruit PIN1 to attract more auxin and again induce a new leaf or flower.

The researchers created a mathematical model that incorporated this proposed feedback mechanism. When they ran computer simulations of the model, auxin peaks emerged at regular distances, capturing the regular patterning of phyllotaxis – a finding published in *Proceedings of the National Academy of Sciences* in 2006.

But mysteries remained, including how the plant cells were sensing local auxin concentrations. The hormone was known to weaken plant cell walls, leading the team to wonder whether mechanical stress might signal to cells that they were adjacent to neighboring cells high in auxin. This, in turn, would recruit PIN1 to the region of the cell membrane nearest the high-auxin neighbor.

In a series of elegant experiments, including ones in which the scientists weakened or obliterated particular plant cell walls with a laser, mechanical stress indeed emerged as the mediator of PIN1's dynamic behavior. They found that as PIN1 directed the flow of auxin from an area of low to high auxin concentration, the cells highest in auxin expanded. In plants, cell walls are shared. So it appeared that mechanical stress on a common wall alerted the cellular neighbor that auxin concentration was high nearby, thus bringing in PIN1.

"Basically, all the observations were in the literature, but no one had thought to put stress into the equation," says Meyerowitz, who published the findings with Marcus Heisler and other colleagues in *PLOS Biology* in 2010.

More recently, Meyerowitz and his collaborators have shown that mechanical stress plays an important role in shaping plant cells beyond the meristem. It turns out that the

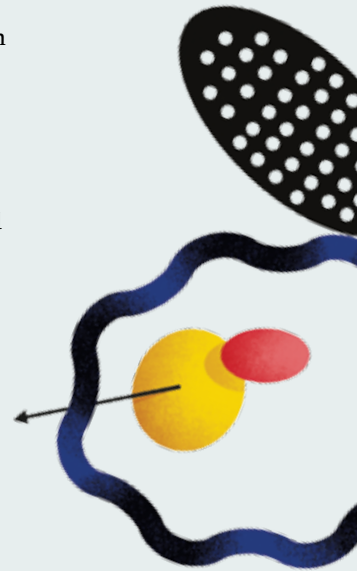
puzzle-shaped pieces of the so-called pavement cells on a leaf's surface create intracellular stresses that cause reorganization of the cytoskeletal proteins called microtubules. These proteins then help dial up the production of cellulose, which, in turn, reinforces the cell walls against the stress, the team reported in *eLife* in April 2014. Combining the microtubule findings with the auxin-related research yields a simple model of feedback driven by physical forces: "Mechanical stress tells cells how to grow, and cell growth creates mechanical stress – and morphology," Meyerowitz and his colleagues wrote in a 2014 *Current Biology* review paper.


Other labs are finding evidence of physical stressors as well. For example, Audrey Creff and Gwyneth Ingram of the Laboratoire de Reproduction et Développement des Plantes in Lyon, France, recently showed that a mechanically sensitive layer of cells in the seed coat of *Arabidopsis* responds to stress exerted by the seed's nutritive tissue, the endosperm, resulting in a fine-tuning of seed size.

The Meyerowitz lab is now looking for cellular stress sensors responsible for the cell wall effects on PIN1 and for the different sensors that mediate the effects of stress on the cytoskeleton. He believes that a greater understanding of the relationship between mechanical force and plant growth is important not just for elucidating a plant's current growth patterns; the research may also lead to techniques for engineering superior food crops – for example, produce with modified leaf arrangements that maximize photosynthesis in a particular growing region.

"Now that we're beginning to understand the feedback between physical stress and growth, it may give us a new way to intervene – or at least predict what would happen if we change things," Meyerowitz says. And for developmental biology in general, bringing mechanics back to the fore may help resolve older mysteries of shape and form.

"As people start to look at things in terms of physical signaling, not just chemical signaling, it may explain quite a bit," he observes. "It seems like we can make some rapid progress in solving old problems." ■





**Marta Zlatic
is as capable of
portraying
the Greek heroine
Hecuba as she
is of pulling back
the curtain on
the neural circuitry
of fruit fly larvae.
At both pursuits,
she's won
wide acclaim.**

All the World's a Stage

BY SARAH GOFORTH



IT'S HARD TO imagine two settings more dissimilar than the vast rotunda of a Greek theater and a small, windowless microscopy room at HHMI's Janelia Research Campus. But Group Leader Marta Zlatic is equally at home in both spaces. At this moment, her microscope's narrow beam is illuminating a *Drosophila* fruit fly larva on its own stage, the semitransparent creature wriggling in a way barely perceptible to the naked eye. To Zlatic, the larva's movements represent a nuanced performance fine-tuned by millions of years of evolution – and a foundation for understanding the neural basis of behavior.

Fifteen years ago, while she was an undergraduate at the University of Cambridge in England, Zlatic herself was in a spotlight onstage – as Hecuba, the ill-fated queen in the Euripides tragedy, *The Trojan Women*. A student of linguistics and neuroscience, Zlatic knew ancient Greek and had attended the audition for the play out of curiosity. Lacking acting experience, she was assigned a role in the chorus. When the lead actress quit halfway through rehearsals, however, the director, impressed by Zlatic's fluency with the language and text, asked her to step in. Audiences and critics cheered the performance, and Zlatic went on to land a string of roles known for their complexity: Electra, Medea, Lady Macbeth, even Oedipus Rex.

"Anyone who has seen a play should wonder: How can this wonderful system, the brain, produce so many different emotions and behaviors?" Zlatic says today, seated in her sparsely decorated office upstairs from the microscopy room in Janelia's east wing. Three empty champagne bottles, one for each major paper published by her lab in its five years of existence, sit next to her computer. The bottles, along with the humble model organism she has chosen as her life's work, make a quiet statement about the patience required of scientists who study such ambitious questions. To go big, you must often start small.

Zlatic leans forward. The human brain, she explains, has 100 billion neurons, roughly equivalent to the number of stars in the Milky Way. Fruit flies have only 100,000, and a fruit fly larva just 10,000. Reduce a nervous system to that relative simplicity, and suddenly it becomes possible to study it comprehensively – not only to describe what it looks like (map it visually), but also to determine which cells connect to one another (map its wiring, or "connectome") and to figure out how those networks listen and respond to signals from the outside world (map its behavior). Zlatic's team, with a constellation of collaborators

at Janelia and around the world, is building something akin to Google Maps for the larval brain, placing these layers of information on top of each other like satellite images overlaid on roadways. They are now beginning to outline, with neuron-by-neuron precision, the neural circuits that determine how a larva responds to its environment and decides what actions to take.

It is no frivolous exercise. "We can look at this much simpler system, and its smaller range of behaviors, and see that many of the things we learn are the same across species," Zlatic explains. For example, *Drosophila* larvae have the same sensory modalities as most animals – sound, light, odor, touch, and heat. Furthermore, they're able to process this information, remember it, and make decisions based on it. How does a nervous system make behavioral choices based on previous experience and a multitude of sensory inputs? What can understanding this simple system tell us about the human brain and our capacity for language, art, and the pursuit of knowledge?

Zlatic wants to know the answers to those questions. By all accounts, her team is already taking steps toward constructing a model for a brain atlas – one goal of the multimillion-dollar BRAIN (Brain Research through Advancing Innovative Neurotechnologies) Initiative that President Barack Obama announced in 2013.

"We are trying to understand what's happening in the brain during sensing and behaving," says Tihana Jovanic, a postdoctoral researcher in Zlatic's lab. "Gaining a better understanding of how the brain works will help uncover insights into how different neural processes are integrated so an organism can function in an ever-changing environment."

A Renaissance Mind

Zlatic was born in Croatia, the only child of a theoretical physicist and a philosophy major who worked at the Ministry of Culture. Her parents were gregarious and ecumenical in their thinking, and she grew up immersed in lively conversation about art and politics, often in mixed languages. Throughout her youth, the family was as likely to be traveling abroad – her father's research career took the family to Germany, England, and California for months at a time – as to be at home in Zagreb.

"I grew up with the idea that I wanted to do biology, because I was fascinated by living beings – especially animals and their behavior," Zlatic reflects. "I also loved literature and art. I always saw these things as complementary." She had both the talent and the opportunity to pick up languages, adding English, French, and German to Croatian. By age 12, she was also studying Latin and ancient Greek in school. At age 30, she spoke six languages, in addition to being literate in Latin and ancient Greek.

"I loved grammar and the rules of language," says Zlatic, "but I was also interested in biology because I wanted to understand how the brain was capable of creating and learning these systems." In high school, she held "the naïve view" that she could someday understand in detail how the brain creates language, and she set that as a goal for herself. She studied linguistics and Russian at the University of Zagreb in Croatia before earning a full scholarship to Trinity College at the University of Cambridge, where she pursued neuroscience. Every summer, she would return to Zagreb to continue her language studies.

"To me, it was like a vacation, to return to Zagreb every summer," says Zlatic. "It didn't occur to me that I was doing something unusual."

In her last year as an undergraduate, Zlatic was inspired by the lectures of Cambridge neuroscientist Michael Bate, who was working to understand how neural circuits form in *Drosophila* embryos. She found irresistible the idea that one could watch how a nervous system comes

into being, could actually witness neurons finding their partner cells and forming connections, known as synapses. She secured a PhD-track position in Bate's lab to study the earliest steps in that process – her idea being that by studying how the nervous system develops, she would gain insights into its function and, ultimately, into the biological roots of behavior.

