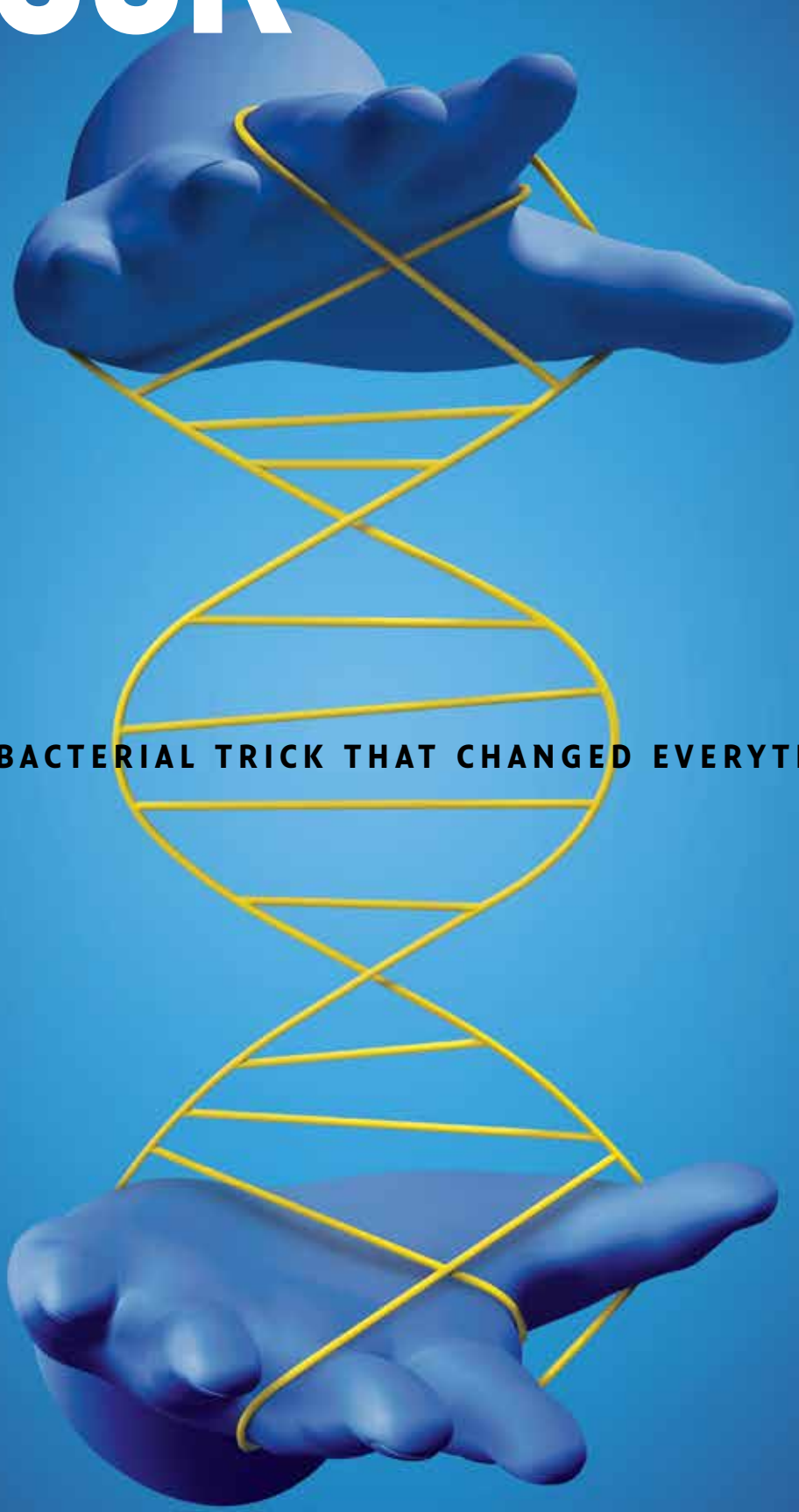
ISSUE
01
SPRING
2017

Seek

THE ROCKEFELLER UNIVERSITY



THE BACTERIAL TRICK THAT CHANGED EVERYTHING

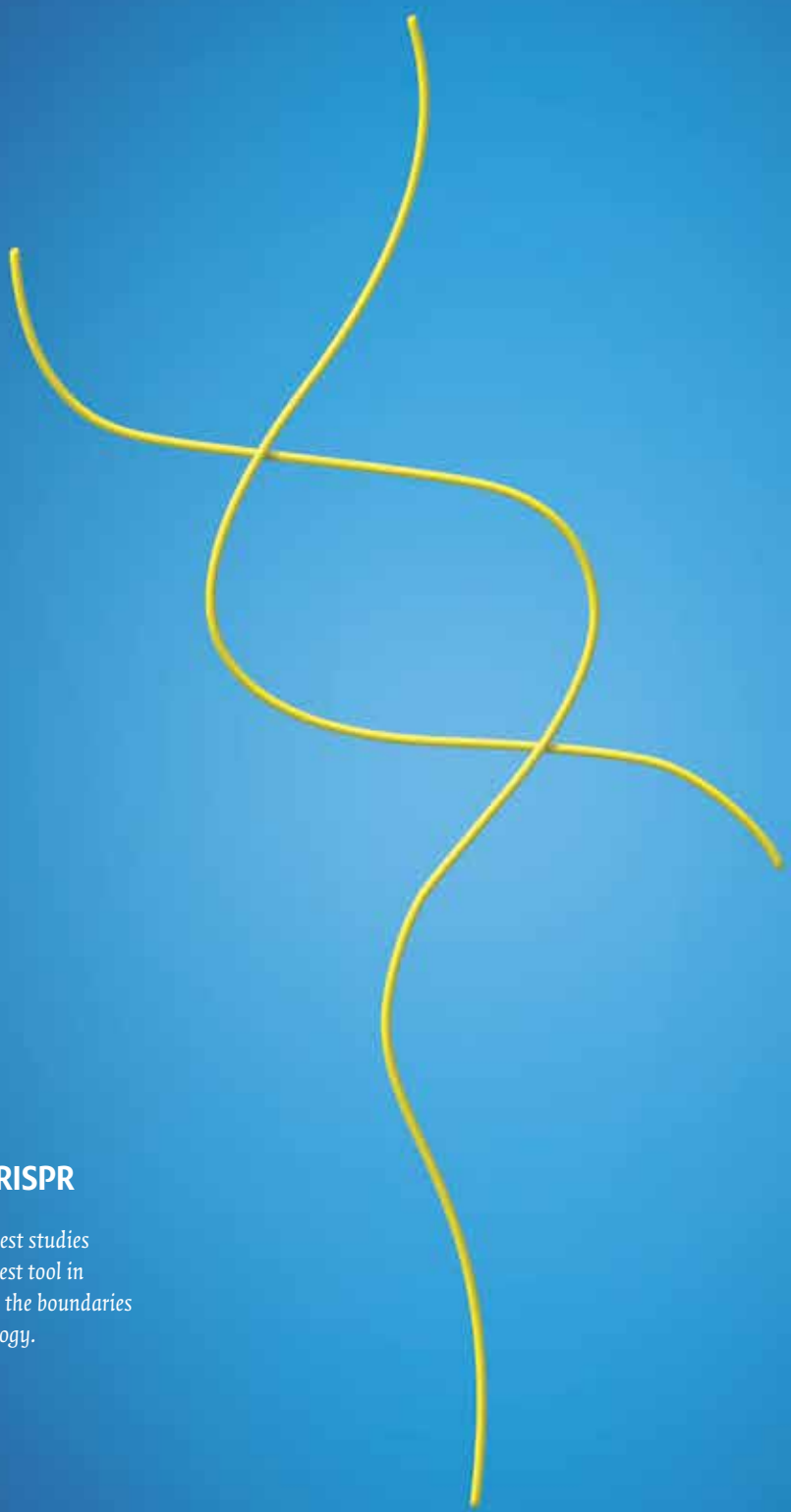
ALSO

An embryo's first two weeks

The radiogenetic mouse

Aspartame on trial

"The story of CRISPR is a testament to the value of basic biology for society."



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Unraveling CRISPR

Emerging from modest studies of bacteria, the hottest tool in biotech has redrawn the boundaries of experimental biology.

Illustrations by Alvaro Dominguez

Seek answers. Seek new questions.

Seek magazine is interested not just in scientific results, but in the people, ideas, and conversations that ignite discovery. In our coverage of bioscience at The Rockefeller University, and beyond, we tell the stories of how new knowledge comes to be. Meet the researchers working at the forefront, and learn how their work is driving science for the benefit of humanity.

Welcome to *Seek*.

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The method is as much genetics as physics, and relies on genes cobbled together from humans, camels, and jellyfish, delivered to mouse neurons via an engineered virus.

Illustration by Ellen Weinstein

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Our origins, observed

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After decades of work, Rice's studies of hepatitis C led to a breakthrough, and a cure. "The most crucial elements were blood, sweat, and tears," he says.

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Why are tornado clusters trending? What's the faintest flicker of light our eyes can detect? And when will Americans reap the benefits of a 50-year-old drug for heroin addiction?

ON THE BACK COVER
"The Rockefeller University" by Aleksandar Savić

LEFT TO RIGHT: ZACHARY VEILLEUX; SARI GOODFRIEND; ANDREW COLIN BECK

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Building by barge Constructing laboratories is easy, at least compared with constructing them over one of Manhattan's busiest roads. That takes a 1,000-ton marine crane, special permits from the Department of Transportation, and a close eye on the tides. Early last summer, the first of 19 preassembled steel modules making up Rockefeller's new $500 million Stavros Niarchos Foundation–David Rockefeller River Campus arrived via barge and was hoisted into place in a single overnight operation, precisely choreographed by a small fleet of tugboats.

PHOTO BY ZACHARY VEILLEUX

Reported by Katherine Fenz, Eva Kiesler, Wynne Parry, and Zachary Veilleux.

FOREFRONT

PHOTONICS

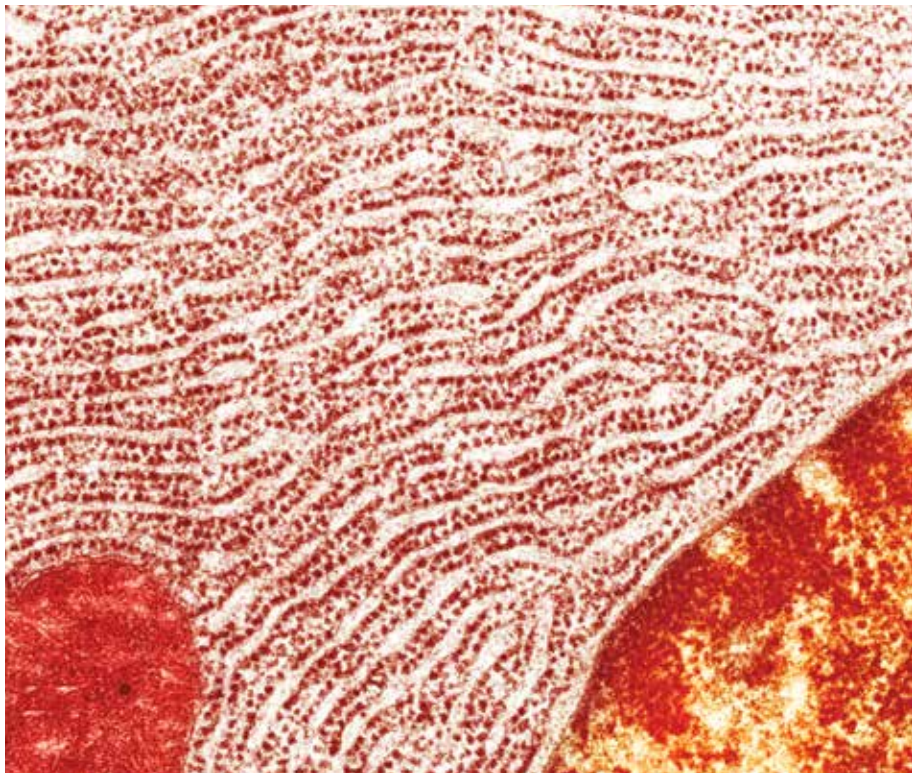
What meets the eye

JUST HOW DARK does it have to get before our eyes stop working? Entirely dark, according to recent experiments. They suggest the human eye can detect the presence of a single photon, the smallest measurable unit of light, once it has been acclimated to the dark.

"If you imagine this, it is remarkable," says Alipasha Vaziri, head of Rockefeller's Laboratory of Neurotechnology and Biophysics, who conducted the research together with scientists at the Research Institute of Molecular Pathology in Austria. "A photon—the smallest physical entity with quantum properties of which light consists—is interacting with a biological system consisting of billions of cells. And the response that the photon generates survives all the way to the level of our awareness."

Illustration by Angie Wang

A growing cell can generate 2,000 or more ribosomes every minute, each assembled by a workforce of some 200 proteins.



AMMRF, UNIVERSITY OF SYDNEY / SCIENCE PHOTO LIBRARY

DRUG INCUBATOR

Fighting yeast? Try aiming for their ribosomes

MAGNIFY A CELL a few ten thousand times, and a pilled-sweater pattern starts to emerge. Some of the cell's internal structures are covered with tiny, dense bumps called ribosomes, molecular machines made of RNA and protein whose job is to manufacture other proteins.

To keep growing, a cell has to produce thousands of new ribosomes every minute. If it fails to do so, it won't be able to divide. So if you want to get rid of certain cells, their ribosome assembly line might make a useful target.

In studying how yeast cells piece their ribosomes together, a team led by Tarun Kapoor has discovered a compound they believe might be developed into an effective antifungal medication. The researchers report in *Cell* that the drug, called Rbin-1, throws a wrench into the ribosome assembly process, halting the proliferation of yeast cells. "Not only does this compound efficiently inhibit the growth of yeast cells, it does so through a unique mechanism," says Kapoor, who is *Pels Family Professor*.

That's welcome news, since the repertoire of antifungal treatments is presently slim. And yeast infections—even generally benign ones like thrush—can be devastating if they spread throughout the body, especially in people with weakened immune systems.

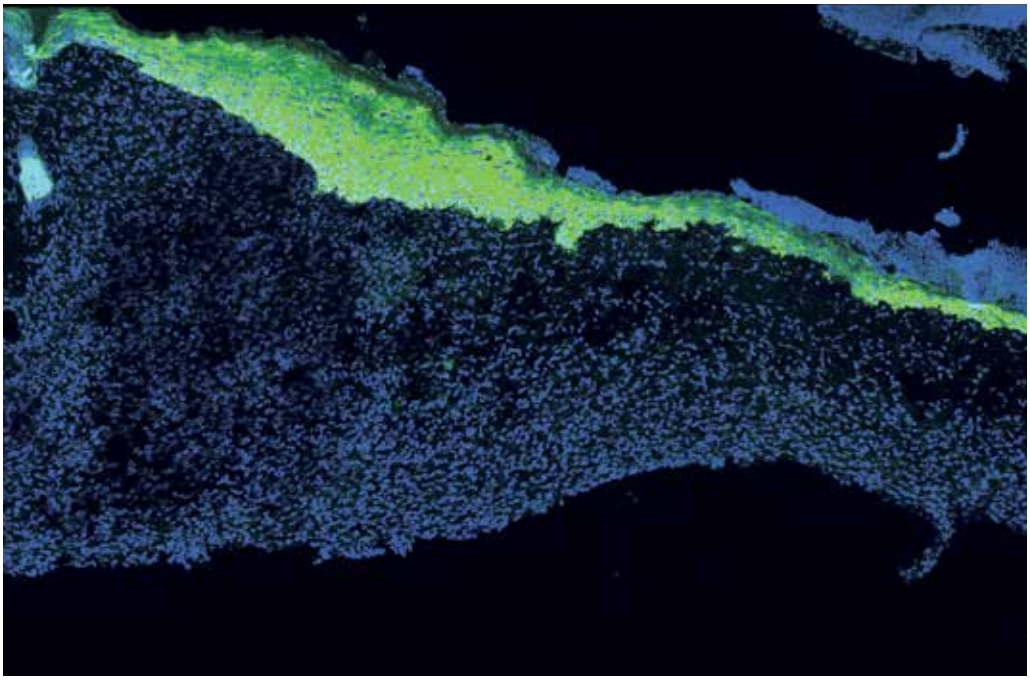
"No antifungals with new mechanisms of action have been approved by the FDA for the past several years," says Kapoor, "and no antifungal currently on the market interferes with ribosomal assembly." ◎

DATA

A 60-watt incandescent lightbulb emits roughly **8.2 quintillion** photons per second.

