ISSUE 10
WINTER 2024

Seek

THE ROCKEFELLER UNIVERSITY

It's our move

Inside the battle between science and antimicrobial resistance

ALSO

My memories, myself

What's new with de novo genes

The end of obesity?

“How did a signature advance of the 20th century become an existential crisis of the 21st?”

16

Facing up to a looming crisis

The dramatic increase of antibiotic-resistant microbes could render us defenseless against common infections, undoing a century of medical progress. Strategies to counter it are emerging—but the clock is ticking.

Illustration by Ibrahim Rayintakath

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CONTENTS

ISSUE 10 WINTER 2024

26
The mechanics of memory

Memory underpins our sense of being a person, a continuous "me" to whom experiences happen and stick. A search to understand its basic machinery led scientists to the center of the brain—and toward new questions about the self.

How does your thalamus decide what's important to you?

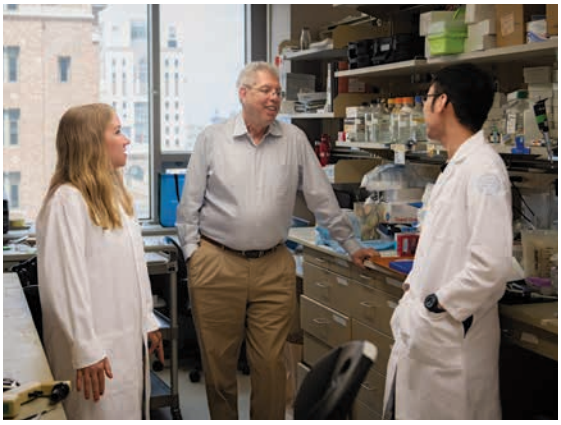
Illustration by Veronyka Jelinek

FEATURES

34

The unfinished business of obesity

The disease has been around for centuries, but only now are scientists learning how to treat it. Drugs like Ozempic may just be the beginning.



"There are books we cannot read that would tell us how to make new antibiotics from scratch."

PAGE 21

43

The future of journals

Results from government-sponsored research will soon be accessible to everyone. How will this policy affect science, and what will it mean for publishers?

12

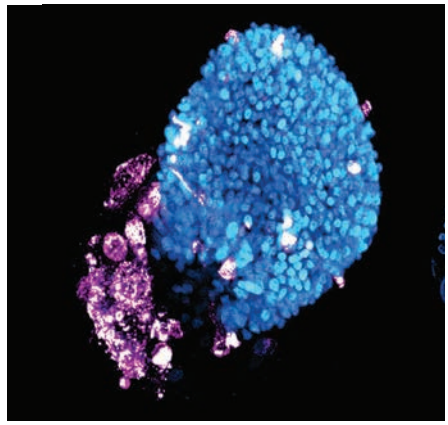
Q&A

Evolution happens very slowly—except when organisms have no time to lose. Li Zhao investigates how individuals and species might shortcut the process.

FOREFRONT

6

Scientists are asking if octopuses dream, how ants smell danger, and **what happens in our lungs when COVID strikes.**



ON THE BACK COVER

The Rockefeller University by Adam Simpson

KHOLOOD EID; ISTOCK; MORDOLFF; CLAIRE HOLT; LABORATORY OF SYNTHETIC EMBRYOLOGY

Seek

Co-Founding Editor and Editor-in-Chief
Eva Kiesler

Editorial Director
Mindy Farabee

Editors & Writers
Joshua A. Krisch
Jen Pinkowski

Associate Editor
Lori Chertoff

Art Direction & Design
Point Five

Production Manager
Kelly McLees

Digital Production
Mary Dang
Maureen Lynch

Contributor
Katherine Fenz

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President
Richard P. Lifton

Executive Vice President
Timothy P. O'Connor

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Contact us:
seek@rockefeller.edu
212-327-7900

Seek Magazine
The Rockefeller University
Box 68
1230 York Avenue
New York NY 10065