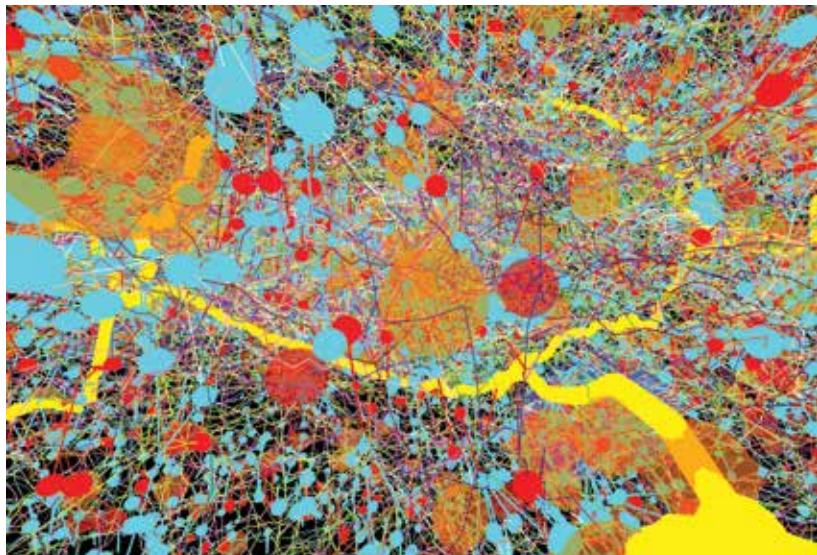
Zlatic focused her attention on sensory neurons in the larvae, studying how they form the extensions, called axons and dendrites, that allow them to communicate with other neurons. Once in place, an axon branches at its tip and connects with another neuron's dendrite, forming part of a circuit. Neurons responsible for different senses, such as the detection of heat or touch, send axons to the region of the fly's nervous system specific to that sensation. Zlatic wanted to understand what was guiding their choice of target. Were the axons being guided by positional cues to terminate at a particular location – a specific “address” in the nervous system – and just connecting with whatever cell they found at that location? Or were they seeking a particular partner, irrespective of the address? “The answer is position, because altering axons' ability to sense positional cues resulted in their overshooting the address where their partners are normally found. They did not appear to care where their partners were,” she says. “But what we couldn't tell was whether their partners cared about *them*,” she adds, because no one had pieced together a neural circuit for the *Drosophila* nerve cord.

Outside Bate's lab, she remained an avid thespian. Onstage, Zlatic was “the absolute star,” remembers her friend Julian Huppert, then a fellow PhD student at Cambridge and now a member of the British Parliament. “She was just spellbindingly good.”

“Some people play sports, other people study. Marta studies. If she is interested in something, usually she is intensely interested in it.”

—ALBERT CARDONA

This digital “map” of a fruit fly larva neural circuit highlights a neuron (yellow) that's able to sense vibrations.



She was no less talented in the lab. With Huppert and two other friends, she won funding in an innovation competition to form a biotech start-up aimed at filling a need apparent from her research – for a “virgin collector,” a way, that is, to more easily collect virgin female fruit flies. The company developed a synthetic pheromone that, in theory, would prevent the insects from mating. The compound suppressed mating most of the time, but not all the time, and the company failed. Zlatic took the bump in stride, says Huppert. “She was trying to fill a need, to solve a problem, and she knew that what she learned would make her better at solving other problems,” he says.

About the time that Zlatic and Huppert were establishing their company, developmental neuroscientist Jim Truman, then at the University of Washington, visited the Bate lab on sabbatical. Truman was studying how stem cells develop into different kinds of neurons in the larval brain – work that would later provide a foundation for Zlatic's brain-behavior mapping. Zlatic, Truman says, stood out: “She was full of pointed questions, tireless in her research and all of her activities. I remember thinking it was exhausting just watching her.”

In 2006, Julie Simpson, one of Janelia's first group leaders, invited Zlatic to join her lab as part of the campus's visiting scientist program. Simpson was using the genetic tools developed by Janelia Executive Director Gerry Rubin and others to study the neural and genetic basis of adult fruit fly behavior. Zlatic – who by then was completing a visiting postdoctoral appointment at Columbia University on a prestigious Trinity College fellowship – joined Simpson's group and began piecing together the first larval behavior map. “I came here with the idea of trying to map circuits and find the function and behavior of the neurons I used to study,” she says. “I quickly realized this was the place to be for the kinds of questions I was interested in – the relationship between structure and function of the nervous system. At that time, no one else at Janelia was working on the larva, but Julie just let me play with larvae anyway.”

A year later, she earned a lab head position at Janelia and started her own group.

The label “MET” on the fruit fly specimens below refers to methoprene-tolerant protein, which controls metamorphosis in the developing flies.



A Marriage of Minds

By that time, Janelia had become a bustling hub for the study of the *Drosophila* brain. Albert Cardona, a young Spanish developmental biologist and neuroscientist with a wide smile and enthusiastic manner, came to the campus for Janelia’s first fly brain conference, organized by Simpson.

“The whole field was excited about the fly lines coming out of Gerry Rubin’s lab,” he remembers, “and there was an energy about what these genetic tools would make possible. But what I remember about that meeting is only Marta.” Two encounters stand out: In one, Zlatic was seated by herself in Janelia’s darkened auditorium before a talk, and she patted the seat next to her. “She said, ‘Sit here,’ and I did,” Cardona laughs. In the other, he had joined her in Janelia’s pub, Bob’s, to discuss data coming out of her lab. “I remember that as we were sitting there together, this guy who was a big-shot conference attendee was there also, talking with us. I remember just thinking, *Go away.*”

Cardona joined Simpson’s lab as a visiting scientist on a pilot project that allowed him to take hundreds of electron microscope images of a segment of the fly larval nerve cord. This was proof of concept that it was possible to reconstruct what a neural circuit looks like in an insect nervous system at the level of single neurons and their synaptic connections. Janelia later committed to completing this wiring diagram for the full larva, under Cardona’s leadership.

Jim Truman, who had come to Janelia in 2007, was by then using light microscopy to image Rubin’s fly strains, each of which had been engineered to allow researchers to study small sets of neurons. The result of both efforts combined was that Zlatic could now manipulate individual neurons and study their effect on behavior. Her ambitious aim was to study not only how neurons affected behavior, but also how they work together to integrate many kinds of information – pain, temperature changes, and odors, for example – and then select a behavior in the animal’s best interest.

“To understand this,” she explains, “we need to know three things: circuit structure; physiological properties of neurons, which we can

understand at the level of single neurons; and the causal relationship between neurons and behavior.”

Meanwhile, Cardona was formulating his own dream project – to lay out the fruit fly larva “connectome,” a map of neural connectivity in the brain. Then he got a job in Zurich. But by that time their relationship was far from just a meeting of scientific minds. They managed to not only stay in touch from afar, but also to get together periodically, with each working as a visiting scientist at the other’s institute, until Cardona was hired as a group leader at Janelia in 2011. They got married – and also cemented their research collaboration. “Some people play sports, other people study,” says Cardona. “Marta studies. If she is interested in something, usually she is intensely interested in it.”

And, notes their colleague Jim Truman, “Albert’s approach to science is very different from Marta’s, in a complementary way. Without his abilities to reconstruct the nervous system of the larva, basically, we would be shooting in the dark.”

A Larva’s Life

Spend much time with Zlatic or anyone on her team today, and you may never look at a maggot the same way. Under the microscope, its wriggles become less random, even purposeful: Out in the world, larvae will sensibly try to approach food and pleasant aromas, notes Zlatic – but at the same time, they have to avoid a range of threatening situations. There are many ways to escape from danger, depending on the context and the animal’s previous experience. In the same way that some mammals can walk, trot, or gallop away, larvae have different means of escape. Some of these means are fast but costly in terms of energy expenditure; others may be slower but less costly. Often, they need to string together sequences of behavior.

Zlatic wanted to understand how the animal’s nervous system chooses among these various behaviors. This is a problem faced by all nervous systems, but in the *Drosophila* larva it is particularly tractable. Her team used a genetic toolkit, developed by Rubin, to activate different sets of neurons and watch what the larvae did in response, like flipping switches in a circuit breaker to test the wiring behind the walls.

Rubin’s lab had developed more than a thousand mutant fly strains with a gene called *GAL4* inserted into specific clusters of neurons. Zlatic’s team crossed those strains with other fly strains that had been engineered to produce, in the presence of *GAL4*, a light-sensitive protein called channelrhodopsin. This gave Zlatic access to the switchboard. By shining light on larvae from each hybrid strain, she could selectively activate whatever neurons had been engineered to include *GAL4*.

Then she and Tomoko Ohyama, a research specialist in her lab, started flipping switches, videotaping the groups of larvae as they performed. Right away, they gleaned some surprising insights, says Ohyama, because they saw variation between individual larvae when the same neurons were stimulated. That indicated to the scientists that even the most discrete behaviors are probabilistic and not 100-percent predictable. Still, clear patterns began to emerge. Zlatic and Ohyama enlisted the help of a team of machine learning specialists and statisticians in the Johns Hopkins University lab of Carey Priebe, who developed an algorithm to classify the behaviors with greater precision than the human eye could discern. In the spring of 2014, the scientists published their work, widely deemed a new framework for mapping behavior to individual classes of neurons, in the journal *Science*. In all, they had analyzed the movements of nearly 40,000 larvae.

Zlatic calls this achievement “just the beginning,” as the paper describes only which neurons evoke a given behavior and not the circuit mechanisms by which it occurs. The next step is to relate this knowledge to the connectome and a neural activity map. “Marta has been a spark plug to keep this going; again, it is her enthusiasm that really makes larval systems work in this cohesive way,” says Truman.



Marta Zlatic manipulates neurons in fruit fly larvae to study their effect on behavior.