Earlier studies had established that the human eye meets its limit at flashes of five to seven photons, but these experiments may have lacked the technology needed to produce such tiny, precise bursts of light. "It is not trivial to design states of light that contain exactly one or any other number of photons," Vaziri says. His team was able to achieve this by implementing a combination of a psychophysics procedure and a quantum light source that can generate single-photon light states. The results, which combined data from more than 30,000 trials, were published in *Nature Communications*. ◎

In young mice, wounds heal quickly. New skin cells, shown in green, have arrived to seal a wound within five days of the injury.



HEALING

For sluggish wounds, a fountain of youth

THE OLDER WE GET, the slower our bodies mend.

Scientists have been intrigued by this aspect of aging at least since World War I, when French surgeons serving at the front line documented that similar-size wounds healed a lot faster in 20-year-old soldiers than in 40-year-olds. Still today, biologists are trying to pinpoint exactly what changes in the body to slow down the wound-healing process.

One explanation is that skin cells called keratinocytes lose their ability to communicate with nearby immune cells.

Whenever the skin is breached, the body's first response is to form a scab, and keratinocytes then travel in under the scab to seal the wound from below. Rockefeller researchers examined this process in 2- and 24-month-old mice—roughly equivalent to 20- and 70-year-old humans—and found that keratinocytes in the older mice took longer to arrive in the scab-coated cut. They also observed that these keratinocytes failed to recruit specialized immune cells, which in younger animals gather around the wound and help.

It turns out that the signals the younger skin cells use to call upon immune cells can be manipulated to boost wound healing in older animals. In experiments done using skin tissue grown in a petri dish, the researchers were able to make aging keratinocytes behave more youthfully by rekindling specific signaling pathways within them.

Elaine Fuchs, *Rebecca C. Lancefield Professor,* led the study, which was published in the November 2016 issue of *Cell*. She says the findings could lead to treatments that stimulate wound healing in elderly people.

"It may be possible to develop drugs to activate pathways that help aging skin cells communicate better with their immune cell neighbors," she says, "and so boost the signals that normally decline with age." ◎

6 MILLION

Number of Americans who suffer from chronic wounds, often as a complication of diabetes or other disease.

Wound healing is one of the most complex processes in the body, involving cells, pathways, and signaling systems that work over vastly different timescales.

LABORATORY OF MAMMALIAN CELL BIOLOGY / THE ROCKEFELLER UNIVERSITY

WHAT'S WRONG

Negative findings that reveal what we don't know, but thought we did

Of clones and colonies

FOR ANTS, status is everything. In any one colony there are queens, workers, and drones—each with unique anatomy and behavior. They look different, act different, and have dramatically different life spans. Yet their genes are identical.

Daniel Kronauer, who keeps dozens of ant colonies in his Rockefeller lab, spends much of his time trying to understand precisely what accounts for this dramatic variation. And although others in the field thought they had a pretty good idea—they implicated a chemical process known as methylation for turning on and off individual genes—Kronauer decided to take another look.

His lab's examination failed to confirm previous results, finding no differences in the amount of methylation between individual ants. The earlier studies, by looking only at averages in methylation levels between different insect types, missed the fact that there was also considerable variation between insects of the same type, Kronauer says.

"It turns out there is nothing there—the current evidence is inconclusive," Kronauer says. "To understand what's really going on we will need to conduct new experiments with higher resolution and more statistical power." ◎

DATA

145,000

Approximate number of ants under study in Rockefeller's Laboratory of Social Evolution and Behavior.

IMAGING PROTOCOLS

The ultimate brain wash

TO WATCH NEURAL processes play out inside the brain, scientists typically have to compromise. They can either zoom in on a thin slice of tissue, producing detailed movies of the neurons firing within it—or view a big chunk of it as a murkier blur.

But Nicolas Renier likes to have the best of both worlds. A postdoc in Marc Tessier-Lavigne's Laboratory of Brain Development and Repair, he is mastering a new imaging technique that allows him to capture—in one crisp snapshot—all the neural activity within an entire mouse brain. He and his colleagues have applied it to study, among other things, the neural pathways that activate when mice are parenting their pups. And last year, Renier got the chance to show off the protocol to New York City Mayor Bill de Blasio, on campus for a biotech conference.

A critical step in the procedure is to make the tissue transparent by "clearing," a chemical soak that takes one to two weeks. The result, as the mayor got to witness, is a see-through brain. Its inner regions can now be exposed by light-sheet microscopy, in which active neurons are captured in three dimensions, snapshot by snapshot (view a single snapshot in "Inside Alzheimer's," page 14). The method can be used to explore how the brain changes in disease, how it responds to a drug, or how it performs sophisticated computations like those involved in decision-making. "You can use it to map anything you want in the mouse brain," Renier says. ◎

de Blasio (left), Tessier-Lavigne, and Renier.

DANIEL KRONAUER; ZACHARY VEILLEUX

TOMORROW'S SCIENTISTS

Say cheese, and swab

IF BIOLOGISTS HAVE a soft spot for microbes, so do cheese makers. On a recent afternoon, high school students from Rockefeller's Science Outreach lab took their microbiology know-how to a cheese cave—a smelly chamber in which the temperature, humidity, and atmosphere are carefully set to agree with lactose-fermenting bacteria used to ripen young cheese wheels.

Collecting samples in a Queens cheese cave.

Hosted by cheese monger Murray's Cheese, the excursion was part of Learning at the Bench, a semester-long after-school program in which students learn to think like scientists and conduct real experiments.

After swabbing different regions of the cave, the students brought their samples back to Rockefeller to isolate strains and extract and genotype bacterial DNA—essential steps in deconstructing a microbial environment.

For more on Rockefeller's Science Outreach program, look for @rockeduteam on Facebook or Messenger.

DATA

Although the U.S. has more tornados each year than any other country, the U.K. has the most per square mile.

ELLEN CRONIN

ALGORITHMS

When tornados unite

WHEN THE 2011 tornado "super outbreak" hit the central plains, it spawned 363 tornados that together caused more than 350 deaths and $11 billion in damage. It was the biggest tornado outbreak ever recorded—and it may be part of a larger trend.

According to Rockefeller's Joel E. Cohen and Columbia University's Michael Tippett, the average number of tornados per outbreak has increased by more than 40 percent since the mid-1950s, though the number of tornados per year has not changed. The two researchers have used mathematical tools to examine six decades worth of storm data collected by the National Oceanic and Atmospheric Administration.

It's not known why serial tornados are getting increasingly common. The researchers reported in *Science* in December that they could not establish a direct relationship between the mounting outbreaks and a warming planet. This could mean that climate change has nothing to do with the increased clustering of tornados, or that scientists don't understand the link.

Cohen, who is a mathematical population biologist rather than a climate scientist, and is *Abby Rockefeller Mauzé Professor*, notes that models from population biology often yield insights into other systems. In this case, he and Tippet applied Taylor's law—a pattern previously verified in ecology and some human diseases—to study tornados. "We've used new statistical tools to put tornados under the microscope," he says. "Analyzing thousands of tornados the way we would study living populations allowed us to discover new features of their outbreaks." ◎

Illustration by Andrew Colin Beck

ADDICTION

Methadone at 50

"We've had a decrease, not an increase, in the number of methadone clinics. Why? Stigma. Why? They don't make money. Why? Not in my backyard."

–Mary Jeanne Kreek on PBS's Frontline, *February 23, 2016*

ALTHOUGH THEY MAY NOT know it, Rockefeller's Mary Jeanne Kreek has been a hero to millions of heroin users. Her 1966 discovery showed that a compound developed as a painkiller could help opioid addicts transition to normal lives. It also launched a new framework for understanding addiction as a disease rather than a moral weakness.

Fifty years later, some things have changed, and some haven't. Kreek's original work on methadone—conducted with clinician Vincent Dole and psychiatrist Marie Nyswander—has given way to sophisticated studies that seek to understand the biological underpinnings of addictions to substances such as cocaine, alcohol, and cannabinoids.

Yet attitudes toward addicts, and political resistance to the use of methadone, remain stuck in the past. In the United States, abstinence-based therapies continue to dominate, partly for ideological reasons. Kreek, who is *Patrick E. and Beatrice M. Haggerty Professor*, has devoted effort throughout her career to championing the implementation of drug treatment programs at home and abroad.

"Addictions are not criminal behaviors, and they are not weaknesses," she says. "They however do respond to treatments—and it's unfortunate that we have tools available to treat opiate addiction, but we're not using them." ◎

Q & A

Richard P. Lifton

Lifton shortly after taking office as Rockefeller's 11th president.

MARIO MORGADO

RICK LIFTON HAS SPENT a career finding connections between diseases and the mutated genes that cause them. After nearly 25 years at Yale, Lifton moved to Rockefeller to become president last fall. We asked him to share his outlook on the present state of bioscience, and his visions for the future.

What's the promise of bioscience at this point in history? We've learned a tremendous amount about health and disease over the past century, yet it seems we continue to fight many of the same battles.
We are in an extraordinary time of scientific opportunity. With the sequence of the human genome in hand, we have, for the first time, a bounded problem. We have the ability to understand what each gene is doing in the context of a living human being, what happens when it is mutated, what happens when it is turned on or off.

This is a galvanizing realization. Linking mutations in each gene to human traits—singly and in combination with environmental factors and other genetic variants—will set the stage for us to understand biochemical and physiologic mechanisms that link genes to specific traits and disease susceptibilities. The knowledge that emerges from this work will define our opportunities to develop preventative and therapeutic strategies for disease for the next 50 years.

This is in many ways a reflection of the availability of new technologies. How important is the development of tools to the practice of biology?
It's not just the development of technology, it's the convergence of multiple technologies, and it's the ongoing evolution of those technologies, which is continuing at an extraordinary pace. We now have large data sets—in many fields, not just genomics—that are richly informative for understanding everything from biological structures and their interactions with small molecules to complex network interactions, at scales ranging from single-cell metabolism to population dynamics. It's increasingly clear that advanced computation is playing a critical role in the development of life science.

Even a decade ago there were many branches of life science in which one could get through an entire career without ever relying on serious computation. Today, trainees in many labs spend as much time analyzing large data sets as working at the bench, a rapid and surprising transformation.

How does Rockefeller best contribute to this type of scientific exploration?
Historically, Rockefeller has been remarkable for bringing together truly brilliant scientists from diverse disciplines in a relatively sparsely populated environment, which has long promoted interactions across disciplines. Technological advances have now eliminated many of the former boundaries between disciplines in science, and most scientists have to be comfortable working in many historically distinct areas. As a result, the rest of the world is coming to some of the collaborative models that we at Rockefeller have done very well for some time.

How can academic institutions like Rockefeller help ensure that science will generate new therapies and other useful innovations?
One of the reasons our country has invested heavily in the life sciences is the recognition that goes all the way back to Rockefeller's founding in 1901: that understanding the fundamental causes of human maladies provides the best opportunity for devising effective approaches to prevent or treat disease.

So coming from that premise, fundamental discovery, which includes much of what we do at Rockefeller, need not have immediate clinical application. Understanding critical principles of biology lays the foundation from which normal and disease biology are subsequently understood. Nonetheless, our collective responsibility as scientists doesn't stop with discovery in basic science. We currently rely largely on a system in which academic institutions do basic science, and translation most commonly occurs in industry. Unfortunately, the assumption that industry fully understands the implications of work in academic labs is not always well-placed. Many outstanding ideas will lack a champion in industry, and their potential will remain unfulfilled. This motivates increased efforts within academia to actively promote potential translational avenues, either alone or preferably in collaboration with industry collaborators.

"With the sequence of the human genome in hand, we have, for the first time, a bounded problem. We have the ability to understand what each gene is doing in the context of a living human being."

There are different ways that clinical translation can happen, and one can argue about where to draw the line as to how far an academic institution such as ours ought to go down the path toward commercialization. Establishing the clinical potential of a target—for example by showing the impact of a small molecule that modulates activity of a gene product or pathway—may often be sufficient to promote a target's in-depth exploration in industry. Programs like those now in place at Rockefeller are well-positioned to move interesting ideas into projects that will become tomorrow's new therapies.

What drew you to science?

I grew up in the space age, and science and technology were important forces in shaping my early thinking about the world. Also, like many young people at that time, I heard John Kennedy's appeal to altruism. He promoted thinking about using science to advance humanity.

But the farther I got into my medical training, the more I became interested in the intersection of medicine, science, and technology. Science is the surest way to illuminate the mysteries of health, well-being, and illness. Making fundamental discoveries about how life works remains a profoundly moving experience, and the clinical impact of some of these discoveries only deepens the experience. In a complex world with often-conflicting motivations, science's commitment to uncovering enduring truths provides a refreshing clarity of purpose. I can't imagine a greater privilege than exploring—and occasionally solving—these mysteries, and having this new knowledge benefit humanity. ◎

DATA

Samples prepared for cryo-electron microscopy are chilled to **-321 degrees Fahrenheit**, eliminating the need for them to be chemically fixed or stained.

JOHN ABBOTT

ATOMIC ARCHITECTURE

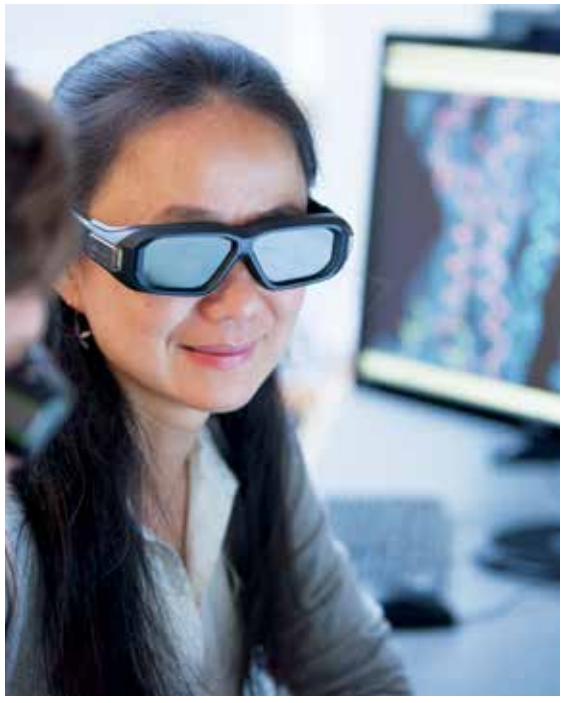
Caught in action: viral squatters

IT'S THE UNWELCOME visitor who never, ever leaves. Once inside the body, herpes simplex 1—the cause of painful and unsightly cold sores—can make its way into nerve cells, hide away within them, and possibly stick around for a lifetime. Until recently, no one knew how the virus manages to lie low for so long.

But with pictures captured at the level of individual atoms, a team led by Jue Chen, *William E. Ford Professor*, has figured out the virus's secret: a sophisticated sabotage of the body's inherent defenses.

The immune system normally recognizes and destroys virus-infected cells based on molecular bar codes—tiny pieces of the virus displayed on the cells' surfaces. Using cryo-electron microscopy, the researchers created molecular images revealing just how herpes interferes with this coding system. The virus, it turns out, clogs a transporter protein responsible for pumping these viral pieces into packaging stations inside cells, from where they are shuttled to the cell's surface. By blocking this transporter, called TAP, the virus effectively hides its own bar code from the immune system.