“There’s something about her that I think is true of a lot of the really good scientists I’ve seen in my career. They’re happy. They’re having fun.”

—GERRY RUBIN

It is also a feat that few scientists can pull off, given that it requires marrying disciplines as diverse as neurobiology, electron microscopy, and machine learning, says Rubin. “A lot of smart people talk themselves out of doing things because they can’t do it all themselves or be in total control,” he says. “That is not Marta’s style. She says, ‘I have this big problem I want to solve, and I know I can’t do it all. I need to get this piece from that person, and this other piece from another person, and together we’ll make it work.’ That’s a skill and a talent – like being a good field general. It’s an approach to science I admire.”

In the meantime, Zlatic and Cardona have also embarked on what she calls the “ultimate act of creativity” – parenthood. Their son, David, now three, is poised to match his mother’s linguistic abilities.

“Marta speaks Croatian to David, and he speaks Catalan or English to me. I understand a bit of Croatian, though, and when I speak it to David he corrects me,” laughs Cardona, a native of Spain’s Catalonia region. The demands of two active laboratories plus a toddler keep the family busy, but they make it work; Zlatic takes the “morning shift,” waking up with David and making him breakfast, while Cardona heads to the campus at sunrise. She works late while he makes dinner, and then the family has an hour or two together before bed. “It works for us,” says Cardona, “because we know what our priorities are.”

And so Zlatic has given up acting in favor of her greater loves – science and family. “What I love most about science is ideas,” she says. “You look at the data, it inspires ideas, then you test them, then you lose them, then you try out other ones. The creative aspect of science is coming up with ideas in dialogue with the past. That’s very beautiful to me.”

“There’s something about her that I think is true of a lot of the really good scientists I’ve seen in my career,” says Rubin. “They’re happy. They’re having fun. They say that to be a successful scientist you have to maintain your childlike curiosity. She exudes that.” ■

Experimental neuroscientists
and theorists at Janelia are joining
forces to make sense of – and improve –
the deluge of data coming out of labs.

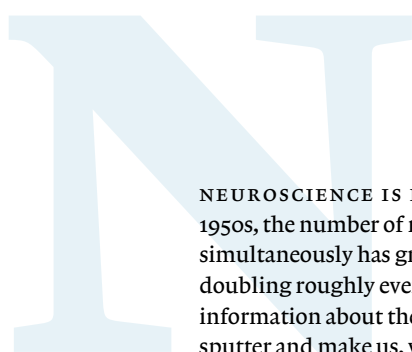
Navigating the Torrent

BY CARRIE ARNOLD



ILLUSTRATION BY CARMEN SEGOVÍA





NEUROSCIENCE IS DROWNING in data. Since the 1950s, the number of neurons that scientists can record simultaneously has grown at an exponential pace, doubling roughly every seven years. To utilize this information about the billions of neurons that spit and sputter and make us, well, human, researchers have had to cope with an exponential growth in data.

“What you would do, back in the day, is maybe look at a couple of neurons in one part of the brain during simple sensory stimulation – a very focused study,” says Jeremy Freeman, a neuroscientist and group leader at HHMI’s Janelia Research Campus.

Now, the pendulum has swung in the opposite direction. Neuroscientists can record the activity of nearly all the neurons in the brains of zebrafish larvae, and in ever increasing portions of mouse and *Drosophila* fruit fly brains.

A single set of experiments can generate terabytes of information. Simultaneous increases in computing power mean researchers can perform more sophisticated analyses, studying relationships between groups of neurons instead of analyzing one neuron at a time. To stay afloat in the deluge of data, scientists need to develop an entirely new way of thinking about experiments – and making sense of the resulting torrent of information.

“It’s really a big change from thinking about what single neurons do to

thinking about what large populations of neurons do. With a single neuron, an experimentalist could use his or her intuition and, in fact, people are quite good at that,” says Larry Abbott, a Janelia senior fellow and a theoretical neuroscientist at Columbia University. “When you have a population of neurons, and they’re all interacting, it’s almost impossible to have that intuition. You really have to make a model of it and figure out how you think it’s going to behave.”

Abbott and Freeman, together with other theoretical and computational neuroscientists at Janelia, are

working to build lifeboats and lighthouses for other scientists to help them navigate the swirling storms of data. Buried in this tsunami of statistics are the patterns and insights that will enable them to crack the biggest mystery in science: how the human brain works.

Computation as Partner

To Janelia Group Leader Kristin Branson, keeping your head above water as data pours in requires computer science as much as it does biology. She joined Janelia in 2010, intending to improve the computer tracking software known as Ctrax that she had built while she was a postdoc at the California Institute of Technology. The premise behind Ctrax was simple. At the time, measuring the effects of neural activity on behavior meant measuring how a fly’s behavior changed after a group of neurons was switched on or off. To make sense of this behavior, a scientist would have to determine what the fly was doing in each frame of video – an impossible task when a single experiment can yield days of video.

Enter Ctrax. Using a variety of computer algorithms that allow a machine to process and analyze images, Ctrax enables researchers to track individual flies even when they congregate in large groups. Unlike other programs available to biologists, Ctrax doesn’t require users to know how to code. Instead, Branson created a GUI (pronounced “gooey” and standing for “graphical user interface”) that allows even non-coders to use the program.

When she first arrived at Janelia, Branson had ideas for improving Ctrax. But the number of other Janelia scientists also working on tracking software gave her pause, as did her realization that the tougher problem would be analyzing the fly’s behavior, not just tracking it. So Branson developed JAABA, the Janelia Automatic Animal Behavior Annotator, which is freely available to all researchers through Branson’s website. Researchers can “teach” JAABA the relevant behaviors to recognize and record, which allows them to begin to figure out what happens to the animal when neural activity is altered.

“The idea is you want to automatically be able to say for every frame and for every fly, Is this fly doing this certain behavior or not?” Branson explains. “Is this fly chasing another fly? Is it walking? Is it turning?”

Branson has begun using Ctrax and JAABA to screen all of the neurons in the fruit fly brain, to link specific groups of cells to behaviors. To begin, she took advantage of the 10,000 lines of fruit flies, created by Janelia Executive Director Gerry Rubin and his lab group, that express the protein GAL4, which scientists use to affect gene expression in different cells. Scientists can select the flies that express GAL4 in specific sets of neurons and then use the fluorescent marker GFP to visualize these neurons with a microscope. Branson crossed 2,000 different lines of GAL4 flies with flies containing *TrpA*, a temperature-sensing gene, which allows her to activate these neurons by raising the fly’s body temperature a few degrees. Placing these flies in an enclosed arena, Branson tracks them with Ctrax and records their behavior with



Jeremy Freeman built an open-source computer platform to help researchers analyze and share massive data sets.

“If you could give me the structure of the circuit, it would serve as crucial inspiration for hypotheses regarding what the circuit is trying to do.”

—SHAUL DRUCKMANN

JAABA. Used together, the systems give her a way to link the activity of specific neurons in flies with measurable behavior differences, such as walking speed.

Machine learning systems have benefits beyond just managing tidal waves of data; Branson has also found that these systems can recognize effects that might go unnoticed by humans.

“It might be a subtle behavioral change that’s happening, but you’re observing it lots and lots of times. So if you analyze a big enough data set, it’s going to come out,” she says. “Maybe it’s not a huge phenotype that you’re seeing – it’s not something that a human would typically notice – but if you compound this with how many times you’ve seen it, it’s something that can’t be ignored.”

Massive Computation

Ctrax and JAABA may be able to help neuroscientists identify and catalog relevant behaviors, but researchers also have other types of data to analyze. The problem, Freeman points out, is that some of these data sets are far too big for a single computer to handle. So researchers are turning to cluster computing, using multiple computers working together to make sense of their findings.

Historically, Freeman says, scientists have worked to solve these types of problems on a lab-by-lab basis. “Individual labs build their own little local solutions to solve problems that are totally customized to their labs and are meant to work on a single machine,” he says. “We’re at

a point where an individual lab is starting to reach a limit of what it can do in a reasonable amount of time.”

This process of continually reinventing the wheel didn’t seem productive, nor did it allow scientists to easily share their data with each other. So Freeman, in collaboration with others at Janelia and elsewhere, built an open-source computer platform that would allow researchers to analyze and share massive data sets. He utilized the Apache Spark platform – an open-source framework able to process large data sets on computer clusters – to create Thunder, a library designed specifically to analyze neural data. Detailed in *Nature Methods* in September 2014, the library is freely available for researchers to use and contribute to. Freeman estimates that Thunder is now being used by some 10 to 20 labs around the world. He’s using it to look at large data sets to gain a more holistic understanding of neural function.

In one of his collaborations, he has been working with fellow Janelia Group Leader Misha Ahrens to image the entire brain of the zebrafish using two-photon microscopy, which can record fluorescing cells at a depth of up to one millimeter. Freeman and Ahrens display a visual pattern in front of the fish. They want to know which neurons fire when the fish sees the pattern, which ones are active as it tries to swim, and whether the animal’s movement is feeding back into the neural activity. Freeman hopes to answer these questions both at the level of the entire brain and on the level of individual neurons. Just an hour of these recordings, however, can generate more than a terabyte of data (the rough equivalent of 16 million books), making the use of Thunder or another type of cluster computing software a necessity.

The analyses performed by Thunder, however, are only as good as the experiments that provide the data. To Freeman, theory and computation aren’t just things you do after you get your results – they need to be integrated into every aspect of the scientific process.

“If people handed me data, and I went away for six months and analyzed it, it would be boring,” he says. “That’s not really the way to progress. You need to be constantly interacting and finding cool stuff in the data together.”

Modeling Networks

The close marriage of computation, theory, and experiment isn’t unique to biology – it’s long been a feature of physics. Perhaps that explains why so many theoretical neuroscientists, including many of those at Janelia, got their start not in biology but in physics. Shaul Druckmann, for example, was all set to start a graduate program in high-energy physics but changed his focus to neuroscience after



Kristin Branson uses computation and machine learning to understand behavior.

This visual representation of neurons from the larval zebrafish brain shows calcium responses in the cells – an indicator of activity – measured using light sheet microscopy. Each circle marks a neuron's position, with colors based on a functional categorization. Curves connecting the circles reflect a measure of neuronal coupling.



hearing a lecture by a theoretical physicist who worked on neural networks. After completing his PhD in computational neuroscience at Jerusalem's Hebrew University, where he concentrated on detailed models of single neurons, Druckmann moved to Janelia for a postdoc position in 2013 and was subsequently hired as a group leader. Since coming to the research campus, he has focused on developing models of working memory, the process that holds information for brief periods of time.

Researchers had previously believed that if you asked mice to remember something for a short period of time, their neural activity would be relatively constant, since all they were doing was remembering. But when scientists actually recorded what the neurons were doing, they found that neural activity in the mice was all over the map, constantly changing. Druckmann wanted to build a model to show why such activity was always shifting. During his postdoc, Druckmann was able to show that a simple model, whereby neurons exchange information between themselves in a specific manner, shows how shifting activity is perfectly consistent with representing information that isn't changing over time.

"The problem in the brain is that typically you have networks of neurons that are

extremely strongly connected to each other, so A triggers B, B triggers C, and then C goes back and triggers A. But it also triggers D, E, and F, and each one of these also triggers X, Y, and Z, and they all feed back into each other," says Druckmann. "And, it's all nonlinear, which means it is considerably more difficult to intuit causes from effects, and all of these models become harder to think through.



Shaul Druckmann focuses on developing models of working memory, the brain's ability to hold information short-term.

Once one does the math, and understands how this can come about, it is straightforward to explain the intuition behind it, but without going through the calculations, it would be hard to reach that intuition."


At the same time, another Janelia group leader, Karel Svoboda, was testing short-term memory in mice, which gave Druckmann a chance to see if his ideas would match experimental data. The memory task presented to the mice was relatively straightforward. First, the researchers trained a mouse to respond to a sensory cue – a pole it could locate with its whiskers. Depending on the location of the pole, the mouse would respond by moving right or left after hearing a beep. Importantly, the sound was not played immediately after the mouse found the pole – the animal had to wait seconds before the beep sounded. This meant that the mouse had to remember both the location of the

pole and the direction in which it needed to move while it waited. Svoboda's team also used optogenetics – a technique that allows scientists to control neural activity with light – to strategically turn off different groups of neurons in the mouse brain cortex. They found that activity in an area of the brain called the anterior lateral motor cortex was crucial to the animal being able to perform the memory task. When scientists switched off that area, performance dramatically declined.

Now that he has this information, Druckmann is revising his models to more accurately reflect Svoboda's data. By understanding these fluctuations in neural activity on time scales of just a few hundred milliseconds, Druckmann hopes to understand what computations are going on during this time period and what kind of mechanisms may be responsible for it. A key piece of information that is hard to get at is the structure of the brain's neural networks, which can tell scientists a lot about how the different parts of the brain function. "If you could give me the structure of the circuit, it would serve as crucial inspiration for hypotheses regarding what the circuit is trying to do," Druckmann says.

Unifying Principles

Cracking just one neural network can give scientists insight into many other networks. Theoretical neuroscientist Sandro Romani, also a group leader at Janelia, spent the early years of his career modeling how primates perform working memory tasks. During his postdoc at the Weizmann Institute of Science in Israel, he worked to understand how humans remember long lists of words. Later, at Columbia University, he went on to study neural circuit dynamics in the hippocampus, a seahorse-shaped region deep in the brain

 To see more of the data being analyzed by Janelia's theorists, go to hhmi.org/bulletin/spring-2015.

“Theory is one of the tools in designing and getting the most out of an experiment.”

—LARRY ABBOTT

that controls navigation and long-term memory. Specifically, Romani was looking at recordings from place cells in the hippocampus of the rat, each of which maps a specific place in the animal's world. Ask a rat to run a familiar maze, and researchers can track where it is located by watching which place cells fire in its brain.

When scientists looked at this firing pattern more closely, however, they found that the signals were even more intriguing than they'd expected. In particular, they noticed a regularly repeating sequential activation of the place cells that moved more quickly than the rat did, indicating that the animal's movement wasn't driving this activity. Instead, the cells' activities were likely predicting the rat's future movements.

Just as a rat explores nearby places – which are similar but not the same – in a sequence, items in memory tend to be recalled according to how similar they are. For example, grabbing a box of cake mix at the grocery store reminds you to pick up frosting as well, instead of triggering your memory that you also need steak. Scientists study this kind of memory by asking people to remember a long list of words in what's known as the free recall task. As the list becomes longer, people can recall more words but the fraction of the total gets smaller.

Romani and colleagues realized that the nervous system may use similar dynamics for both sequences of places and sequences of words, and they developed a neural network model to show that this can be done with a realistic neural architecture.

At Janelia, Romani has worked with Group Leader Eva Pastalkova on a modified version of the model to account for some of her experimental results on a group of hippocampal neurons she has dubbed “episode” cells. These neurons are active in a sequence when a rat stops its motion through the environment while it is engaged in a memory task.

“One contribution that theoretical neuroscience can bring to the table is to step back a little from the phenomena and try to find unifying mechanisms and principles behind them,” Romani says.

Theoretical models, Abbott points out, are another set of tools for people designing and carrying out experiments – and should be treated as such. “In the past, you generated a whole bunch of data and you just threw it at a theorist and said, ‘Well, what does it mean?’ But it's better to have people with different expertise *in* the process,” he explains, “the same as you might have an expert microscopist in the process and say, ‘How do we design this experiment so we can get

the best use out of the tools?’ Theory is one of the tools in designing and getting the most out of an experiment.”

It's a process Abbott himself uses while trying to understand how an animal responds to stimuli. For example, the olfactory system in a fly helps it to recognize different odors in its world, everything from nutritious food to the presence of potential mates. But the fly's brain doesn't stop at just recognizing odors; it also helps drive the fly's behavior in response to those odors. A fly might move toward healthy food but away from food that is less nutritious – responses that Abbott, working with the lab groups of Gerry Rubin at Janelia and HHMI Investigator Richard Axel at Columbia University, has begun to identify in the olfactory circuit.

“You see this transformation from a representation that's dominated by the outside world – many odors come in and activate cells – to something that's internal to the animal,” Abbott says. “It depends on their experience, whether they're hungry or not, those kinds of things.”

The task now, he says, is to work with other scientists to design experiments to identify those experiences and internal factors that drive choices. All the theoretical and computational neuroscientists at Janelia agree that their work is iterative.

“What I hope will happen at Janelia is that there will really be this close shoulder-to-shoulder interaction with experimental labs: the development of models based on experimental observations, which in turn generates predictions to be tested with new experiments,” Romani says.

This partnership among theory, computation, and experiment in neuroscience gives researchers an enhanced ability to devise effective ways to peer into the mysterious depths of the brain. As better experiments yield a clearer understanding of neural processes, these scientists are helping researchers to navigate the turbulent sea of neural activity and row toward the truth. ■



Sandro Romani studies the ability of the brain's hippocampal cells to store sequences of places and words.

Perspectives & Opinions

The Theory Connection

Neuroscientist Jeremy Freeman believes scientific theory is all about making connections: between people, between labs, between biological systems. And he considers Janelia Research Campus, where he's a group leader, to be an ideal environment for fostering those relationships.

WE ALL LEARNED the scientific process. Formulate a hypothesis, collect exactly the right data to test the hypothesis, and then evaluate it. But it's never that straightforward. More often, we collect not-really-the-right data and end up swimming in observations, many of them unexpected.

Some are curious, some are trivial, some are groundbreaking. How do we sort through them? How do we plan the next experiment? How do we put it all together?

To me, theory is a collection of strategies, tools, and concepts for making sense of experimental data. It's the halfway point between data and ideas. By design, I rarely enter a collaboration or experiment with a preconceived notion of what I hope or expect to find. I let my thinking be driven by the data, and allow myself to be surprised. But data analysis quickly suggests hypotheses

and more targeted experiments. I do my best to sit in the middle of that loop.

This approach becomes all the more pressing as we study increasingly complex systems. The magnitude of data being collected and the experimental capabilities, at Janelia and elsewhere, are outpacing our ability to analyze the data, let alone develop principles for what they mean and what experiments to do next. We have unprecedented access to the inner workings of the nervous system and can do experiments that a few years ago would have seemed impossible. To keep up, we need to modernize our analysis, making it more efficient, more collaborative. And do it fast enough to adjust our experiments in real time.

One great example is my lab's collaboration with Nick Sofroniew, in Karel Svoboda's lab. Nick has designed an incredible virtual reality system that simulates a tactile environment through which a real mouse can navigate, running on a ball while walls move back and forth stimulating its whiskers. We can monitor neural responses over increasingly large portions of the animal's brain while

it performs this behavior.

The complexity is daunting – not only of the data themselves, but the sheer number of possible behavioral paradigms and experimental manipulations. One way to start paring down that list is to analyze the data online.

This lets us make experimental decisions or manipulate neurons

based on the measured data, all while the mouse is still on the ball. Some of what we're developing is specific to this system, but much of it is general – both the techniques and the ideas – and we're actively collaborating with others to develop similar approaches in other systems.