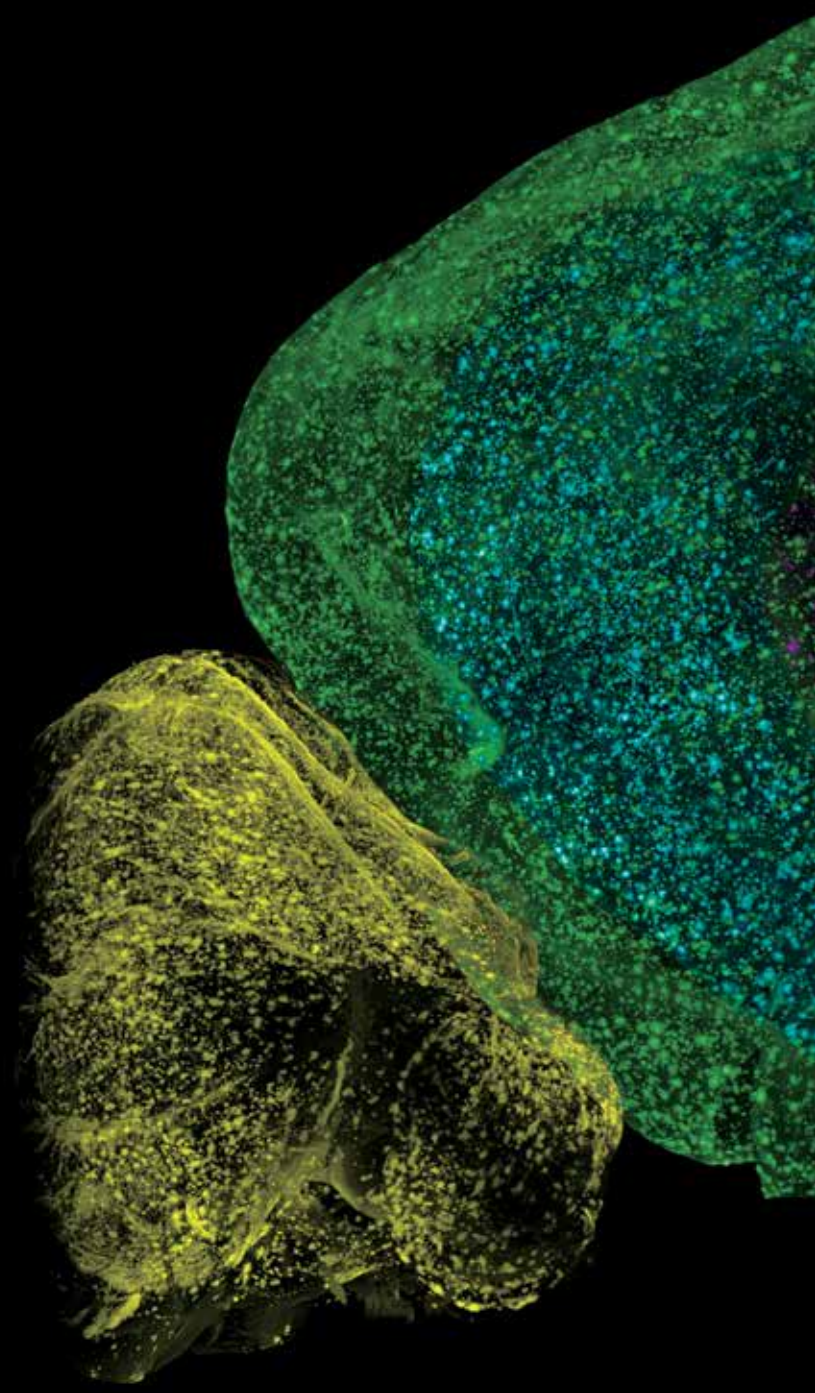
"We haven't been able to figure out how to inhibit these transporters," Chen says, "but by studying how viruses do it, we might learn some good strategies." ◎



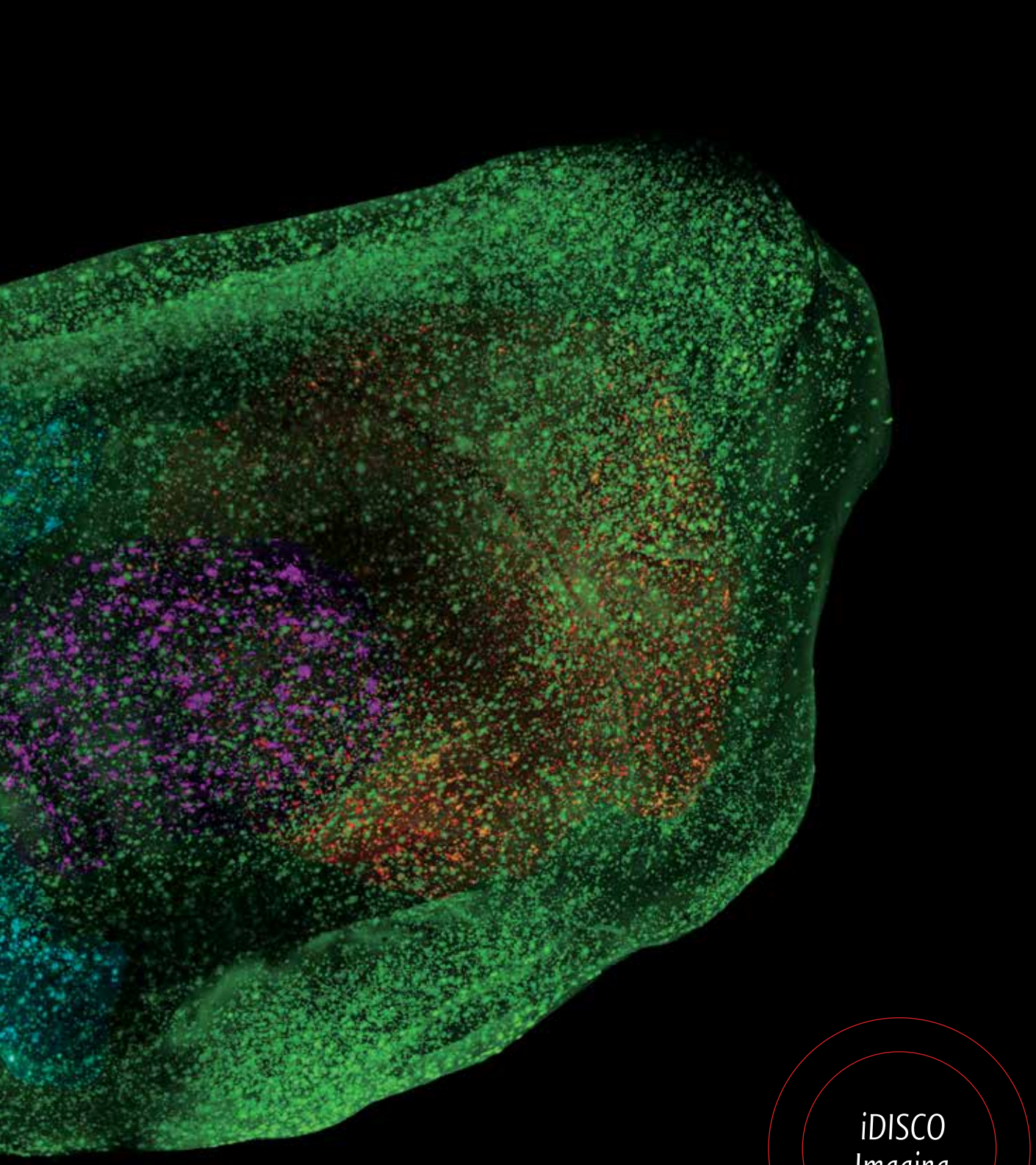
3-D glasses allow Chen to easily see renderings of transporter molecules from different angles.

Inside Alzheimer's

A BRAIN RAVAGED BY Alzheimer's disease is like no other: it contains deposits of a gunky protein called amyloid-beta, shown as bright speckles in this image of a mouse brain. To get the shot, researchers from Paul Greengard and Marc Tessier-Lavigne's laboratories used a technique called iDISCO, which involves making the brain tissue transparent so that deep-seated molecules can fluoresce within it.



LABORATORY OF MOLECULAR AND CELLULAR NEUROSCIENCE / THE ROCKEFELLER UNIVERSITY


iDISCO
Imaging

Working with worms, a graduate student set out to understand what memories are at their most basic level. Getting the data turned out to be the easy part.

Xin Jin

By Eva Kiesler

"SO, WHAT'S THAT?"

Xin Jin had been staring blankly at her computer screen for a good 10 minutes when the question, spoken from above her shoulder, made her rattle in her seat. Mildly troubled by the data she was analyzing, she hadn't noticed the approach of her advisor, neuroscientist Cori Bargmann, who appeared to have materialized behind her, wizard-like, to assist in her rumination.

It was early 2013, and Jin was halfway through her graduate thesis project, trying to understand how *C. elegans,* a flea-size worm that lives in the soil, uses its tiny brain to learn and remember things. On this particular day, she was looking at the first results from months of experiments designed to determine how a specific neural circuit in the worm generates a memory and then later recovers it. But as eager as Jin was to get these results, she couldn't quite make sense of them.

Neither could Bargmann. "It's weird," she had agreed, still gazing at the bar charts and figures on Jin's monitor. "But it's also really interesting, don't you think?"

It would take Jin and Bargmann at least two more years to fully understand these weird but interesting findings, which seemed to suggest that the cells that are critical for shaping a worm's memory have no stake in reviving it later. Because what Jin didn't know on that day four years ago—but eventually found out and reported last year as the first author of an extensive paper in the journal *Cell*—is that even in *C. elegans*, whose brain contains only 302 neurons, the formation and retrieval of a memory are separate processes, performed by distinct neural circuits residing in different parts of the brain.

This and other discoveries she made during her thesis work suggest that—at least in the context of learning—the humble worm is remarkably sophisticated, and more similar to us than researchers had previously imagined. And ultimately, her findings will help scientists dig deeper into the cognitive processes that all animals' brains are capable of.

"HONESTLY, BIOLOGY IS OFTEN messy and confusing to me," says Jin, gleefully. Today, she is a freshly graduated Ph.D. and a junior fellow at Harvard. Of the many things she learned while training at Rockefeller, she says one of the most important is to be open-minded—to never get too attached to an idea or insist on proving a particular hypothesis.

"At one time," Jin says, "Cori looked me in the eye and said, 'you have to love the data.' What she meant is, there can always be 20 mundane explanations for why your data looks the way it does—maybe it isn't showing anything new, or maybe it's an artifact. Still, you need to listen to it; it might be trying to tell you something."

The worm has something unique to offer scientists like Jin and Bargmann, who want to understand the very basics of the brain and

Photograph by Sari Goodfriend

how it generates behavior. It's a neurological microcosm in which each nerve cell has been painstakingly mapped out, and can be genetically manipulated or induced to fluoresce under the microscope.

"We need a very simple system to ask very basic questions," Jin says. "What exactly is a memory? What changes in the brain when we make one?" During her graduate work, she was able to pinpoint the cells and genes required for a process called imprinting, which allows young worms to form lifelong memories in response to certain traumatic experiences.

In nature, *C. elegans* feeds on bacteria, and relies on its sense of smell to decide which species of microbe to eat and which to avoid. In the lab, Jin set up experiments in which she placed worm eggs to hatch on a lawn of poisonous bacteria, which made the baby worms sick. After half a day, she switched the young worms over to plates stocked with more gourmet bacteria. And three days after that, she reexposed the animals—now adults—to choose between the toxic diet and the good one, and examined their decision-making. It turns out that worms that have had this unpleasant experience early in life will remember it for good. When they smell the same strain of bacteria as grown-ups, they flee, and this behavior stays with them throughout their lives (never mind that their lives only last for a few days—the worm considers it a good run).

Jin next did genetic experiments to find out how a particular brain cell called an interneuron, which processes olfactory information, turns a smell signal into a memory—and how it retrieves that memory later, when the adult worm is reacquainted with the smell of bad food. One by one, she silenced interneurons in the worm to identify those responsible for memory formation, storage, or retrieval. However, in doing these experiments she discovered that the different stages of the learning process didn't actually confine themselves to one group of cells, or even to one area of the brain. Two of the interneurons she tested did generate an olfactory memory, as she had expected, but they did not seem to be required for retrieving the memory later. Instead, the retrieval task appeared to be handled by a separate group of interneurons residing elsewhere in the worm's brain.

C. elegans is not unique in using separate neural circuits for memory formation and retrieval. A similar phenomenon has been observed in humans—for instance, in the case of the legendary epilepsy patient "H.M.," who in the 1950s had his medial temporal lobe surgically removed. After losing this part of the brain, the patient was able to retrieve old memories, but had difficulty forming new ones.

"So we were excited to discover that, even for worms, learning isn't a one-neuron job," Jin says. In subsequent experiments, she was able to uncover the link between the learning and memory retrieval processes: a signaling substance called tyramine—the *C. elegans* version of adrenaline—that is made by a learning neuron and sensed by a retrieval neuron. "When the learning cell excretes tyramine," Jin explains, "it's like it's telling the rest of the brain, 'Wake up! Remember this!'"

Jin with her grandparents in 1990. Desun Jin, right, a botanist, was an early inspiration.

Bargmann, who is *Torsten N. Wiesel Professor*, adds that Jin's thesis work has provided new insights into our earliest evolutionary history. "It has taught us that the brain has been a learning machine from its very origin," she says, "and it suggests that learning and memory are not some fancy innovations of a large brain—they are fundamental features of all nervous systems."

Jin grew up in the southeast of China, where her grandfather Desun Jin was a professor and the deputy director of the Fujian Institute of Subtropical Botany. After Jin came home from school, her grandfather often took her for a stroll in the botany garden, which has thousands of plant species on display. He would produce a pen and notebook, and teach her how to illustrate a plant and appreciate its individual anatomy—the woodiness of a stem, the shape and venation of a leaf, the delicate arrangement of a root system. He also planted in her young head a curiosity for other aspects of living things, too small to be seen.

"Grandpa made a point of emphasizing that morphology isn't everything," Jin says. "He showed me species of plants that looked almost identical, and explained that while one could be used as a medicinal herb, the other might be poisonous. Since then, this idea has always fascinated me: that the smallest differences between molecules and cells can have such a huge impact on biological function."

She says this idea also motivated her to study chemistry in college, at Peking University, and to subsequently embark on a daring journey: At 19, Jin decided to leave China and move, on her own, to the United States, to continue her chemistry studies at MIT. Two years later, as she was finishing up her undergraduate degree, she was preparing to go on to earn a higher degree while switching gears from chemistry to biology. A top student, she interviewed and received offers from a number of prestigious graduate programs around the country; deciding where to pursue her Ph.D. wasn't easy.

"Also, it was scary," Jin says. "All my peers seemed so successful and confident. And being a chemistry student, I knew almost nothing about biology, not even the basic terminology."

But then she interviewed at Rockefeller, where researchers have been trespassing academic boundaries for generations. Among other scientists, she met with Roderick MacKinnon, a former physician and biochemist who in the 1990s, determined to solve

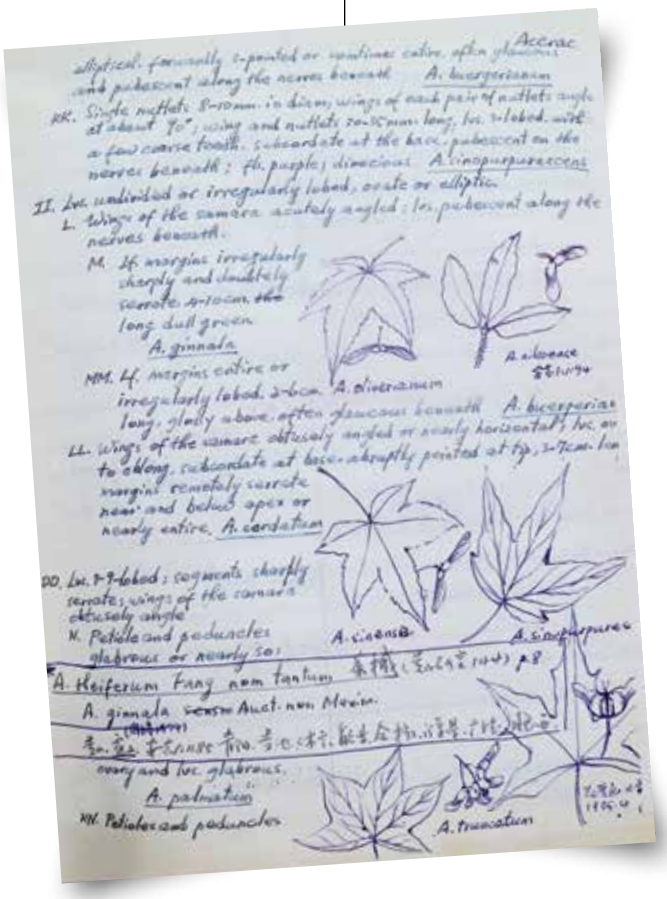
"One thing has always fascinated me: that the smallest differences between molecules and cells can have a huge impact on biological function."

a particular problem, taught himself x-ray crystallography, a notoriously finicky technology. (MacKinnon used this technique to determine the structure of the potassium channel, work for which he was later awarded a Nobel Prize in chemistry.) She spoke at length with Leslie B. Vosshall, a geneticist who has switched her lab's focus from flies to mosquitoes, and developed a range of tools to study these disease-carrying insects (see "All the world's genes, at our fingertips," page 20). And then, of course, there was that decisive interview with Bargmann, a former cancer biologist turned neuroscientist.

"All these great scientists were so supportive and enthusiastic about teaching me biology," Jin says. After these interviews, she no longer felt that switching fields would be a problem.

"I'm moving to New York," Jin wrote on her Facebook page.

"See you in September!" Bargmann replied.