Establishing a computational collaboration starts with making a personal connection. We need to open and maintain conversations, between theorists and experimental groups, and among different groups who might find it

useful to be exposed to each other's ideas. I don't think it's a surprise that most of the great discoveries in science have two or more names attached to them, and that will only continue as we tackle larger and more complex problems – together.

Janelia is a phenomenal place to do computation and theory because there are almost no barriers to building these relationships. Everyone is so open to looking at the experimental data with others. Janelians want to collaborate.

The physical space in which we work matters a lot, especially when it comes to proximity. Janelia is relatively small, so it's easy to run into people. On a big, sprawling campus it would be harder to get collaborations off the ground. At Janelia, if I just sit all day by the coffee bar, I'll have a chance to talk with all of my collaborators – and probably end up with new ones.

Theorists' activities at Janelia are diverse, but one thing we have in common is that we work across different systems. Theorists at Janelia have a unique opportunity to sit at the intersection between labs, revealing principles that span animals and modalities, and sharing ideas and techniques. Part of my own work is building platforms for analysis of neural data that make it easier to collaborate and coordinate. As that effort grows beyond our campus and into the broader neuroscience community, Janelia itself has been a model for what openness and collaboration can, and should, look like.

I often hear that neuroscience needs more theorists. Maybe it's not that we need more; it's just that we need to dissolve the barriers to making connections. All scientists should work closer to the intersections. We'd all be thinking a little more theoretically, a little more computationally. And that's where the big ideas will come from.

–Interview by Nicole Kresge

Jeremy Freeman

—
Jeremy Freeman is a group leader at the Janelia Research Campus.

Jeremy Freeman
applies computational
approaches to
data analysis and
experimental design.



Perspectives & Opinions

Q&A

What book have you read that influenced your work, and what effect did it have?

Books have the ability to take us places we've never been, to teach us concepts we didn't know, and to introduce us to people we've never met. Often, long after we've closed a book, its contents will stay with us. Here, four HHMI investigators recall some of their memorable reads.

–Edited by Nicole Kresge



Nicole King

HHMI Investigator
University of California, Berkeley

When I was in graduate school at Harvard, paleontologist Andy Knoll handed me a copy of *The Evolution of Individuality* by Leo Buss, which opened up an entirely new way of thinking about evolution and development for me. The book provides a beautifully written and compelling framework for thinking about the meaning of “individuality” – a topic that is central to my lab’s work on multicellularity and the interactions between eukaryotes and environmental bacteria. It has become required reading for all new members of my lab.



Oliver Hobert

HHMI Investigator
Columbia University

A disparaging remark often heard from reviewers and editors is that a piece of work is “too descriptive.” This statement never ceases to perplex me. Javier DeFelipe’s wonderfully illustrated book *Cajal’s Butterflies of the Soul: Science and Art* – its poetic name is derived from Cajal’s description of pyramidal neurons in the neocortex – serves as a brilliant reminder of the importance of descriptive science. The book conveys the beauty of such work and encourages me to keep pursuing several highly descriptive lines of research.



Erich Jarvis

HHMI Investigator
Duke University

I had my lab read George Gopen’s *Expectations: Teaching Writing from the Reader’s Perspective*, a book about writing skills focused on what scientists and other professionals expect to read in articles, grant proposals, and books. Gopen, a Duke University professor who studies writing, claims that our prose can be more successful if we consider the common reader’s perspective and expectations. After I started using Gopen’s suggestions, I found that my grants and papers were more understandable. It didn’t mean that my readers agreed with me, but at least they grasped what I was trying to explain.



Jeannie Lee

HHMI Investigator
Massachusetts General Hospital

In George Orwell’s *Animal Farm*, I came to understand that one of Snowball’s seven commandments – the transfiguration of “Four legs good, two legs bad” to “Four legs good, two legs better” – was a sign to validate animal models. I am once again embracing human cellular models.

Chronicle

34 SCIENCE EDUCATION

From Insight to Action

36 TOOLBOX

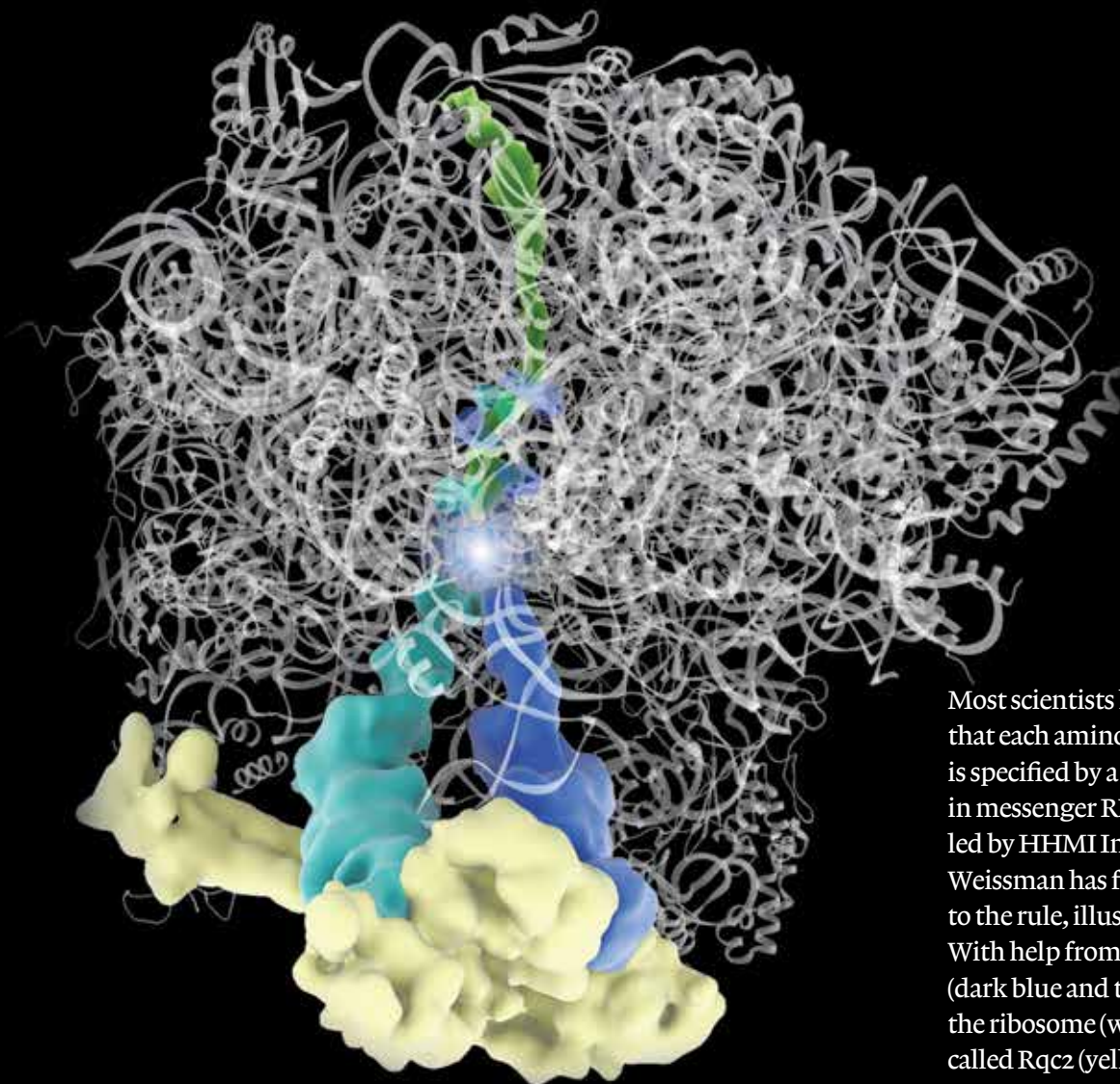
Barcoding Bacteria

38 LAB BOOK

T Cell Burnout

Got Thirst? Here's Why

Salvaging SNAREs



Most scientists have long believed that each amino acid in a protein is specified by a three-letter code found in messenger RNA (mRNA). But a team led by HHMI Investigator Jonathan Weissman has found an exception to the rule, illustrated in this structure. With help from a cell's transfer RNA (dark blue and teal) and part of the ribosome (white tangles), a protein called Rqc2 (yellow) can assemble a protein (green) in the absence of an mRNA blueprint. But there's one caveat. Rqc2 recognizes only alanine and threonine (abbreviated "A" and "T"), so it creates what Weissman refers to as "CAT tails." *Read more about the research in "A CAT Tale" on page 38.*

Chronicle / *Science Education*

From Insight to Action

PULSE, a national network of science education leaders, is helping transform college-level science teaching and student learning.

SOMETIMES THE BEST way to solve big, national problems is to start by talking to your neighbor just down the road. Take Jenny McFarland, for example, a biology faculty member at Edmonds Community College in Lynnwood, Washington. Many of her science students continue their studies at nearby four-year universities and colleges – but seldom do faculty members from the various schools interact with each other. Yet if professors don't coordinate their teaching of key concepts, like the structure of cells or the growth of organisms, that can hurt students who transfer.

Recently, McFarland joined faculty teams from 14 schools in the Northwest for a three-day workshop to think about how the institutions could learn from one another for the benefit of their students. “We wanted to look at our region as a system,” says McFarland. “How could we work together? What resources could we draw from each other?” For some institutions – even those just

a few miles apart – it was the first time their faculty members had talked to one another.