A page from Desun Jin's sketchbook.

COURTESY OF XIN JIN

LAST SUMMER, AFTER graduating from Rockefeller, Jin joined the Harvard University Society of Fellows, where she will once again be venturing into uncharted territory. This prestigious program offers junior fellows the chance to pursue research without formal requirements. "It basically means I could study whatever I want, be it biology or economics or medieval art," Jin laughs. Yet generally speaking, she remains a neuroscientist at heart, and what she wants to do next is to tackle the vast complexity of the mammalian brain.

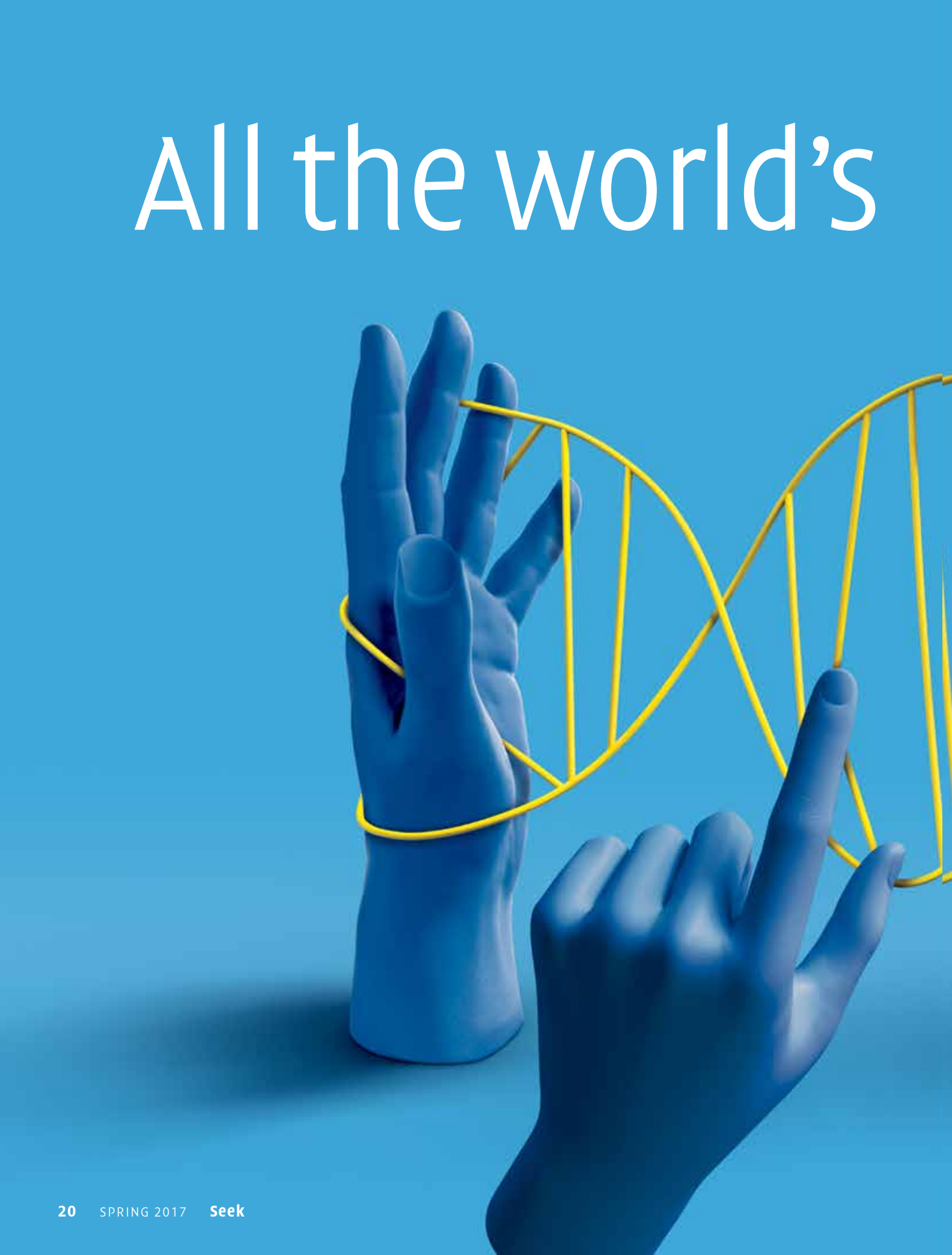
"Why was our brain scaled up this much?" Jin says, referring to the evolution that led from the 302-neuron worm brain—through fish, amphibians, birds, mammals—to the 10-billion-neuron human brain. "We don't quite know how many different cell types there are in the mammalian brain, or what they do. For example, can differences in cell-type composition tell us something about how one person's brain differs from another's?" She plans to seek answers to these questions working with Paola Arlotta, a developmental neuroscientist at Harvard, and through collaborations with bioengineer Feng Zhang's lab at MIT.

The project she intends to pursue is daunting, she says, and she is sometimes seized by doubt that it will work. Yet again, she recalls something she learned as a grad student, concerning her own mind's penchant for critical thinking: it needs to take a break sometimes.

"There are times when we need to see the big picture, and ask ourselves whether what we're doing in our research is relevant or worthwhile," Jin says. "But then at some point, we have to commit to a project. We have to just burrow down and do the experiments, and trust in the results." ◎

EVA KIESLER *is the founding managing editor of* Seek *and part of Rockefeller's Communications and Public Affairs team. She has a Ph.D. in molecular biology from Stockholm University.*

All the world's



genes, at our fingertips

A bit of genetic trickery, borrowed from bacteria, has made gene editing easy. The question now is how to make good use of CRISPR.

By Alexander Gelfand

Illustration by Alvaro Dominguez

Only a few years ago, and seemingly by chance, scientists stumbled upon a powerful way to manipulate genes. The result is a cheap and versatile set of tools that is already transforming biomedicine—and whose promise to advance human health raises tough ethical questions.

Standing before a collection of plastic vials and petri dishes, Luciano Marraffini, head of the Laboratory of Bacteriology, surveys the tools that he uses to study bacterial evolution.

Some of the vials, Marraffini explains, contain plasmids: circular bits of DNA that travel from one bacterium to another, spreading useful genes as they go. (Bacteria don't reproduce sexually, so they must rely on other means of refreshing their gene pools.) Other vials contain viruses known as bacteriophages—phages, for short—that kill bacteria, yet may also pass them genes that increase their virulence.

As Marraffini talks, Philip Nussenzweig, a graduate student in the lab, pulls a petri dish from a refrigerator hidden beneath a countertop. The dish is loaded with *Escherichia coli*, the gut bacterium that causes traveler's diarrhea and, on rare occasions, kidney failure; and also with *Staphylococcus aureus*, a microbe that produces deadly infections of the heart, blood, lungs, and bones.

Lurking inside them all is yet another tool. It is called CRISPR (pronounced "crisper") and it is the most powerful means of manipulating genes that the world has ever seen.

Biologists have long been able to alter genes using a number of techniques, and those methods of genetic engineering have played a vital role not only in laboratory research, but also in applications such as the creation of genetically modified crops, and of plants and microorganisms capable of producing drugs and vaccines.

Around a decade ago, however, scientists discovered a new class of gene-editing tools that allowed them to make highly specific DNA changes much more accurately and efficiently than ever before. And CRISPR, which exists in nature and was adapted only

Luciano Marraffini

CRISPR is not the first technique to modify genes, but it's by far the most flexible and useful.

MARIO MORGADO

recently to form the basis of a new gene-editing technology, has provided biologists with the most flexible and widely applicable gene editor thus far. Over the past several years, researchers have used it to tweak the DNA of dozens of organisms, from wheat and trees to cows and chimpanzees.

At Rockefeller alone, laboratories working on a vast range of human diseases keep finding new uses for the technology (see "Three ways CRISPR could advance medicine and help people," page 27). Meanwhile, Marraffini, who played an important role in the development of CRISPR, continues to conduct experiments on bacteria and phages to elucidate the inner workings of the system.

"In a way, we're playing with this thing," he says. "And that's what makes it fun."

That may be. But it is a kind of play that has already revolutionized biological research. And the fun is only just beginning.

In the late 1980s, researchers at Osaka University in Japan identified curious repeated sequences in the DNA of *E. coli*—sequences that were interspersed with other, non-repetitive stretches of genetic material.

Those repeated sequences and their intervening spacers were dubbed CRISPR, for "clustered, regularly interspaced, short palindromic repeats." Scientists soon found CRISPR in many different bacteria and in other single-celled organisms called archaea; and they eventually figured out that these CRISPR systems formed part of a bacterial immune system that fended off attacks from phages.

For example, investigators determined that the spacers represented snippets of DNA captured from phages, plasmids, and other foreign sources of genetic material. They surmised that those snippets functioned like genetic mug shots that allowed bacteria to recognize phages that had previously attacked them. And they gathered that somehow, the infected bacteria were able to render these returning phages harmless with the help of special CRISPR-associated (Cas) proteins (see "CRISPR: How it works," page 25).

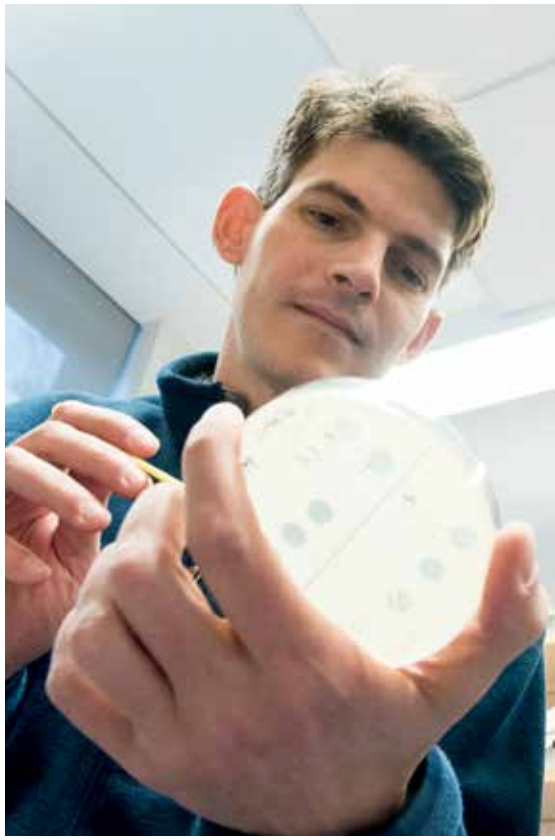
Nonetheless, they still did not know quite how these CRISPR-Cas systems—and there are many different types—accomplished all of that. Their best guess was that CRISPR-Cas neutralized invading phages by targeting their RNA, a close chemical relative of DNA that phages rely upon to replicate themselves. The idea initially seemed plausible, but in 2008, Marraffini published work demonstrating that the CRISPR-Cas system he'd identified in the bacterium *Staphylococcus epidermidis* was in fact targeting DNA.

This research—which Marraffini began in his spare time while completing his Ph.D., and which subsequently formed the basis of his postdoctoral work with molecular biologist Erik Sontheimer, at Northwestern University—represented a major breakthrough in understanding how CRISPR-Cas actually worked. And in a phrase that today seems remarkable both for its restraint and for its prescience, Marraffini and Sontheimer suggested that, if it could be put to use elsewhere, the ability of CRISPR-Cas to target DNA might have "considerable functional utility."

Less than a decade later, the utility of CRISPR-Cas is no longer a matter of speculation. And Marraffini is regarded as one of the leading figures in a gene-editing revolution that is poised to transform fields ranging from medicine to industrial agriculture.

Marraffini's 2008 prediction turned out to be prophetic, a self-fulfilling prophecy, really, since after coming to The Rockefeller University in 2011, he himself would help establish that a particular CRISPR-Cas system called CRISPR-Cas9—so named for the Cas9 protein, which cuts through double-stranded DNA as neatly as a molecular scalpel—could be modified in the lab to make precise edits in the genome of any organism.

In 2012, Marraffini and his team at Rockefeller, along with Feng Zhang at the Broad Institute, were among the first to



demonstrate that this programmable form of CRISPR-Cas9 could be used to edit genomes in mice and human cells. They used it to alter the function of specific genes or put them out of action altogether by making multiple edits to their DNA sequences; and they inserted chunks of new genetic material into existing genomes.

These experiments helped show that CRISPR-Cas9 could be further modified to repress or activate genes without actually cutting them—a potentially useful trick for studying organisms in which different genes are switched on and off at different times, and for designing synthetic ones that do the same.

In yet other experiments, Marraffini and his team at Rockefeller programmed CRISPR-Cas9 to target only virulent and antibiotic-resistant strains of *S. aureus*—the same bacteria that cause antibiotic-resistant infections in hospital patients—in lab mice, thereby demonstrating that the technology could be used to selectively kill bad bacteria while leaving good bacteria alone. They even used it to stop the bacterium *Streptococcus pneumoniae*, a potentially lethal microbe that can cause pneumonia, meningitis, and a host of other infections, from switching from its non-virulent form to its virulent one.

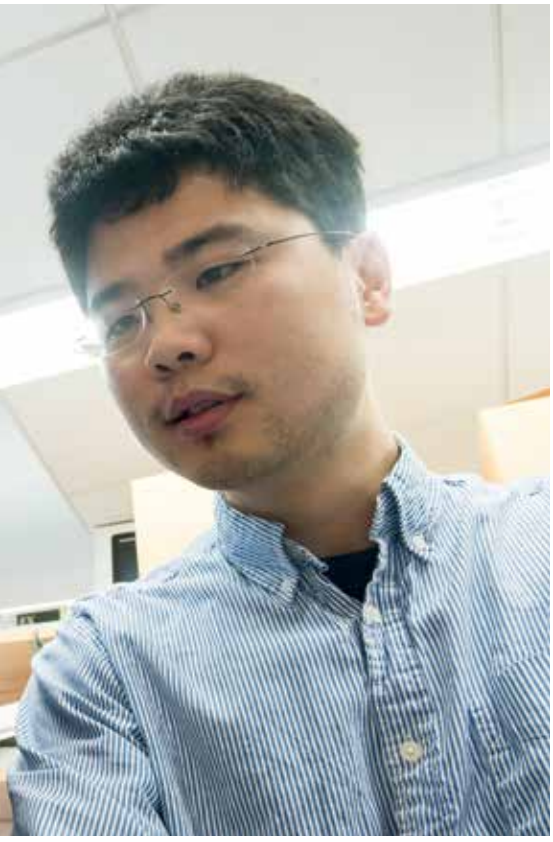
Scientists have since employed CRISPR-Cas9 to cure muscular dystrophy in mice; make human stem cells immune to HIV; and overcome at least some of the genetic challenges that have thus far prevented doctors from transplanting pig organs into people. CRISPR-Cas9 is not the first tool to make it possible to tinker with patients' genes in order to cure diseases; other forms of gene therapy have been developed, and some are already being tested in clinical trials. But none of its competitors are as versatile or as easy to use as CRISPR-Cas9. As a result, just about everyone with an interest in manipulating DNA seems to have jumped on the CRISPR bandwagon.

"I have enough gray hair to look back 30 years, and this is just astonishing," George Q. Daley, a stem cell biologist at Harvard Medical School, said of the field during a

Will the rapid progress of techniques to engineer living things outstrip efforts to determine whether doing so is safe or ethical?

Marraffini (left) with former graduate student Wenyan Jiang.

ZACHARY VEILLEUX



seminar about CRISPR that took place at Rockefeller last year.

When asked how long it might take to see CRISPR-driven therapies in the clinic—treatments that in the near term could be used for sickle cell anemia, and that someday might eradicate genetic diseases like cystic fibrosis or reduce the risk of complex ones like Alzheimer's—Daley, who directs the Stem Cell Transplantation Program at Boston Children's Hospital and is himself using the technology to seek better therapies for patients with bone marrow disease, did not hesitate.

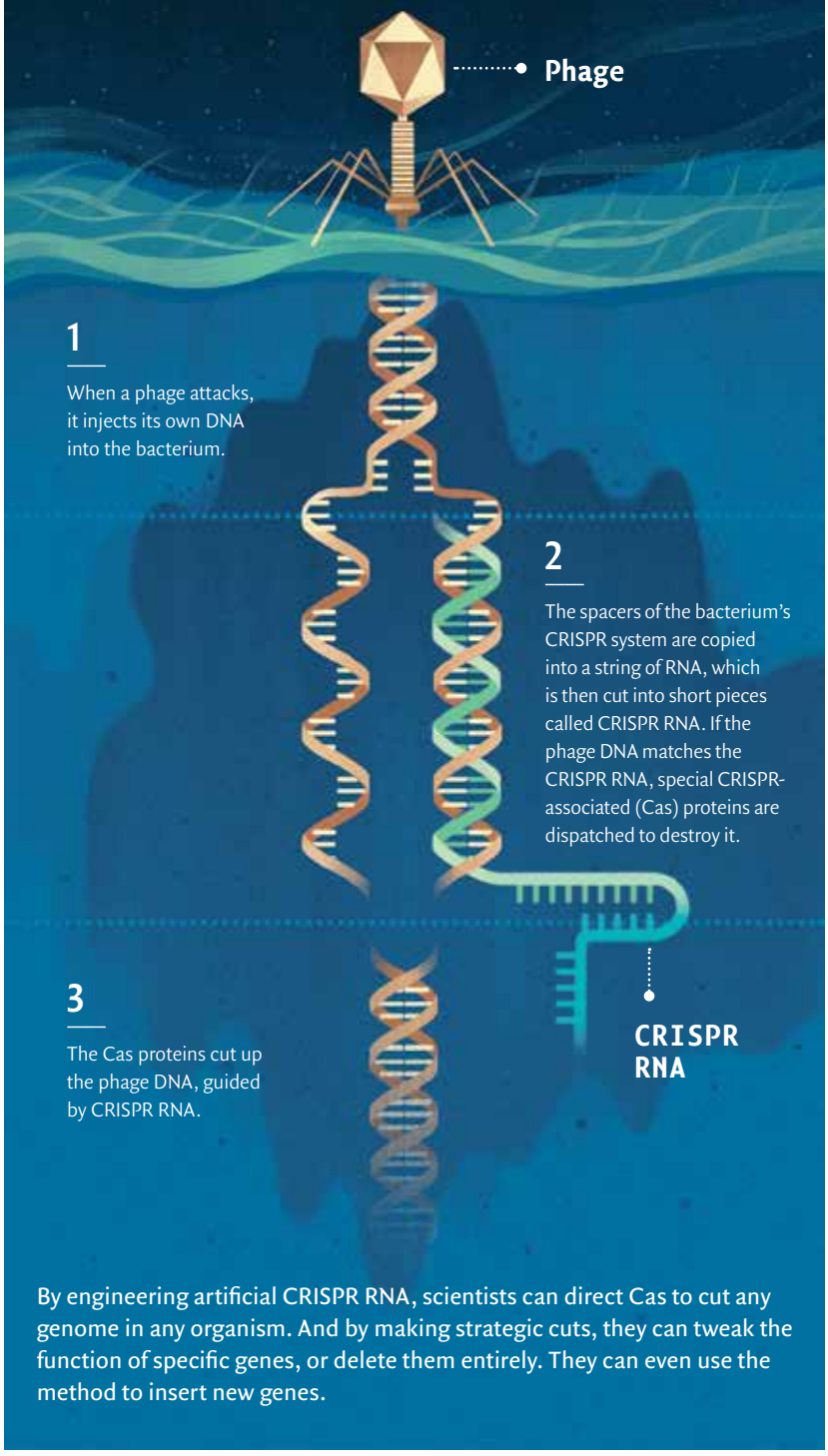
"Maybe even within two years," he responded. "But certainly within five."

In fact, the rapid progress of scientists' ability to use CRISPR-Cas9 to engineer living things is raising concerns that it may be outstripping society's ability to determine whether doing so is safe or ethical.

The scientific community is itself divided over this issue. Some researchers, for example, are keen to use CRISPR-Cas9 to modify animals such as mosquitoes in

CRISPR: How it works

In nature, CRISPR-Cas systems protect bacteria from viruses called phages. Every CRISPR-Cas system uses repeated sequences of DNA separated by non-repeating spacers. The spacers contain DNA snippets from phages that the bacteria have previously encountered.



By engineering artificial CRISPR RNA, scientists can direct Cas to cut any genome in any organism. And by making strategic cuts, they can tweak the function of specific genes, or delete them entirely. They can even use the method to insert new genes.

Illustration by Jasu Hu

28

Number of scientific publications mentioning CRISPR in 2008, the year Marraffini published his first groundbreaking discovery about the system.

2,282

Number of publications mentioning CRISPR in 2016.

Many microbes, including some of the most common types of bacteria, use CRISPR to store memories of past invaders. Scientists still have a lot to learn about how the system evolved and how it functions in nature.

order to combat public health threats like malaria and Zika. Others, however, worry that releasing such modified organisms into the wild could have unforeseen consequences for natural ecosystems.

And then there is the issue of determining how, and when, to use CRISPR-Cas9 in people.

In March 2015, for example, a group of prominent biologists called for a moratorium on using CRISPR-Cas9 to modify human eggs, sperm, and embryos. Such modifications, known as germline edits, could be passed down to future generations. And they raise the specter of what Daley calls "the dark side" of CRISPR-Cas9: the prospect of a new era of eugenics, of a brave new world divided between genetic haves and have-nots. Yet in April of that same year, Chinese researchers revealed that they had already used CRISPR-Cas9 on human embryos. (The embryos themselves were nonviable, and could not have gone on to become babies.)

In response, leading scientists in the field, under the auspices of a number of international scientific organizations, convened a global summit on human gene editing in Washington, D.C., in December 2015. In addition, the U.S. National Academy of Sciences and the National Academy of Medicine commissioned a comprehensive study by an international committee, of the legal, ethical, and social implications of the technology. The committee is preparing a formal report that will include policy recommendations on its use and regulation (see "The future opportunities—and conceivable dangers—of CRISPR," page 28).

Yet despite these caveats, movement toward human applications proceeds apace: Last June, the Recombinant DNA Advisory Committee, which reviews all human gene therapy protocols in the United States, approved the first such protocol involving the use of CRISPR-Cas9. And the technology continues to generate widespread excitement over the promise it holds for both basic scientific research and for a broad range of applied fields including drug development, public health, and agriculture.

To Marraffini, the story of CRISPR is "a testament to the value of basic biology for society." He was drawn to the topic, which had no obvious applications for improving human health, out of sheer curiosity: a desire to understand how bacteria defend themselves, acquire genetic material, and evolve. Yet now, in what by scientific standards is the mere blink of an eye, the practical applications of CRISPR seem almost without limit, and Marraffini's own work with bacteria exemplifies the dual promise of the technology: In addition to explaining how bacteria evolve, his research could one day lead to better antibiotics, or help impede the spread of antibiotic resistance.

Much the same might be said of the CRISPR-driven work being done in Leslie B. Vosshall's lab, where fundamental research on a major disease vector could eventually help control one of the world's great scourges.

Three ways CRISPR could advance medicine and help people

Rockefeller scientists are using CRISPR to study many diseases, including:

1 **ALZHEIMER'S:** A team led by Marc Tessier-Lavigne, *Carson Family Professor*, has created a CRISPR-based method that allows them to more easily generate neurons carrying genetic defects seen in Alzheimer's patients. The advance should promote the development of treatments for this and many other diseases.

2 **HEART ATTACK,** stroke, and other thrombotic conditions: The laboratory of Barry S. Coller, *David Rockefeller Professor*, is using CRISPR to study the biology of platelets, cells that help the blood coagulate. Their goal is to discover new therapeutic targets for platelet-related diseases.

3 **ZIKA,** dengue, and other mosquito-borne diseases: In the lab of Leslie B. Vosshall, *Robin Chemers Neustein Professor*, scientists apply CRISPR to explore how mosquitoes smell and seek out human prey. Their work might create new opportunities to curb the spread of illnesses that together cause millions of deaths each year.

OPPOSITE PAGE: ZACHARY VEILLEUX

Q & A

The future opportunities—and conceivable dangers—of CRISPR

Despite its promise, CRISPR-Cas9 raises a number of safety issues and ethical concerns. Barry S. Coller, *David Rockefeller Professor* and Rockefeller's vice president for medical affairs, is a member of the Human Gene Editing Study Committee organized by the National Academy of Sciences and the National Academy of Medicine. Here, he discusses some of these concerns, how they might be addressed, and what scientists and society at large still have to figure out.

What are some of the risks posed by CRISPR-Cas9?

CRISPR and a number of other gene-editing technologies can be used to make cuts in specific DNA sequences, but up until recently, these methods have been associated with quite significant off-target cleavages—breaks that occur where they should not. And most off-target cleavages may well wind up inactivating things or altering things in unpredictable ways.

The technology keeps improving, and people are getting much better at reducing the unwanted effects. But they'll probably never get to zero. And if you make a modification that's never before been seen in the human genome, that will open up the issue as to whether or not the alteration might have unforeseen consequences.

So how can scientists and regulators determine when to use the technology?

We're always weighing risk and benefit case by case. In the past, ethics committees have often ruled to accept a greater risk in exchange for the possibility of doing something for an otherwise untreatable and lethal disease.

All of the decision-making needs to be put in that context. So, there aren't going to be absolutes.

What about the ethical concerns surrounding potential therapies that would introduce changes to the human germline—curing diseases by repairing defective genes in sperm or egg cells, for example, or in early embryos? Wouldn't such edits be passed down from parents to their children?

I think there are a number of very profound issues with germline editing.

One is that of informed consent. Generally speaking, we have a lot of precedent for letting parents make medical decisions that can impact their children. For example, if a child is sick and the doctor recommends an experimental therapy, it's the parents—with the assent of the child, when possible—who ultimately decide whether to pursue that treatment or not. But with germline editing, there are no precedents for obtaining informed consent for the future generations that may be affected.

Vosshall, who is *Robin Chemers Neustein Professor*, and her team study *Aedes aegypti*, the mosquito that transmits the yellow fever, dengue, West Nile, and Zika viruses. Gram for gram, it is one of the most dangerous animals on the planet. And it finds its human prey, as well as the standing water in which it lays its eggs, by relying on a complex system of sensory cues that include humidity and heat, the chemicals in our sweat, and the carbon dioxide in our breath.

Historically, mosquitoes have been extremely difficult to modify genetically. Today, however, Vosshall and her team are using CRISPR-Cas9 to investigate how *Ae. aegypti*'s biology drives its behavior: how the insect is drawn to us by specific molecules in our body odor; how it detects the presence, and even the quality, of the water where it deposits its offspring; and why it is attracted by the lactic acid we excrete but repelled by a chemical such as DEET.

But in manipulating one mosquito gene at a time, they are not only unpacking the mysteries of an exquisite piece of biological machinery that has evolved over millions of years. They are also generating insights that could be used to make better mosquito repellents, or limit the number of these blood-sucking disease vectors. And they are developing broadly applicable methods that other researchers are already adapting to other insects, such as ants, which were previously inaccessible to genetic modification.

In the past, Vosshall and her team relied on other gene-editing technologies with obscure names (TALENs, zinc-finger nucleases) to create mutant mosquitoes that lacked particular genes, such as the ones that regulate the ability to detect carbon dioxide or certain kinds of odors. They then ran experiments on the insects in order to determine what role those missing genes played in guiding *Ae. aegypti* to us. Would odor-blind mutants still find and bite people, for example? Or would they be rendered harmless? (The answers to those questions turned out to be "yes" and "no," respectively.)

But where those earlier methods were tricky, slow, and expensive, CRISPR-Cas9 is straightforward, fast, and economical. Ben Matthews, a postdoctoral fellow who leads the CRISPR-Cas9 program in the Vosshall lab, recalls attending a genome-engineering conference in 2013, soon after Marraffini and others began publishing the first papers describing the many practical applications of CRISPR-Cas9. Even at that early date, Matthews's colleagues told him that the technology was "too easy not to try."

MARIO MORGADO

Vosshall and Matthews tending to their vast collection of mutant mosquitoes.

"CRISPR has opened the door to experiments we couldn't have dreamt of 10 years ago."

They were right. Obtaining the materials from a commercial lab to make a mutant mosquito using earlier techniques cost between $5,000 and $25,000 and took three months. Today, preparing the equivalent materials in-house using CRISPR-Cas9 takes one week and costs roughly $25. "It's ridiculously cheap," Vosshall says.

The insectary where Vosshall and her team rear their research subjects—a warm, humid room packed floor-to-ceiling with water-filled trays containing larval *Ae. aegypti* and mesh boxes containing adults—is now chock-ablock with mutant mosquitoes that have had various genes knocked out or edited in more complex ways. Being able to operate on that kind of scale, and to make so many different kinds of mutants so easily and so quickly, says Matthews, "opens the doors to experiments that we couldn't even really have dreamt of 10 years ago."

One of Vosshall's goals, for example, is to insert a gene into *Ae. aegypti* that would cause the mosquito's neurons to glow when stimulated by sensory input—a complicated bit of genome editing that would bring her lab closer than ever to cracking the code of how the insect's intricate neurosensory system responds to odors, humidity, and other cues.

"We've made halting attempts in the last five years," she says. "But it looks like there will soon be a breakthrough."

TWO FLOORS BELOW Vosshall's mosquito reservoir, Marraffini continues to play with his own, considerably smaller mutants: modified versions of the plasmids, phages, and bacteria that led to the CRISPR revolution in the first place. And discoveries continue to accrue: Just recently, he and his team identified a CRISPR-Cas system that targets not only DNA, but RNA, as well—an ability that would appear to confer an evolutionary advantage upon the bacteria that enjoy it.

At the moment, Marraffini sees no obvious practical application for this switch-hitting form of CRISPR-Cas. But then again, the researchers who first discovered CRISPR nestled in the DNA of *E. coli* nearly 30 years ago had little inkling of its considerable functional utility. And as Marraffini points out, if there's one thing that working with CRISPR has taught him, it's never say never.

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ALVARO DOMINGUEZ (LEFT); SARI GOODFRIEND

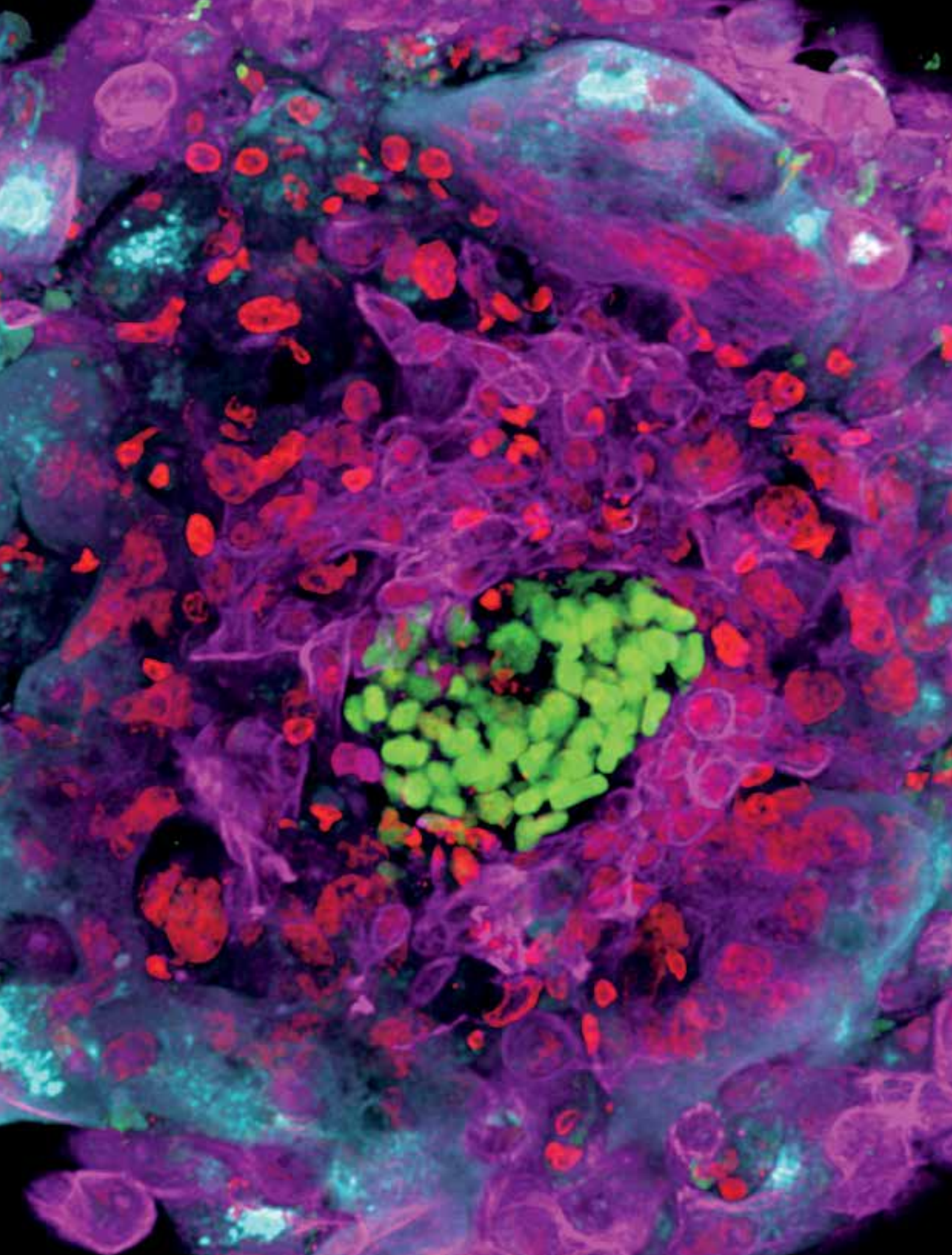
In an Embryo's Second Week, a Surprise

BY W. WAYT GIBBS

Rockefeller biologists opened a window into the mysterious period when a human embryo first attaches to its mother's uterus—and what they saw amazed them.