The workshop was part of a regional initiative conducted by the Partnership for Undergraduate Life Sciences Education (PULSE), a group formed after the 2011 publication of a report titled *Vision and Change in Undergraduate Biology Education: A Call to Action*. Produced by the American Association for the Advancement of Science, with support from the National Science Foundation (NSF), the report offered a number of recommendations to make higher-education science teaching more effective – including giving students more research experiences, urging faculty members to move beyond the traditional lecture format, and catalyzing departmental-level change. To help turn these ideas into concrete actions, officials at HHMI, the NSF, and the National Institute of General Medical Sciences worked together to found PULSE.

“We wanted to make sure that this report didn't just gather dust on everybody's shelves,” says HHMI's Cynthia Bauerle, assistant director of undergraduate and graduate programs (and a coauthor of *Vision and Change*).

The effort got under way in 2012 with the appointment of 40 PULSE Fellows; one of them was McFarland, and all were mid-career or senior life science faculty members at schools ranging from community colleges to large research universities. Since then, PULSE has made significant progress in identifying, measuring, and supporting schools' efforts to implement Vision and Change principles for exceptional life sciences education.

One of the group's first projects was developing robust regional networks. These networks focus on area-specific interests and opportunities. For example, in the Southeast, teams of campus leaders from community colleges, small private colleges, research institutions, and historically black colleges met last summer to discuss strategies that could help underrepresented minorities persevere and succeed in the sciences. Up until that meeting, interaction between the region's historically black colleges and predominantly white institutions had been relatively rare, says Bauerle.

A second key initiative is a program to recognize institutions that have done

exceptional work implementing Vision and Change recommendations. This process digs deep into schools' curricula and approaches: Are concepts such as evolution taught explicitly and implicitly throughout the curriculum? Are more than half of science students able to take advantage of mentored research opportunities? Are classrooms small and designed for real interaction between students and faculty members, as opposed to cavernous, theater-style lecture halls?

Group members recently completed a successful pilot study with eight schools to see if the criteria they'd developed accurately measured whether schools were achieving Vision and Change goals. The results, which will be shared on the PULSE website later this spring, were promising, says Kathy Miller, a PULSE Fellow and chair of the biology department at Washington University in St. Louis. The group will likely assess more schools in the near future, she adds.

The recognition program will both commend top-performing schools and help all schools understand smart next steps, says McFarland. “If people are assessing their departments with these rubrics, and they want to see ways that they can improve, they have a roadmap,” she says. People trying to make a case for investment in teaching, she continues, “aren't just saying, ‘I have an idea that I want to try in the classroom.’ They're making the case with rubrics that have been standardized, vetted, and validated.”

A third major initiative is the creation of Vision and Change Ambassadors – teams of

“We wanted to make sure that this report didn't just gather dust on everybody's shelves.”

—CYNTHIA BAUERLE



faculty trained to guide conversations about reform at other institutions. “The ambassadors meet faculty members on their own turf and try to kick-start departmental change,” explains McFarland, “whether that’s to get things started, help them change directions, or guide them in some way.”

As these various initiatives within PULSE take shape, a growing online community – now numbering some 1,500 members, at pulsecommunity.org – is actively sharing its successes, challenges, and teaching practices. It’s proving that a strong vision still allows for

varied, effective teaching approaches, says Jim Collins, an ecologist and evolutionary biologist at Arizona State University. “I’m seeing a lot of diversity in the way that individuals are moving away from the ‘sage on the stage’ model,” he says. “They might use clicker [technology] to gather information, they might try flipping the classroom with video-based lectures that students watch on their own time, they may work with smaller groups. There’s so much richness, and that’s great.”

As the members of PULSE look ahead, they hope to continue to foster close

connections among institutions, to scale up the recognition program, and to provide increased support to institutions and individuals who want to implement Vision and Change principles in their classrooms and departments, says Chuck Sullivan, a program director for the NSF’s Division of Biological Infrastructure. “PULSE is expanding its circle of influence, like the concentric rings you see after a stone is thrown in a pond,” he says. “More people are getting involved and reforming their courses. And that’s what we hoped would happen.” – Erin Peterson

Chronicle / *Toolbox*

Barcoding Bacteria

DNA barcodes and a half-century-old equation help scientists track infection in the gut.

FOR THE BACTERIA that cause cholera, the journey through a host's gut is no picnic. The first stop is the stomach, filled with gastric juices so deadly that the vast majority of *Vibrio cholerae* bacteria won't make it out alive. Survivors burrow into the wall of the small intestine, hoping to avoid the tide of digested food rushing by. The lucky ones will

reproduce; the less fortunate will be attacked by the immune system or, in some cases, killed by antibiotics.

These ebbs and flows in population size have long fascinated biologists, who can use the information both to garner clues about the genetic fitness of an organism and to pinpoint the best times to overpower a pathogen.

"From a theoretical perspective, it's much easier to fight a very small population than a big population," explains Pia Abel zur Wiesch, a postdoctoral fellow who works with Ted Cohen, an infectious disease expert at Brigham and Women's Hospital in Boston. "If you have an antibiotic or vaccine acting when the population is especially vulnerable and small, you have a much higher chance of success because it's easier to eliminate a few bacteria than a lot of them."

But how can researchers track a population of microscopic bacteria inside an animal's gut? Zur Wiesch and her husband, Sören Abel, also an infectious disease postdoctoral researcher, were pondering this question at dinner one night, shortly after he joined the lab of HHMI Investigator Matthew Waldor, also at Brigham and Women's Hospital. The two postdocs decided to combine her mathematical expertise with his microbial knowledge to answer the question. Their solution involved coupling 500 DNA barcodes with a 50-year-old mathematical formula to produce a technique that can track the ups and downs of pretty much any cell population – pathogen or otherwise.

The technique is called STAMP, for "sequence tag-based analysis of microbial populations." It's a two-part procedure that boils down to tacking a kind of barcode onto bacteria and then tracking the barcode frequency to calculate the size of a founding population.

The barcodes are short stretches of DNA that help the scientists distinguish one bacterial cell from another. "The labels are just like last names," says zur Wiesch. "They don't really change anything about the individuals. If you think about a few people founding a village, for example, and you just follow their last names, you can infer how many people there were to begin with."

To deduce the size of the founding population from the barcodes, zur Wiesch made use of a mathematical equation established nearly a half-century ago. In 1971, geneticists Costas Krimbas and Spyros Tsakas showed that it was possible to use markers to infer the size of a population at two different time points. Krimbas and Tsakas used their equation to follow the effects of an insecticide on the population dynamics of fruit flies that fed on treated olive trees.

In a similar fashion, Abel, zur Wiesch, and their colleagues used STAMP to trace the growth and decline of *V. cholerae* infection in rabbits. Abel added about 500 different barcodes to the genomes of a batch of *V. cholerae*. He infected rabbits with the labeled bacteria and then collected samples from the animals' guts at various time points. New deep DNA sequencing technology allowed him to determine how many of each type of tagged bacteria were in the samples.



With those numbers in hand, the researchers used Krimbas and Tsakas’s equation to trace the population dynamics of the tagged *V. cholerae* bacteria. They compared the relative abundance of the tags in the injected bacteria to the numbers in the different intestinal samples. A sample with a large change in tag frequencies most likely experienced a bottleneck – some event that drastically reduced the population size and, in the process, altered the distribution of tags. Conversely, a sample with a smaller change in tag frequencies probably went through a more benign pathway that had a minimal effect on the bacteria, resulting in a population very similar to the one that had initially infected the rabbits.

The calculations yielded some surprising information about the bacteria’s journey through the gut. “Frankly, when we applied STAMP to *Vibrio cholerae* in rabbits, we thought it would be kind of a boring experiment,” says Waldor. “We thought the pattern of bacterial migration from the stomach to the anus would be a foregone conclusion. But that’s not how it turned out.” Instead, the team discovered that, after a certain point in the infection,

the bacteria changed direction, going up the intestinal tract instead of heading down.

“We couldn’t have figured that out with any other method, because we wouldn’t have known the identity of the bugs,” says Waldor. Though the scientists aren’t sure why the *V. cholerae* reversed course, they’re investigating a few ideas.

The researchers’ technique, published in the March 2015 issue of *Nature Methods*, isn’t limited to bacteria, according to Waldor. “STAMP is applicable, in principle, not only to all pathogens – bacteria, viruses, and parasites – but also to commensal organisms and even to eukaryotic cells,” he says. “For example, you could use this in cancer metastasis studies, to determine how many cancer cells metastasize to a new site.”

The importance of the method, says Waldor, is that it enables scientists, in a retrospective fashion, to figure out how many cells or bacteria were the founders of a particular population and to infer where bottlenecks occur. This is good news for humans, but bad news for *V. cholerae* and other pathogens, now that scientists can pinpoint when they’re most vulnerable. –Nicole Kresge

“STAMP is applicable, in principle, not only to all pathogens, but also to commensal organisms and even to eukaryotic cells.”

–MATTHEW WALDOR

T Cell Burnout

Some immune cells function better after taking a break.

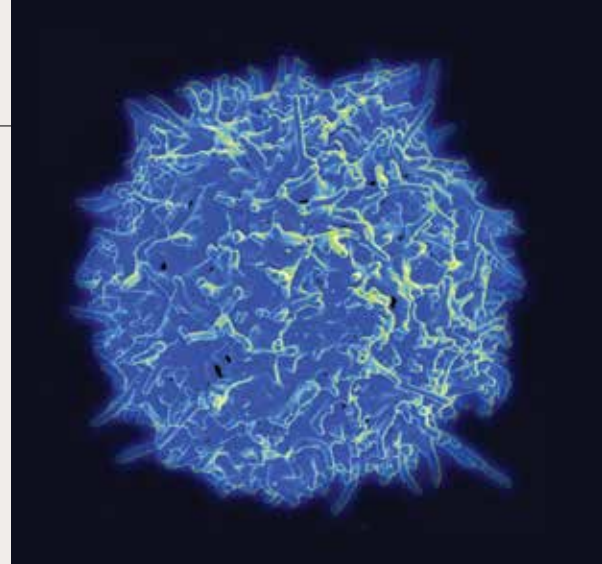
EVERY LIVING BEING needs to rest. Even our immune cells enter a dysfunctional state called T cell exhaustion if they're overworked. In fact, this fatigue and the ensuing downtime are important parts of the immune process.