An attached human embryo 12 days after fertilization.

LABORATORY OF STEM CELL BIOLOGY AND MOLECULAR EMBRYOLOGY / THE ROCKEFELLER UNIVERSITY / NATURE PUBLISHING GROUP



"These are pictures of our own origins that no one has ever seen before."

WE ALL BEGIN AS A TINY, hollow ball of about 150 cells, rolling around in the uterus. Whether conception happens in a test tube or the old-fashioned way, the embryonic ball, called a blastocyst, has to accomplish a crucial early task to earn a shot at life: It must stop rolling and attach itself to the walls of the uterus, where it will be supplied with food and oxygen for the next 40 weeks.

Many embryos fail this test. For reasons that remain mysterious—scientists have never witnessed the magical moment of implantation in humans—a substantial fraction of embryos either don't stick at all or take more than nine days to implant, with each additional day reducing their chances of survival.

"We know absolutely nothing about what goes wrong," says Ali H. Brivanlou, *Robert and Harriet Heilbrunn Professor* and head of the Laboratory of Stem Cell Biology and Molecular Embryology at Rockefeller. "Embryos' failure to attach is the biggest problem that fertility doctors see when they perform in vitro fertilization."

But Brivanlou's team recently pulled back the curtain on this momentous event in human development. By applying the Rockefeller group's special expertise in working with embryos of many kinds, from frogs to humans, Brivanlou and research associates Alessia Deglincerti and Gist Croft, and research specialist Lauren Pietila, were able to accomplish what no one had done before. They established a reliable technique to sustain human embryos in petri dishes all the way through the second week after fertilization, and to record in detail how the blastocysts attach to a plastic scaffold (in lieu of a womb), flatten into discs, and begin to develop under the control of an internal program.

It's a breakthrough that gives biologists a remarkable opportunity to fill in many of the missing pieces in the puzzle of the earliest stages of human life. That more complete picture could in turn lead to concrete medical advances, starting with insights into why so many pregnancies fail before implantation or within the first two months afterward.

"If someone had told me when I was in grad school that I would ever be able to witness this stage of human development, I would have thought they were crazy," Brivanlou says.

CONSIDER, FOR INSTANCE, one of the most fundamental questions in early human development: whether human embryos are able to develop properly without being connected to the maternal uterus, which supplies vital biochemical signals. The new research shows that all of the information necessary to control attachment and the next few steps of development resides in the embryo itself. While hormones and other signals from the mother certainly become crucial at some point in development, that point occurs later than biologists had assumed.

ZACHARY VEILLEUX

Brivanlou works to understand how embryonic structures take shape early in development. His lab works with many kinds of embryos, including frog and human.

"The fact that communication between the mother's tissues and the embryo isn't necessary for attachment to happen was a big surprise to the field," Brivanlou says.

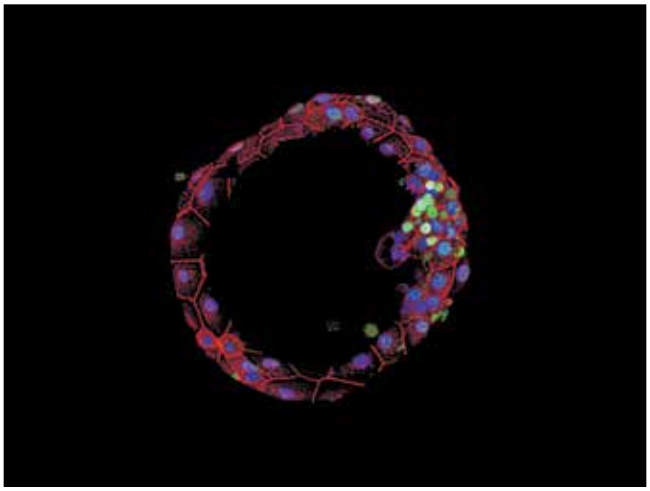
The research has other important implications. By labeling the embryos with molecular markers that can be lit up with lasers, and monitoring the order in which genes are turned on and off during this crucial period of development, scientists are now able to detect when things go wrong on a molecular level. "Genetic errors and other defects can start to pile up. Even if the embryo survives, such errors could potentially cause disease or complications later in life," Brivanlou says. If so, it would be good to know how to detect, prevent, or correct the defects.

The researchers are also excited about the emerging potential of using embryonic stem cells to model diseases of many kinds in the lab. A subset of those 150 cells in the blastocysts, embryonic stem cells have the ability to regrow brain tissue, to reconstitute the immune system, or to differentiate into any of the body's many cell types. But scientists need more knowledge, specifically from human cells, for such approaches to become effective, says Croft. "We have to understand where embryonic stem cells are coming from, and what decisions they've made or are about to make," he says. "Only then will we be able to control their ability to become the cell types that are useful for drug screening and transplantation."

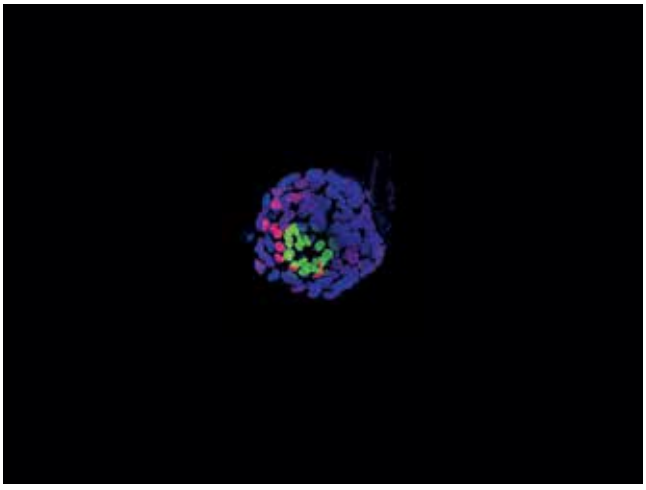
Currently, the use of cells derived from human embryos is limited by both ethical and practical constraints. One big problem, Brivanlou notes, is that when researchers grow new tissue from these stem cells, success is largely a matter of trial and error because we don't understand well enough how the processes of differentiation work in the body—how a stem cell naturally gives rise to bone cells, for example. "Our new method may let us observe, for the first time, how the human body does this itself,"

An intimate look at our beginnings

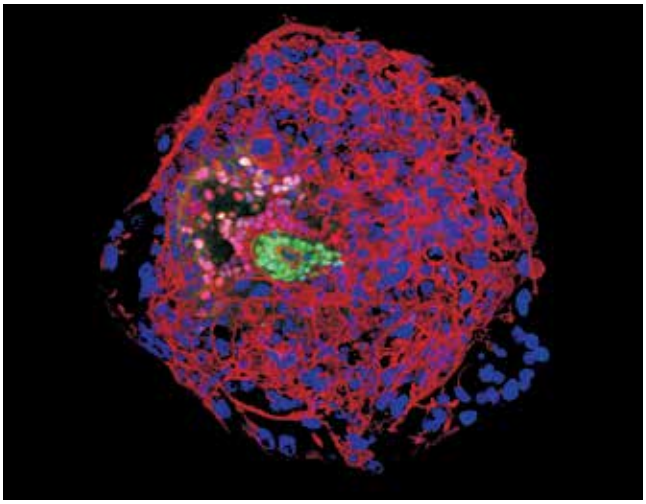
Once scientists had learned how to sustain human embryos in a dish for two weeks, their next challenge was to visualize the complex processes that take place within them before, during, and after implantation. "The bigger the embryo gets, the harder it is to image," says Gist Croft, who optimized advanced imaging collection and processing techniques to be able to track each cell type in three dimensions, optically slicing his way through the test-tube embryos.



DAY 6: A few hundred cells form a hollow sphere called a blastocyst, shown here in cross section. In pregnancy, a subset of the green cells, called epiblasts, would eventually become the fetus and ultimately carry on as adult tissues. The rest of the cells would create the placenta and other structures that support the embryo.



DAY 8: The sphere has collapsed and attached to the dish. The epiblasts (green) begin to separate from primitive endoderm cells (red) that soon will give rise to the yolk sac, a structure that normally supplies blood to the embryo.



DAY 10: All cell types are dividing at tremendous speed and attempting to form embryonic structures. On the left is the yolk sac (pink), which in pregnancy would be one of the first structures detectable by ultrasound. The green epiblasts have formed the amniotic cavity, whose left side would eventually become the fetus. The right side of the cavity would produce the amniotic membrane surrounding the fetus.

LABORATORY OF STEM CELL BIOLOGY AND MOLECULAR EMBRYOLOGY / THE ROCKEFELLER UNIVERSITY

he says. While others work on the applications, Brivanlou and his team are focusing on further improving the technique so that it will reproduce more faithfully how embryos develop during normal pregnancy.

DEGLINCERTI ADAPTED the new approach from a similar method used to study fetal development in mice, established by a colleague at the University of Cambridge, Magdalena Zernicka-Goetz. "We carefully manipulate the embryos in a dish, and use chemicals to peel off a thin envelope that surrounds the embryo at this early stage," Deglincerti says. "We then bathe the naked blastocyst in hormones and growth factors." With this combination, the group was able to grow the embryos through attachment and up to the 14th day of development.

They stopped there. According to international ethical guidelines for conducting experiments on human embryos, 14 days is the cutoff. But those ethical guidelines were set up many years ago, when the 14-day limit seemed well out of reach. Now it may be time to reevaluate them, Brivanlou says, since his group has shown that it's technically feasible to culture embryos to the 14-day limit, and possibly beyond.

"Today we would not want to attempt going past day 14, even if the regulations allowed it," he says, "because anatomical abnormalities would start to appear in the embryos." However, scientists may soon find a way to keep development proceeding normally for longer than two weeks. And at that point, technology will no longer be the limiting factor, yet the ethical concerns will remain. "We need to have a debate throughout all society about whether in certain cases it could be acceptable to allow embryos to develop further in vitro in order to gain more insight into human development," Brivanlou says.

WHILE THERE IS no predicting what discoveries could follow from allowing expanded research, the very first glimpses through this

Deglincerti (left) and Croft used advanced techniques to acquire stunning images of human embyros.

ZACHARY VEILLEUX

Now that technological barriers are lifting, it may be time to open a debate on ethical guidelines that were established decades ago.

new window between days 9 and 14 turned up something quite unexpected. While sifting through the mass of results from the many tests they had run on individual cells inside the embryos, the team stumbled upon a new kind of cell, which they named yolk-sac trophectoderm cells.

"Nobody has seen this cell type in any animal before," Brivanlou says. "We reproduced our experiments 10 times to convince ourselves. It is there, and it is specific to humans. But we have no clue what these cells are doing. Are they like the tail and gills—structures that appear in the womb but then vanish before birth? Or do they give rise to something that stays with us throughout life?"

Questions like these often pop up when scientists crack open a black box and peek inside. It makes you wonder: What else is in there? ◎

W. WAYT GIBBS *is a freelance writer and a contributing editor to* Scientific American. *His stories about science, technology, and engineering can be found in* Nature, Science, *and* Wired, *among other publications. Wayt lives near Seattle with his wife and son.*

FLIPPING A SWITCH INSIDE THE HEAD

With new technology, scientists are able to exert wireless control over brain cells of mice with just the push of a button. The first thing they did was make the mice hungry.

By W. Wayt Gibbs

READY YOUR TINFOIL HATS— mind control is not as far-fetched an idea as it may seem. In Jeffrey M. Friedman's laboratory, it happens all the time, though the subjects are mice, not people.

Friedman and his colleagues have demonstrated a radio-operated remote control for the appetite and glucose metabolism of mice—a sophisticated technique to wirelessly alter neurons in the animals' brains. At the flick of a switch, they are able to make mice hungry—or suppress their appetite—while the mice go about their lives normally. It's a tool they are using to unravel the neurological basis of eating, and it is likely to have applications for studies of other hard-wired behaviors.

Friedman, *Marilyn M. Simpson Professor*, has been working on the technique for several years with Sarah Stanley, a former postdoc in his lab who now is assistant professor at the Icahn School of Medicine at Mount Sinai, and collaborators at Rensselaer Polytechnic Institute. Aware of the limitations of existing methods for triggering brain cells in living animals, the group set out to invent a new way. An ideal approach, they reasoned, would be as noninvasive and non-damaging as possible. And it should work quickly and repeatedly.

Although there are other ways to deliver signals to neurons, each has its limitations. In deep-brain stimulation, for example, scientists thread a wire through the brain to place an electrode next to the target cells. But the implant can damage nearby cells and tissues in ways that interfere with normal behavior. Optogenetics, which works similarly but uses fiber optics and a pulse of light rather than electricity, has the same issue. A third strategy—using drugs to activate genetically modified cells bred into mice—is less invasive, but drugs are slow to take effect and wear off.

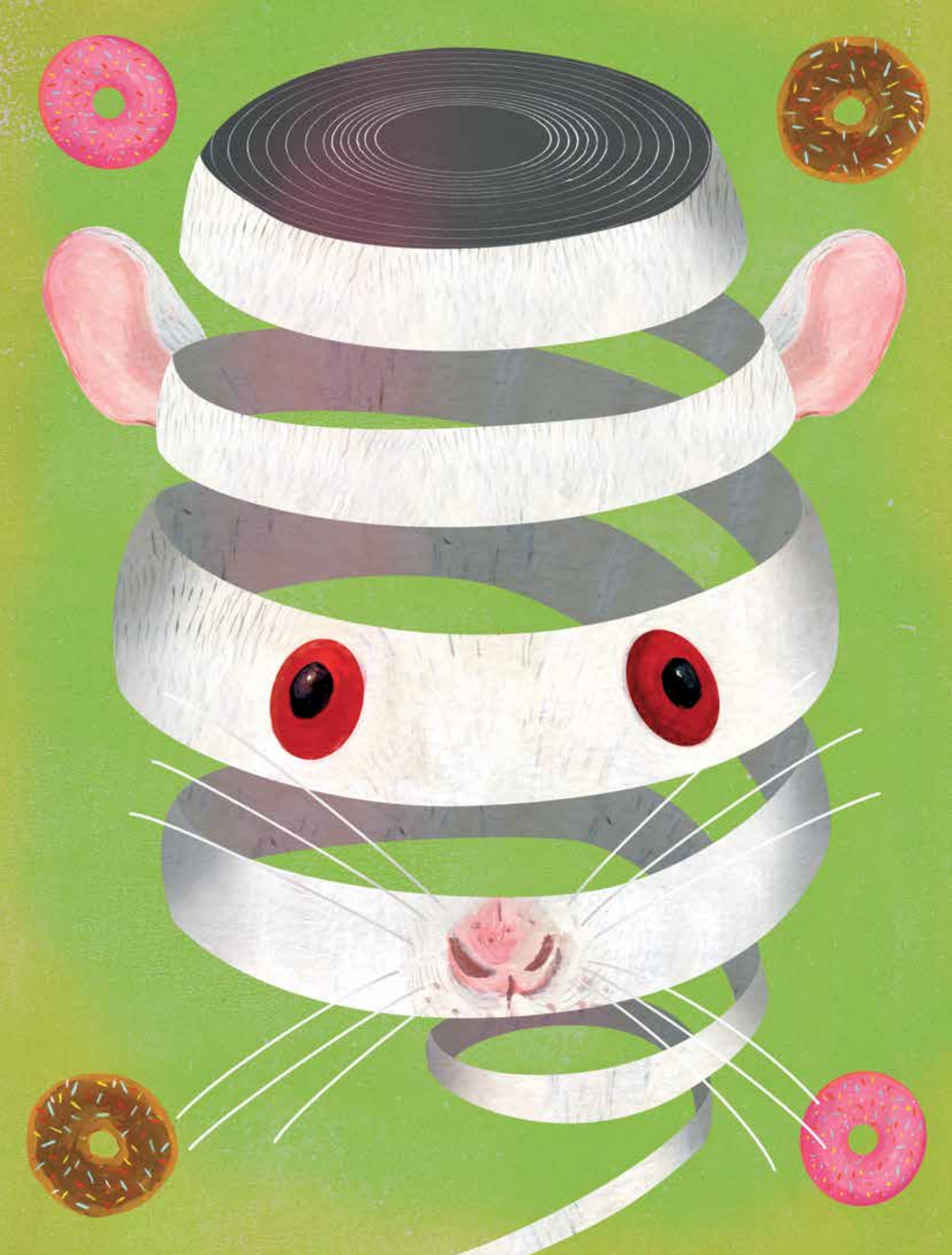
The solution that Friedman's group hit upon, referred to as radiogenetics or magnetogenetics, avoids these problems. With their method, published last year in *Nature*, biologists can turn neurons on or off in a live animal at will—quickly, repeatedly, and without implants—by engineering the cells to make them receptive to radio waves or a magnetic field.

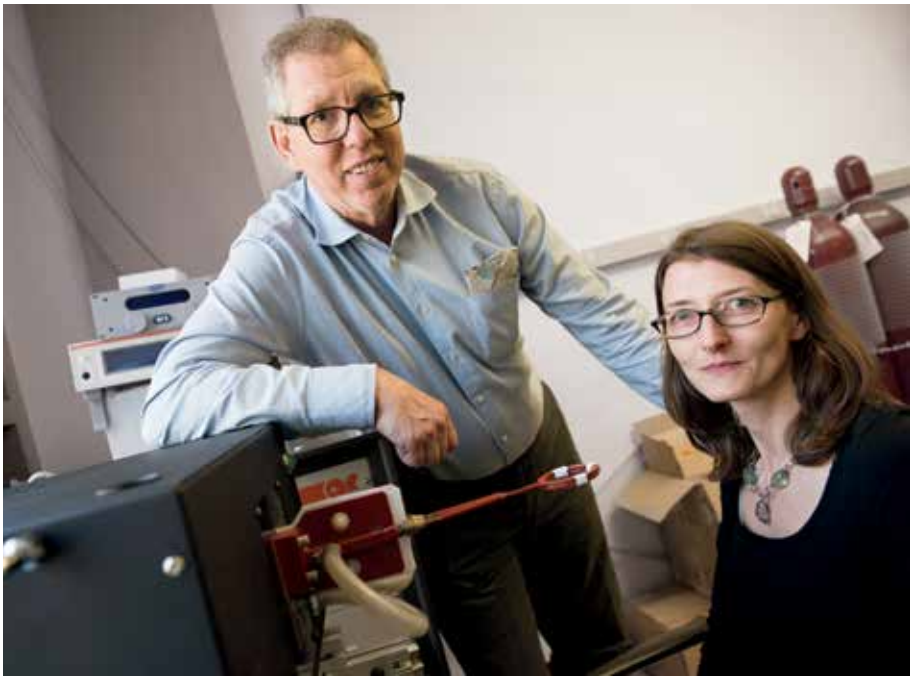
"We've combined molecules already used in cells for other purposes in a manner that allows an invisible force to take control of an instinct as primal as hunger," Friedman says.

The method links five very different biological tools, which can look whimsically convoluted, like a Rube Goldberg contraption on a molecular scale. It relies on a green fluorescent protein borrowed from jellyfish, a peculiar antibody derived from camels, squishy bags of iron particles, and the cellular equivalent of a door made from a membrane-piercing protein—all delivered and installed by a genetically engineered virus. The remote control for this contraption is a modified welding tool (though a store-bought magnet also works).

The researchers' first challenge was to find something in a neuron that could serve as an antenna to detect the incoming radio signal or magnetic field. The logical choice was ferritin, a protein that stores iron in cells in balloon-like particles just a dozen

Illustration by Ellen Weinstein





Friedman and Stanley, with equipment they use to send radio waves.

nanometers wide. Iron is essential to cells but can also be toxic, so it is sequestered in ferritin particles until it is needed. Each ferritin particle carries within it thousands of grains of iron that wiggle around in response to a radio signal, and shift and align when immersed in a magnetic field. We all have these particles shimmying around inside our brain cells, but the motions normally have no effect on neurons.

Friedman's team realized that they could use a genetically engineered virus to create doorways into a neuron's outer membrane. If they could then somehow attach each door to a ferritin particle, they reasoned, they might be able to wiggle the ferritin enough to jostle the door open. "The 'door' we chose is called TRPV1," says Stanley. "Once TRPV1 is activated, calcium and sodium ions would next flow into the cell and trigger the neuron to fire." The bits borrowed from camels and jellyfish provided what the scientists needed to connect the door to the ferritin (see "How to outfit a brain for radio control," right).

Once the team had the new control mechanism working, they put it to the test. For Friedman and Stanley, whose goal is to unravel the biological causes of overeating and obesity, the first application was obvious: Try to identify specific neurons involved in appetite. The group modified glucose-sensing neurons—cells that are believed to monitor blood sugar levels in the brain and keep them within normal range—to put them under wireless control. To accomplish this, they inserted the TRPV1 and ferritin genes into a virus and—using yet another genetic trick—injected them into the glucose-sensing neurons. They could then fiddle with the cells to see whether they are involved, as suspected, in coordinating feeding and the release of hormones, such as insulin and glucagon, that keep blood glucose levels in check.

> "In effect, we created a perceptual illusion that the animal had a drop in blood sugar."

Once the virus had enough time to infect and transform the target neurons, the researchers switched on a radio transmitter tuned to 465 kHz, a little below the band used for AM radio.

The neurons responded. They began to fire, signaling a shortage of glucose even though the animal's blood sugar levels were normal. And other parts of the body responded just as they would to a real drop in blood sugar: insulin levels fell, the liver started pumping out more glucose, and the animals started eating more. "In effect," Friedman says, "we created a perceptual illusion that the animal had low blood glucose even though the levels were normal."

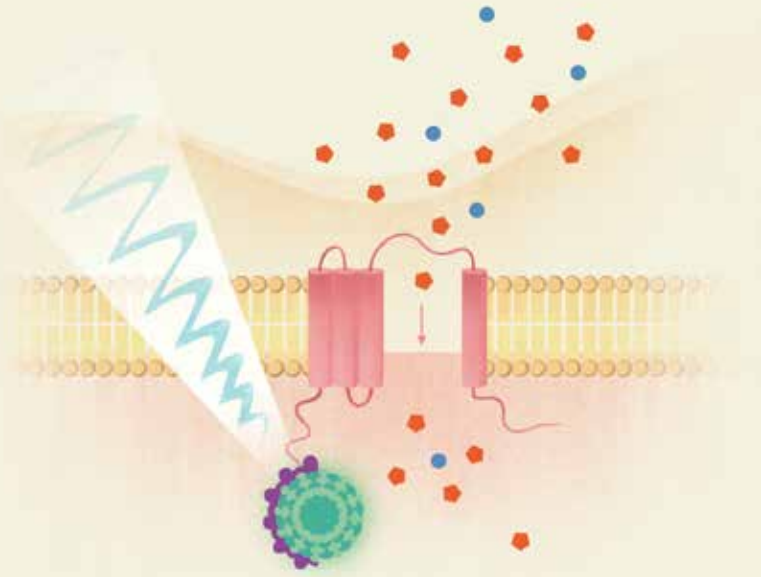
Inspired by these results, the researchers wondered if magnetism, like radio waves, might trigger ferritin to open the cellular doors. It did: When the team put the mice cages close to an MRI machine, or waved a rare-earth magnet over the animals, their glucose-sensing neurons were triggered.

Stimulating appetite is one thing. Could they also suppress it? The group tweaked the TRPV1 gene so it would pass chloride, which acts to inhibit neurons. Now when they inserted the modified TRPV1 into the neurons, the rush of chloride made the neurons behave as if the blood was overloaded with glucose. Insulin production surged in the animals, and they ate less. "This seems to indicate clearly that the brain as well as

ZACHARY VEILLEUX

How to outfit a brain for radio control

Scientists have come up with a clever way to control neurons via radio by cobbling together genes from humans, camels, and jellyfish. They use an engineered virus to install a door into each target neuron's outer membrane, then jostle the door open using ferritin particles that respond to strong radio signals. Once the door opens, calcium ions pour into the cell and trigger the neuron to fire.



1

To install the radiogenetics system into neurons, the scientists equipped an adenovirus with the various genes needed to make the system work. Then they squirted the modified virus onto the brain cells they wanted to alter.

2

One of the added genes produces TRPV1, a protein that normally helps cells detect heat and motion. Within each neuron, the TRPV1 protein (pink) embeds itself into the cell's outer membrane. Like a door, it can change shape to open or shut an ion channel. To add a knob to the door, the researchers stitched TRPV1 to a "nanobody" (violet)—an unusually simple variety of antibody found in camels.

3

Iron-filled ferritin particles (green) serve as the system's sensor. To allow them to grab onto the nanobody doorknob, the researchers tacked on a gene for GFP—a jellyfish protein that glows green under ultraviolet light. By design, the nanobody and GFP stick together tightly.

The system is now connected. When exposed to strong radio waves or magnetic fields, the ferritin particles wiggle, the ion channel opens, and calcium ions (red) flow in to activate the cell.

Illustration by Jasu Hu

the pancreas is involved in glucose regulation," Friedman says.

Friedman and Stanley hope that biologists will be able to use the remote-control system to tackle a range of neural processes other than appetite. And beyond being a basic research tool, the method could potentially lead to novel therapies for brain disorders.

For example, one could imagine using it to treat Parkinson's disease or essential tremor—conditions that are sometimes treated by deep brain stimulation, via wires implanted into patients' brains and connected to a battery pack tucked into the chest. Potentially, it would be less invasive to inject the crippled virus into the same spot of the brain and let it permanently modify the cells there, making them responsive to wireless control.

In theory, it might also be possible to make a patient's own cells receptive to electromagnetic waves by removing them from the body, delivering TRPV1 and ferritin, and then putting the cells back, Friedman says. This would be a protocol not unlike those currently used in stem cell treatments and some cancer immunotherapies, in which patients' own cells are engineered and reimplanted back into their bodies.

At this point, however, the system's clinical usefulness is a question of speculation. "We are a long way from using it in humans for medical treatments," Friedman says. "Much would need to be done before it could even be tested." ◎

A Sugar Bomb in Disguise

It's a time-honored, if sometimes ill-advised, tradition in medical research: Try it first on yourself. Thomas Huber's own weakness for diet soda led to his search for evidence that chemical attempts to fool the human sweet tooth may have unanticipated effects. Now, he is conducting a clinical trial to better understand if artificial sweeteners alter metabolism.

By Wynne Parry

INÉS DIELEMAN / TRUNK ARCHIVE

AMERICANS LOVE SUGAR. We put it in bread, breakfast cereal, yogurt, pasta sauce, salad dressings—all kinds of things that don't really need it. We invented the waffle cone sundae and the 44-ounce Slurpee. We consume 12 million tons yearly, about 75 pounds per person.

In Europe, the sugar cult is less intense. When Thomas Huber lived in his native Germany, where even the doughnuts are not very sweet, he drank sparkling mineral water every day. But after he moved to the United States, he couldn't find it as easily. Seeking a bubbly fix, he turned instead to the sappy-sweet, flavored sodas that fill American supermarket aisles. But he was wary of sugar calories, so like many others before him, Huber made a compromise. He turned to diet soda.

It had flavor, it had bubbles, and it didn't have sugar. Before long, he was working his way through two liters a day.

It wasn't exactly a healthy choice, Huber knew, but it couldn't be too bad.

Huber's move from Germany, where he grew up, happened 16 years ago. And since he switched to diet soda, he's been consuming aspartame—one of a number of chemicals that beverage makers use in their diet products to take the place of sugar—in large quantities on a daily basis for much of that time.

Huber is a scientist, and it so happens that he studies the receptors on cells that detect things. Things like nutrients, and sweetness. Even so, like the rest of us, for years, he had no reason to doubt that aspartame did exactly what it was supposed to do: trick those receptors into registering sweetness, then pass on through the body without being converted to calories the way sugar is.

But what if things were more complicated than that?

SUGAR FUELS THE BODY but can be toxic at high concentrations. We crave it—before modern conveniences like cupcake ATMs it signified foods such as fruits and roots, which are nutritious and provided our hunter-gatherer ancestors with quick bursts of energy. But today we eat way too much of it. It causes obesity, hypertension, and diabetes, and public health experts really wish we would cut back.

Artificial sweeteners promise better living through chemistry. They have all of the sweetness and, supposedly, none of the consequences. Although a few studies have suggested a link between sweeteners and cancer or neurological problems, the FDA deems aspartame, as well as five other artificial sweeteners, safe. The substances are also cautiously endorsed by medical advocacy groups including the American Heart Association and the American Diabetes Association, who say they can be effective ways to reduce calorie intake and help prevent disease.

There is mounting evidence, however, that artificial sweeteners may have subtle effects on metabolism, the process by which the body breaks down and uses food. In fact, a widely publicized 2014 study in the journal *Nature* linked artificial sweeteners to disruptions in the body's ability to control blood sugar, a hallmark of diabetes.

Huber reads *Nature*. He saw the study, and it made him think of sweetness receptors. It turns out they are not confined to the tongue. Scientists have found them lodged in many organs, including the small intestine, where they help maintain blood sugar at safe levels by detecting the arrival of an energy-rich meal.

"I thought, what if my gut receptors had been activated by artificial sweeteners?" he says. "And, what if by consuming so much artificially sweetened soda on a regular basis, I had overstimulated my metabolic control system, preventing it from responding normally to food?"

HUBER HAD HIS HYPOTHESIS. To test it, he decided to launch a pilot study, on himself. He gave himself an oral glucose tolerance test, typically used to diagnose gestational diabetes in pregnant women. The tools were simple enough: a bottle of water mixed with 75 grams of glucose (the equivalent of 20 sugar cubes) and a $30 blood glucose meter kit from the drugstore.

"Either the results would come back normal and I could happily continue drinking my soda," Huber figured, "or, if they didn't, I would stop to benefit my health, and I would have a fascinating project. It was a win-win scenario."

After fasting overnight, he drank the wince-inducing sugar water and pricked his finger five times over two hours, charting the numbers as his body struggled to control the sugar.

The results were unambiguous. Huber immediately knew the magnitude of this chart's arc was abnormal. But what he didn't realize at the time was that his peak blood sugar levels were up by over 30 percent.

He reasoned that if his test results were indeed the result of too much aspartame—if the aspartame had overstimulated the sweetness receptors in his gut—then the effect should be reversible. The lining of the gut renews itself periodically, so over time, the skewed receptors would be replaced with new ones. As long as the new receptors weren't exposed to aspartame, his metabolism should return to normal.

He moved to phase two of his experiment. For the first time in a decade and a half, he stopped drinking diet soda. He drank sparkling water instead. He avoided anything artificially sweetened, but otherwise ate normally. He went about his business. And three weeks later, he retook the test.

Sure enough, his blood sugar was back to normal.

HUBER, A RESEARCH ASSISTANT professor in molecular biologist Thomas P. Sakmar's lab, showed these observations to his boss, who studies cellular signal transduction. Sakmar, who tends to be fascinated by any finding related even tangentially to his work, was indeed fascinated. A clinical trial was born.

They brought in John Paddock, a Weill Cornell Medicine student who was completing a research rotation in Sakmar's lab, and with assistance from staff at The Rockefeller University Hospital, they designed and launched a trial using volunteer subjects who already have a diet soda habit.

Molecular biologists like Huber and Sakmar don't often find themselves running patient studies. "As scientists at Rockefeller, we have the amazing leeway to start with an interesting observation or idea and run with it," says Sakmar, who is *Richard M. and Isabel P. Furlaud Professor*. "Even people like us who don't typically run experiments that involve humans can get the support we need to set up a clinical trial."

At its outset, the study seeks to link regular consumption of aspartame with problems controlling blood sugar. To this end, the researchers recruit volunteers like Huber—healthy people who drink at least three cans of diet soda a day. Participants take an initial test—an extended version of the glucose tolerance test Huber gave himself—and those showing abnormally elevated numbers are invited back.

This is where it gets interesting. The trial's second phase investigates whether these abnormalities in blood sugar control can be reversed. For five weeks, the participants move into The Rockefeller University Hospital's inpatient unit, where they eat and sleep under the supervision of specially trained research nurses. Here, researchers are able to control every calorie and every nutrient they take in. Their meals are prepared in a special kitchen where each ingredient is measured and recorded with precision. Nothing goes into their bodies that Huber, Sakmar, and Paddock don't know about.