Killer T cells get their name from their function. They target sickened cells involved in chronic infections, killing them before

the viruses inside can replicate and spread. But chronic infections can be lengthy, and after a few weeks of sustained action, cellular exhaustion often sets in. The T cells become less effective at slaying their targets and begin producing proteins that prevent them from recognizing infected cells.

One of these proteins is called programmed cell death protein 1, or PD-1. Susan Kaech, an HHMI early career scientist at Yale University, discovered a feedback loop by which PD-1 activation leads to an increase in a protein called FOXO1. FOXO1, in turn, produces factors that promote T cell exhaustion, including more PD-1. Kaech suspected that eliminating FOXO1 might curtail T cells' exhaustion and make them better killers.

What she found was the exact opposite. When her team created mice lacking the FOXO1 gene, the rodents' T cells did produce less PD-1 compared to animals with the gene. But the cells weren't better at controlling the viral infection. Instead, without the rest afforded them in their exhausted state, the cells died, and viral replication increased.



The immune system's T lymphocytes, like this one, need downtime to do their job properly.

Kaech concluded that T cells need this respite to function properly. "They have to turn down their response or else they'll get over-activated, and we think that causes the cells to deteriorate and die," she says. "We're starting to appreciate that exhaustion is an important process that is helping to maintain this precious pool of T cells."

The findings, published November 20, 2014, in *Immunity*, could lead to drugs that modulate FOXO1 and help reinvigorate the immune response of patients being treated for chronic viral infections or even cancer. "There may be a point where you can stop the cells from fully entering the exhausted state – where you suppress PD-1, but not so much that the cells die," Kaech says. —Nicole Kresge

IN BRIEF

SEEING RED

As a fish swims, nerve cells fire in its brain, sending signals racing along a neural network ending in muscles that make its fins flap and its tail swish. By using a molecule called CaMPARI to permanently mark neurons as they fire, scientists can now watch as signals light up such neural networks in live animals.

CaMPARI came out of a collaborative project spearheaded by Eric Schreiter, a senior scientist in Group Leader Loren Looger's lab at the Janelia Research Campus. The team started with a protein called Eos, which emits a green glow until it's exposed to violet light. The light changes the molecule's structure, causing it to glow red. By combining Eos with the calcium-sensitive protein calmodulin, the researchers were able to couple Eos's color change to the burst of calcium that accompanies



neuronal signaling. The resulting molecule indelibly tags firing neurons with a red glow in the presence of violet light.

"Ideally, we would flip the [violet] light switch on while an animal is doing a behavior that we care about, then flip the switch off as soon as the animal stops the behavior," Schreiter explains. "So we're capturing a snapshot of neural activity that occurs only while the animal is doing that behavior."

The scientists published their results February 13, 2015, in *Science*. Although they are still tinkering with CaMPARI to make it more sensitive and reliable, they've already made it available to scientists on Addgene and the Bloomington Drosophila Stock Center. Janelia Group Leader Misha Ahrens is also distributing CaMPARI-expressing zebrafish.

IMMUNE RALLY CRY

Like a Good Samaritan, a cell that's been attacked by a virus warns

neighboring cells to shore up their defenses. These alerts are sent via a family of proteins called interferons, which are produced when surveillance proteins in the infected cell detect a pathogen. Although several different surveillance proteins scout for signs of pathogens, new research shows how the proteins all activate a single molecule called IRF3 to turn on interferon production.

There are three known pathways that trigger type 1 interferon production. In each case, the individual pathway's unique surveillance protein uses its own adaptor protein to relay the message that an invader is present and interferon is needed. Siqi Liu, a graduate student in HHMI Investigator Zhijian "James" Chen's lab at the University of Texas Southwestern Medical Center, noticed that all three adaptor




proteins have similar stretches of five amino acids that became tagged with phosphate groups. As the team reported March 13, 2015, in *Science*, the addition of a phosphate molecule to that stretch of amino acids causes the adaptors to activate IRF3.

Now that they know how the interferon pathways converge, Chen and his team are examining them in more detail. Eventually, they hope to develop small molecules that treat immune disorders by interfering with the pathways.

A CAT TALE

A central tenet of biology is that each amino acid in a protein is specified by a three-letter code found in messenger RNA (mRNA). That may be true most of the time, but HHMI Investigator Jonathan Weissman at the University of California, San Francisco (UCSF), along with Onn Brandman at Stanford University and UCSF

 Watch a mouse begin to drink thirstily when prompted by a blue light. Go to hhmi.org/bulletin/spring-2015.

Got Thirst? Here's Why Scientists have pinpointed the neurons in the brain that control thirst.

HUMANS CAN TYPICALLY survive only three to five days without water. But we've all said "I'm dying of thirst" well before we've been waterless that long – albeit with little understanding of the mechanism behind the urge. But now the brain signals that govern thirst are no longer a mystery. A team led by HHMI Investigator Charles Zuker has figured out that the motivation to drink water is controlled by two sets of neurons: one that provokes the stimulus to sip and one that quenches it.

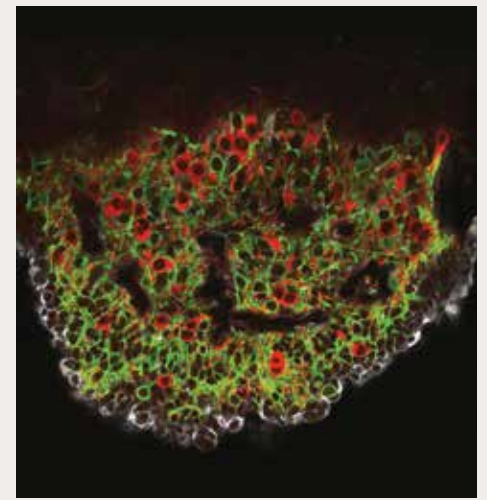
Researchers have long suspected that the signals driving animals to drink originate in the brain's subfornical organ, or SFO. Located outside the blood-brain barrier, where it has the opportunity to directly sense the electrolyte balance in body fluids, the SFO shows increased activity in dehydrated animals. Yuki Oka, a postdoctoral fellow in Zuker's Columbia University lab, took a closer look at the SFO in mice and identified two types of nerve cells: excitatory CAMKII-expressing neurons and inhibitory VGAT-expressing neurons. Using optogenetics, Oka added a light-sensitive protein to the cells in the animals' SFOs, allowing him to selectively activate them with blue light. When he flipped the switch and activated the CAMKII neurons, the rodents drank with gusto.

"You have a water-satiated animal that is happily wandering around, with zero interest in drinking," says Zuker. "Activate this group of SFO neurons, and the mouse just beelines to the water spout. As long as the light is on, that mouse keeps on drinking." Oka showed that the animals became such avid drinkers that they consumed as much as 8 percent of their body weight in water – the equivalent of 1.5 gallons for humans. When Oka used the same technique to stimulate VGAT neurons, thirsty animals immediately stopped drinking and reduced their water

intake by about 80 percent. The researchers published their findings online January 26, 2015, in *Nature*.

According to Zuker, the opposing neurons likely work together to ensure animals take in enough water to maintain fluid homeostasis, including blood pressure, electrolyte balance, and cell volume. It remains to be seen whether the same circuit controls thirst in humans; if so, the findings could one day help people with an impaired sense of thirst. – *Nicole Kresge*

The mouse brain contains CAMKII neurons (red) that trigger thirst and VGAT neurons (green) that quench thirst.



colleague Adam Frost, has discovered an exception to the rule.

Generally, protein synthesis requires three components: an mRNA template, a ribosome to read the template and assemble the protein, and transfer RNA (tRNA) molecules to supply the necessary amino acids. Weissman and his colleagues discovered that sometimes, when protein synthesis stalls, elongation can still continue without the mRNA template. This type of synthesis relies on only two components: a part of the ribosome called the 60s subunit and a protein named Rqc2 that recruits tRNA molecules. With these components, cells will continue to randomly add amino acids to the stalled peptide, with one caveat. Rqc2 recognizes only alanine and threonine (abbreviated "A" and "T"), so it creates what Weissman refers to as "CAT tails."

"This is a mode of protein synthesis that we've missed for 50 years. It's totally unprecedented," says Weissman. He and his colleagues

published their findings January 2, 2015, in *Science*. They don't yet know the function of CAT tails, but they have some ideas they're pursuing. For example, the extra amino acids may trigger a stress response in the cell, or they could tag an incomplete peptide for destruction.

HOX PARADOX NO MORE

During development in animals, the Hox gene family directs the formation of segment-specific anatomy along the head-to-tail axis. The genes are responsible for putting the head, thorax, abdomen, and other body parts in the right places. Surprisingly, all Hox proteins bind with high affinity to very similar stretches of DNA, which begs the question: how can Hox proteins find and activate their target genes if all binding sequences look essentially the same?



Janelia Group Leader David Stern recently revealed the answer to this long-standing paradox.

Stern's team, in collaboration with Richard Mann's lab at Columbia University Medical Center, figured out that Hox proteins activate genes via weak interactions at previously unrecognized DNA binding sites. The scientists discovered this by showing that a Hox protein called Ubx controls expression of a gene called *shavenbaby* by binding weakly to two enhancer regions on DNA near the gene. These low-affinity binding sites conferred *shavenbaby*'s specificity for Ubx over other Hox proteins.