At first, the volunteers continue to drink diet soda. Then, after a few weeks, they stop, and a few weeks later they start again. All the while their precisely controlled diets remain steady, and the research nursing staff document their levels of blood sugar as well as incretins, hormones that help regulate glucose metabolism.

Sweetness through chemistry

Aspartame isn't alone. Food chemists have found several ways to give us the experience of sweetness without delivering energy content, and calorie-conscious consumers worldwide ingest nearly 17 million tons of these types of artificial sugar substitutes. These are the most common:

SACCHARIN
Benzoic sulfimide
Available since: 1879
Up to 700 times sweeter than sugar per gram, it's commonly used in beverages, toothpaste, and chewing gum.

ASPARTAME
Methyl L-α-aspartyl-L-phenylalaninate
Available since: 1981
It's 200 times sweeter than sugar and is a common ingredient in soft drinks, pudding, and cereal.

ACESULFAME POTASSIUM
Potassium 6-methyl-2,2-dioxo-2H-1,2λ6,3-oxathiazin-4-olate
Available since: 1988
About 200 times sweeter than sugar, it's found in frozen desserts, candy, and baked goods.

SUCRALOSE
Trichlorosucrose
Available since: 1998
Up to 1,000 times sweeter than sugar, it's commonly used in beverages, desserts, and baked goods.

OPPOSITE PAGE: ZACHARY VEILLEUX (TOP); LEVI BROWN / TRUNK ARCHIVE

For more information about clinical trials underway in The Rockefeller University Hospital, visit www.rockefeller.edu/hospital.

"The ultimate irony is that aspartame may be contributing to the obesity epidemic, not mitigating it."

—THOMAS HUBER

AS IT HAPPENS, incretins are a critical part of this story. They are the keystone in Huber's hypothesis as well as the subject of much other research on the effects of artificial sweeteners. When activated, intestinal sweet receptors trigger the release of incretin hormones, which in turn prompt the pancreas to release insulin, a hormone that tells cells to take in sugar.

This system helps to ensure good control of blood sugar. Huber suspected that by excessively triggering the gut's sweetness receptors, artificial sweeteners may be somehow interfering with the incretin response, ultimately leading to problems metabolizing sugar. In other words, the issue may not be with insulin production itself, but with the signaling molecules that turn it on and off.

Others scientists who are not involved in Huber's study have come to the same hypothesis. Kristina I. Rother, a pediatric endocrinologist at the U.S. National Institute of Diabetes and Digestive and Kidney Diseases, conducts clinical research on the effects of artificial sweeteners, and her work has linked a combination of two artificial sweeteners to increased incretin release when they are consumed before a glucose tolerance test like the one Huber gave himself.

"I am among those who strongly believe that artificial sweeteners do disrupt metabolism; however, until we have long-term studies in human beings, we won't have proof," Rother says. "By looking at what happens over the course of weeks, when aspartame is withdrawn from heavy consumers in a controlled environment, Huber's study has the potential to fill an important gap."

Meanwhile, experiments by Susan E. Swithers, a professor of psychological sciences at Purdue University, have turned up something quite different from Rother's work with people. Using rats, she is examining how artificial sweeteners might affect the learned relationship between sweetness and calories, and has noted that after consuming artificial sweeteners, the animals show a decreased incretin response to sugar.

"For a long time, it was assumed that artificial sweeteners were inert; they passed through your gastrointestinal system, and didn't engage it. We don't believe that is true any longer," says Swithers. "Some of us in the field are starting to see that these sweeteners do have consequences. But we really don't understand what those consequences are or how they come about."

FIRM ANSWERS TO complicated questions don't come easily, and running month-long tests on volunteers is slow and expensive. Just finding people who qualify for the study—men and women age 18 to 45 who already have a three-can-a-day diet soda habit—has proved challenging. But Sakmar and Huber believe it's the best way to settle a question that has enormous public health implications. Aspartame, after all, isn't found just in soda. It's in yogurt, chewing gum, ice cream, pudding, breath mints, and all kinds of packaged baked goods.

"The ultimate irony may be that, by interfering with the gut's ability to properly perceive sweetness, aspartame and other artificial sweeteners may be contributing to the obesity epidemic—not mitigating it," Huber says. "I suspect the relationship between this unconscious and conscious perception in the brain will be key to fully understanding the effects of these sweeteners."

Huber himself isn't waiting for the results. After seeing his blood sugar return to normal on his second test, he gave up his diet soda for good. "I once tried one again, but found it incredibly sweet," he says. "I am now back where I started: sparkling water, maybe with a splash of fruit juice, if I want to indulge." ◎

WYNNE PARRY *is a science writer on Rockefeller's Communications and Public Affairs team. She has a bachelor's in biology from the University of Utah and a master's in journalism from Columbia University.*

When experiments keep failing, is there a time to call it quits? Meet the scientist who spent decades of his life chasing after hepatitis C before his efforts helped produce a cure.

Charles M. Rice

By Jessica Wapner

CHARLIE RICE FIRST BEGAN work on the hepatitis C virus in 1989, when it seemed like a straightforward project. The hope was that a vaccine could be modeled after one that had successfully eradicated yellow fever from much of the world.

But the virus proved inscrutable. The vaccine never materialized, and it took decades of study before an effective treatment could be created—a combination of antiviral drugs that became available in 2013 and are credited with saving hundreds of thousands of lives.

For his pivotal role in the development of this therapy, Rice, *Maurice R. and Corinne P. Greenberg Professor in Virology*, was awarded the Lasker–DeBakey Clinical Medical Research Award last fall alongside two other scientists. We spoke with him about the highs and lows of his 30-year quest to cajole the virus into a form scientists could work with.

When you first began researching the hepatitis C virus, did you know what a vexing foe it would prove to be?

Yes and no. Certainly the mystery of identifying the virus in the first place hinted at the complicated future to come. For many years, we knew that there existed some entity in blood that was neither hepatitis A nor hepatitis B. But until that entity was found, we could only refer to it as non-A, non-B hepatitis. That cumbersome name really foreshadows how hard this virus would be to pin down.

Yet there were reasons to think we could readily stop the virus in those early years, too. When the hepatitis C virus (HCV) was finally identified, in 1989, scientists saw that it was a member of the same family as the virus that causes yellow fever. The yellow fever vaccine is one of the safest and most effective immunizations available, so it was easy to imagine that creating a vaccine for HCV would not be too arduous.

In fact, back in the late 1980s, when I was at the Washington University in St. Louis, my lab was working on a molecular clone of the strain of the yellow fever virus that was used in the vaccine, and my group, in concert with others, tried to use that clone to create an HCV vaccine. It seemed like a logical approach, considering the similarity between these two pathogens.

So what happened?

As it turned out, the features that distinguish HCV from yellow fever and other viruses also make it a challenging target for a vaccine. More than 20 years later, we are still waiting for an HCV vaccine.

When did you turn your attention to searching for a cure?

We realized that if we wanted any chance at finding a treatment for HCV, we needed to be able to study it outside of people—in cell cultures and animal models. But when we tried to coax the virus to replicate in cells in the lab or in animals, it mostly didn't work. We used all the classic techniques, which

Photograph by Sari Goodfriend

used the genome of the virus to initiate replication, but failed.

Eventually we started to wonder: Were we actually working with the correct genome sequence for the virus? RNA viruses like HCV won't replicate if a portion of the genetic sequence is missing. And indeed, when we reevaluated the sequence, it turned out we'd been missing a portion that was essential for replication. A Japanese group working on the same problem came to the same conclusion at around the same time.

Was that realization enough to clear the way toward finding a treatment?
No. The shifty nature of HCV proved to be another significant hurdle. The replication mechanism of the virus is prone to error. That tendency constantly gives rise to new versions of the virus, enabling it to avoid attacks by the immune system, which may not recognize a particular mutant as an invader. And creating a treatment that works on all versions of the virus was a daunting proposition. How do you know that the genetic target you home in on is exactly the same in all strains of the virus?

Does the diversity of the virus mean that people living with hepatitis C could be housing a virus whose genome differs from the one you were studying in the lab?
Yes, and that was yet another problem we needed to solve. To overcome this issue, we created our own version of the genome, in which each building block of the RNA genome was the one most commonly found in a person infected with the virus. This lab-created version of the virus proved capable of replicating in the liver, just like natural HCV.

This was a critical breakthrough. Using our "consensus" model virus, we were able to show that some viral proteins that people suspected would make good drug targets were indeed essential for HCV to replicate and spread. It was about this time that I moved to The Rockefeller University to establish the Center for the Study of Hepatitis C.

What was still missing at that point?
Despite its ability to replicate in the liver, our model HCV genome failed to replicate in cell culture. It was incredibly frustrating. We desperately needed a way to study the virus in the lab if we were to make any headway against this devastating illness. The next breakthrough came from taking a consensus HCV genome, chopping out the part that coded for the proteins making the virus particle, and inserting a marker that could be used to select for HCV replicating in cells.

We later found that such HCV "replicons" actually have adaptive mutations that make their propagation in cells more efficient. By making new replicons with these mutations, we were able to provide the first cell-based systems for studying how HCV amplifies its genome inside a host cell. These systems also turned out to be useful for evaluating and discovering new anti-HCV drugs. In the hands of biotech and pharma, they enabled the discovery of drug combinations that can completely eliminate the virus from the body.

But even though HCV replicons could replicate, derivatives that contained the whole HCV genome initially failed to make infectious virus. It was overcoming this last hurdle that got us an efficient cell culture system in which, for the first time, every step in the HCV life cycle could be studied.

Work by many scientists around the world—including Volker Lohmann and Ralf Bartenschlager who first reported on the replicon system—has made this progress possible.

What technological advances were most essential to this success?
Although many technologies contributed, I think the story of HCV is really one about persistence. The most crucial elements were blood, sweat, and tears. We had to continue believing that success was possible, and continue trying different approaches even when we repeatedly encountered failure.

How were you able to sustain such determination?

"The story of hepatitis C is really one about persistence. We had to continue believing that success was possible, and continue trying different approaches."

Since 2011, 13 new drugs for hepatitis C have been approved.

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Rice (right) at Washington University in 1989.

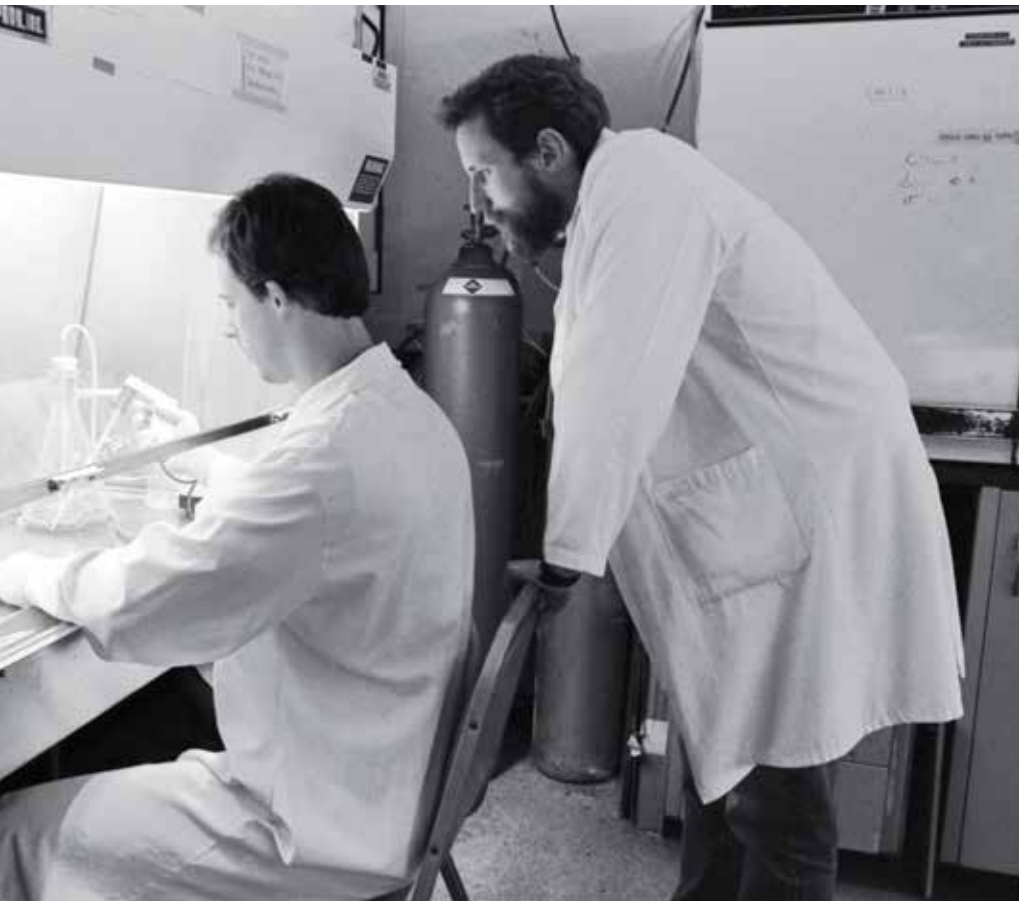
One standout memory was hearing from a woman whose adopted daughter was infected with HCV. That was nearly 20 years ago. We met and remained in touch over the years. The daughter was treated as a teenager. She's now married and has a family of her own. When the Lasker Award was announced, I received an email from her thanking me and others in the field. That's a very rare event for a basic scientist.

We always had in mind the fact that existing treatments were not very good, and people were suffering. Hepatitis C is a global epidemic. Up to 700,000 people were dying each year from liver diseases related to this pathogen. Until we could create something better, patients were limited to treatment with interferon, which often failed and caused harsh side effects.

What discoveries from your HCV work are proving useful in the fight against other infectious diseases?

The methods for determining the missing sequences of the genome, the concept of creating a consensus clone, finding a reliable method for creating a functional replication system for a virus with so much variation—all of these could be useful in other arenas.

Perhaps more relevant, though, is the fundamental importance of supporting scientists in becoming skilled before the need for those skills arises. In my case, my involvement with hepatitis C happened only because of my prior work on yellow fever. No one knows where the next virus threat will emerge or what expertise might be called upon, and no one can predict what research will yield the next biomedical breakthrough. So to enable rapid progress against dangerous new pathogens, we need to continually foster diversity in science.



What is your outlook for the future of HCV?

We actually still have a lot of work to do on this virus, which is why my lab and many others continue to study it. We don't yet understand much about the pathogenesis mechanisms of HCV infection—how the virus triggers liver scarring or cancer. Although this is becoming increasingly rare, we still need salvage therapies for people for whom the current medications fail.

Ending HCV will also require work beyond the laboratory. Bringing the curative treatment now available to people infected with the virus turns out to be more complicated than we would want. I would like us to do better, nationally and globally, implementing the necessary measures so that anyone infected with the virus can be treated.

How much do these remaining achievements depend on basic science?

A great deal. We all want bench research to lead to new treatments and other meaningful benefits, and I believe any scientist supported by the public good must keep this concern upfront. But it's also important to recognize that these clinical advances depend on a deep knowledge of fundamental biology. If we don't support basic science, then a vast world of potential progress will remain unexplored. ◎

JESSICA WAPNER *covers healthcare and medicine for* The New York Times, Scientific American, *and* Slate, *among other outlets. Her 2013 book* The Philadelphia Chromosome *made the* Wall Street Journal*'s top-ten list of nonfiction titles. Jessica lives with her family in Brooklyn, New York.*

The Fly Treadmill

THE SECRET to an effective fly treadmill is getting the air pressure right. You need just enough to keep the fly's walking surface—a smooth ball about the size of a pea—spinning freely, but not so much that it starts to gyrate.

Gaby Maimon's Laboratory of Integrative Brain Function didn't invent the fly treadmill. But their work, alongside others' in the field, has taken it to the next level, enclosing it in a visual virtual-reality environment and training cameras on the ball that allow them to observe *Drosophila melanogaster*'s navigational decisions while simultaneously recording neural activity in neurons.

Members of the Maimon lab carve the ball out of foam using a homemade tool akin to a melon baller, then ink the markings with a Sharpie and balance it atop a custom-machined air jet nozzle that lets it rotate freely. Tracking software, adapted from code written by colleagues in Australia, is fed by cameras trained on the ball's markings.

The treadmill setup allows Maimon and his colleagues to ask sophisticated questions about how the brains of their tiny subjects calculate angles, keep track of where they've been, and avert danger.

Photograph by Mario Morgado

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