The findings, published January 15, 2015, in *Cell*, explain why scientists have been unable to predict where Hox proteins bind to DNA simply by looking at its sequence. "They're not regulating genes by binding to the sites that everybody thought they were – they're binding to these

sites that don't look like good Hox binding sites," explains Stern.

A TARGET FOR DYSKINESIA

Some human diseases occur episodically in people who appear otherwise healthy. One of these disorders is paroxysmal nonkinetic dyskinesia (PNKD). The neurological disease's symptoms – involuntary movements in the limbs, torso, and face – are triggered by coffee, alcohol, and stress. In 2004, HHMI Investigator Louis J. Ptáček at the University of California, San Francisco, discovered the gene that causes PNKD. Now, just over a decade later, he's figured out what the gene does.

To deduce the function of the PNKD gene, Ptáček's team raised antibodies against the PNKD protein. This allowed them to isolate PNKD and its binding partners from mouse brain tissue. From these isolates, they discovered that PNKD interacts with two other proteins, called RIM1 and RIM2, that are involved

Chronicle / Lab Book

Salvaging SNAREs

Scientists catch a protein-recycling machine in the act.

CELLS ARE HUGE proponents of recycling. They reuse everything from tiny electrons to massive molecular complexes. Despite this penchant for salvage, scientists have never been able to see one form of cellular recycling in action – until now. A group led by HHMI Investigator Axel Brunger recently captured a protein in the act of pulling apart a spent cluster of membrane fusion molecules.

“Membrane fusion is a process akin to the merging of soap bubbles into larger ones,” says Brunger, a structural biologist at Stanford University. “But that’s where the analogy ends, since biological membranes do not merge all that easily.” To facilitate the process,



A cell's NSF complex uses SNAP proteins (orange) to grasp and unwind membrane-embedded SNARE proteins (red and blue helices).

cells use membrane-bound SNARE proteins. During fusion, SNAREs located on opposing membranes zip together into a stable complex, linking the membranes. When the two membranes have become one, a protein called NSF recycles the SNARE components.

Brunger's team used a technique called single-particle electron cryomicroscopy to freeze NSF molecules bound to SNARE complexes at several stages and then capture images of them. The resulting snapshots provide near-atomic or better detail of the SNARE disassembly process. Like a series of movie stills, the images show NSF latching onto a SNARE complex, then NSF bound to ATP – the molecule that powers the salvage operation. Yet another image captured NSF after it had finished working, bound to an energy-depleted form of ATP, called ADP.

The SNARE complex resembles a rope with a left-handed twist; the team's images revealed that NSF uses adapter proteins called SNAPs to grasp the “rope” in multiple places. The SNAPs wrap around the SNARE complex with a right-handed twist, suggesting that the disassembly occurs via a simple unwinding motion that frees the zipped SNARE proteins.

The results, published February 5, 2015, in *Nature*, raise other questions the team is eager to pursue. “There is a lot to be done in order to understand the motions and conformational changes needed to disassemble the SNARE complex,” says Brunger. “Our electron microscope structures now enable us to design follow-up biophysical experiments to answer these questions at a very deep level.” – Nicole Kresge

IN BRIEF

in neurotransmitter release. As the scientists reported March 10, 2015, in the *Proceedings of the National Academy of Sciences*, PNKD normally suppresses neurotransmitter release, but the mutant form found in the disease does so less effectively. The result is excessive synaptic transmission, which leads to the involuntary movements experienced by people with the disease.

According to Ptáček, RIM1 and RIM2 may be important targets for developing better medications for PNKD and other dyskinesias, such as those seen in Parkinson's disease.

THE LONG LOST RECEPTOR

In our cells, there is a widely expressed secreted factor called pigment epithelium-derived factor (PEDF), which affects a bewildering array of bodily processes. The cellular receptors that bind PEDF are at the root of many useful activities, such

as preventing the formation of new blood vessels, protecting cells in the retina and brain from damage, and stopping cancer cells from growing. Despite considerable effort by scientists in academia and industry, the identity of the receptors had, until recently, remained a mystery. This long-standing puzzle was so intractable that it took HHMI Early Career Scientist Hui Sun and his team at the University of California, Los Angeles, seven years to solve.

“We were initially intrigued by this problem because of PEDF's broad medical value in treating major diseases,” says Sun. “But the hunt for the receptor turned out to be an extremely challenging adventure.” His team methodically searched for PEDF's binding partner by looking at various native tissues and cells, as well as in databases of molecules with unknown functions. Finally, they found two proteins that fit the bill – PLXDC1 and PLXDC2.

As Sun's team reported

December 23, 2014, in *eLife*, these two proteins confer cell-surface binding to PEDF, transduce PEDF signals into distinct types of cells that respond to PEDF, and function in a cell type-specific manner. Because of the critical role of these cell-surface receptors in PEDF signaling, they have high potential as therapeutic targets for cancer and other diseases.

UNIVERSAL PROTEIN SYNTHESIS

The process of gene expression is, at its core, universal. But different organisms use different methods to carry out the “DNA makes RNA and RNA makes protein” recipe. For example, the signals used to recruit ribosomes and start protein synthesis in bacteria are not the same signals used by eukaryotes. Yet much of the structure and function of the ribosome, the molecular machine that translates RNA into proteins, is conserved in both types of organisms. This made HHMI

Early Career Scientist Jeffrey Kieft at the University of Colorado Denver wonder if a universal start signal existed that could be recognized by both eukaryotic and prokaryotic ribosomes.

A structured region of an RNA molecule dubbed an internal ribosome entry site (IRES), which is used by some viruses to initiate translation in eukaryotes, fit the bill. Taking a close look at the molecule, Kieft's team discovered that it could also initiate protein synthesis in prokaryotes. This particular IRES, it turns out, binds both bacterial and eukaryotic ribosomes in a similar structure-dependent manner, but the bacterial interaction appears transient and weaker.

The findings, published March 5, 2015, in *Nature*, suggest there might be other naturally occurring structured RNAs that can initiate bacterial protein synthesis. One of Kieft's next steps will be to determine if other such structures do indeed exist, and, if so, what they mean in terms of evolution and gene regulation.



Observations

With Theory Comes Order

The gray matter of the human brain may not be as physically vast as the dark matter of outer space. But the challenge of crunching astronomical data pales compared to the complexity of parsing today's deluge of data about the brain. Making sense of that torrent requires more than banks of supercomputers, says computational neuroscientist and essayist Olaf Sporns. To achieve fundamental insights, he posits, neuroscientists must do as astronomers do and apply a theoretical framework to their immense quantities of empirical data.

Neuroscience, it turns out, is on the brink of its own “big data” revolution. Supplementing more traditional practices of small-scale hypothesis-driven laboratory research, a growing number of large-scale brain data collection and data aggregation ventures are now underway, and prospects are that this trend will only grow in future years. Mapping the roughly one hundred trillion synaptic connections of a human brain would by some estimates generate on the order of a zettabyte of data (a zettabyte corresponds to one million petabytes). For comparison, that's about equal to the amount of digital information created by all humans worldwide in the year 2010. And recently proposed efforts to map the human brain's functional activity at the resolution of individual cells and synapses might dwarf even these numbers by orders of magnitude. The coming data deluge is likely to transform neuroscience, from the slow and painstaking accumulation of results gathered in small experimental studies to a discipline more like nuclear physics or astronomy, with giant amounts of data pulled in by specialized facilities or “brain observatories” that are the equivalent of particle colliders and space telescopes.

What to do with all this data? Physics and astronomy can draw on a rich and (mostly) solid foundation of theories and natural laws that can bring order to the maelstrom of empirical data. Theories enable significant data reduction by identifying important variables to track and thus distilling a torrent of primary data pulled in by sophisticated instruments into interpretable form. Theory translates “big data” into “small data.” A remarkable example was the astronomer Edwin Hubble's discovery of the expansion of the universe in 1929. Integrating

over years of observation, Hubble reported a proportional relationship between redshifts in the spectra of galaxies (interpreted as their recession speeds) and their physical distances. Viewed in the context of cosmological models Albert Einstein and Willem de Sitter formulated earlier, these data strongly supported cosmic expansion. This monumental insight came from a dataset that comprised less than fifty data points, compressible to a fraction of a kilobyte. When it comes to applying theory to “big data,” neuroscience, to put it mildly, has some catching up to do. Sure enough, there are many ways of analyzing brain data that are useful and productive for extracting regularities from neural recordings, filtering signal from noise, deciphering neural codes, identifying coherent neuronal populations, and so forth. But data analysis isn't theory. At the time of this writing, neuroscience still largely lacks organizing principles or a theoretical framework for converting brain data into fundamental knowledge and understanding.

From *The Future of the Brain: Essays by the World's Leading Neuroscientists*, edited by Gary Marcus and Jeremy Freeman. © 2015 by Gary Marcus and Jeremy Freeman. Reprinted with permission from Princeton University Press.

Plant Pavers

Jigsaw puzzle-shaped cells bulging with chlorophylls (red) cover the surface of leaves in many flowering plants. The fanciful pattern is more than just a whimsical quirk of nature, however. HHMI Investigator

Elliot Meyerowitz and his team have shown that, as plants grow, the indented regions and lobe-like outgrowths of these so-called pavement cells create internal stresses that reorganize cytoskeletal proteins. These microtubule proteins align along the cell walls (green), a process that in turn reinforces them against stress – evidence of a subcellular mechanical feedback loop. Learn more about the role of force in the development of animals, as well as plants, in “Show of Force,” page 12.