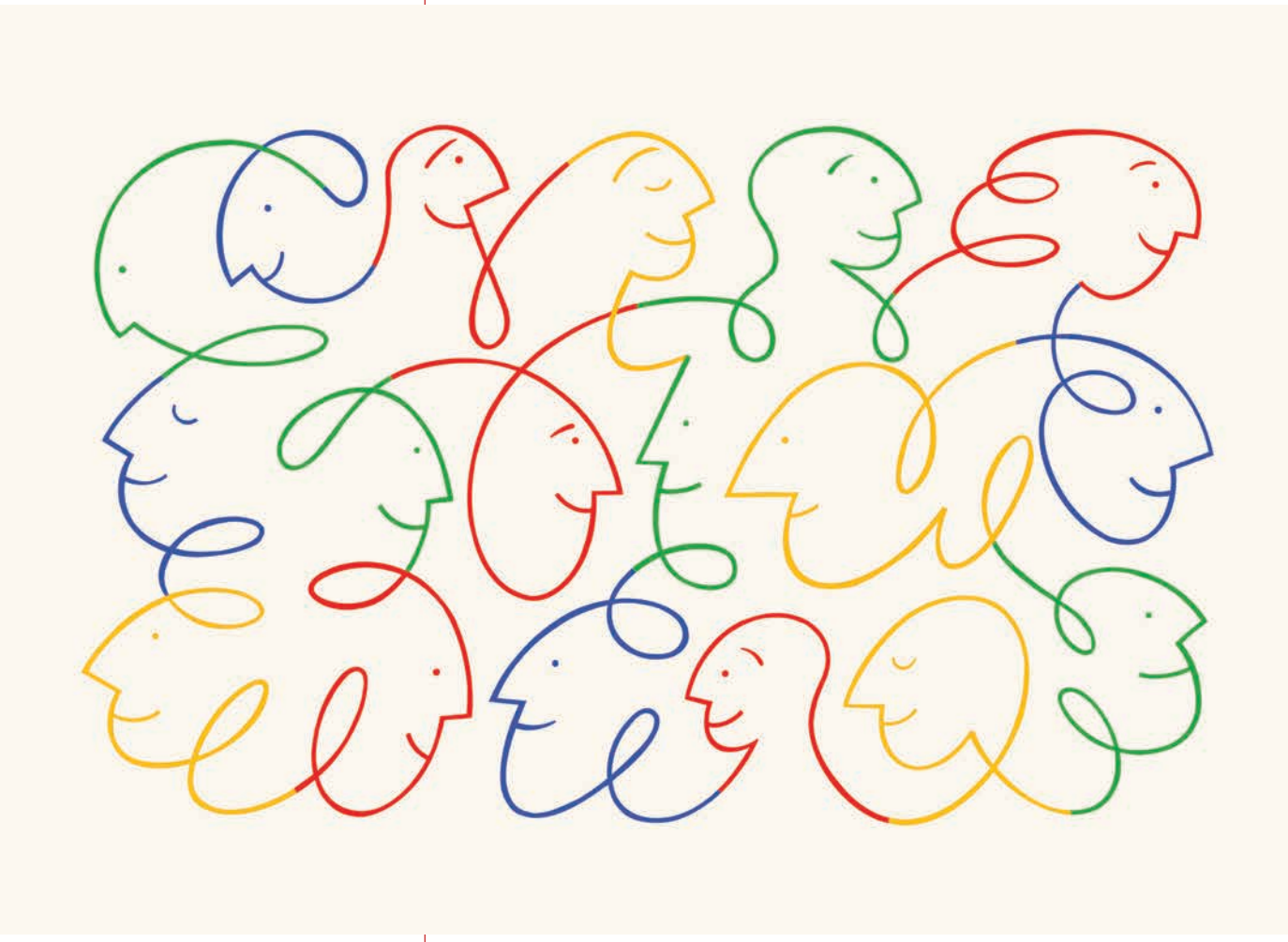
seek.rockefeller.edu

A garden where kids can grow. There are few spaces in this dense city where children can make mud pies and pluck raspberries, let alone learn STEM basics by planting and tending flora. But a campus garden has been a periodic Rockefeller feature since 1911, at one point swelling to two acres. Nowadays, 102 toddlers in the university's childcare and preschool enjoy a smaller (albeit large for Manhattan) 706-square-foot plot. Lovingly tended by teachers, parents, and the kids themselves, the harvest is used in countless classroom science projects—when it isn't eaten right off the vine.

PHOTO BY MATTHEW SEPTIMUS

Reported by Lori Chertoff, Mindy Farabee, Katherine Fenz, Eva Kiesler, Joshua Krisch, and Jen Pinkowski.

FOREFRONT



PANGENOMES

Genetic diversity comes into focus

THE HUMBLE WILD cabbage has had one since 2016. A lowly gut bacterium has had one even longer. Now, courtesy of the Human Pangenome Reference Consortium—an international alliance whose leaders include Rockefeller's Erich D. Jarvis—we *Homo sapiens* finally have one too: a genetically diverse pangenome that promises to dramatically increase our understanding of human disease and expand access to personalized medicine.

When the human genome was first released in 2003, scientists were already working to improve it. Over the next two decades, technology advances made it possible to fill in gaps and correct errors, but a substantial problem remained: Two-thirds of the DNA in the original reference genome came from a single person. As a result, many genetic variants found in non-European populations, such as people of African or Asian descent, weren't included.

This lack of representation can lead to biases in biomedical data that may in turn contribute to inequities and health disparities between different groups, Jarvis says. Among people with European ancestry, researchers have discovered countless genetic variants that predispose to specific illnesses, influence the severity of disease, or affect responses to particular drugs—knowledge that can provide powerful tools for physicians

Illustration by Pierre Buttin

DATA

The fraction of human DNA that varies from person to person.

to diagnose diseases, predict health outcomes, and tailor treatments for individual patients. Such discoveries have yet to be made for populations whose variants were excluded from the reference genome in the first place.

The human pangenome that Jarvis and his colleagues unveiled earlier this year is a first step toward tackling these problems head-on. Obtained with DNA from 47 people from around the world, it reveals nearly 120 million new DNA data points. Most are related to so-called structural variations, genetic differences that arise when long stretches of the double helix are duplicated, deleted, or rearranged.

"Structural variations can have dramatic effects on trait differences, disease, and gene function," Jarvis says. "There will be a lot of new discoveries to come that weren't possible in the last 20 years."

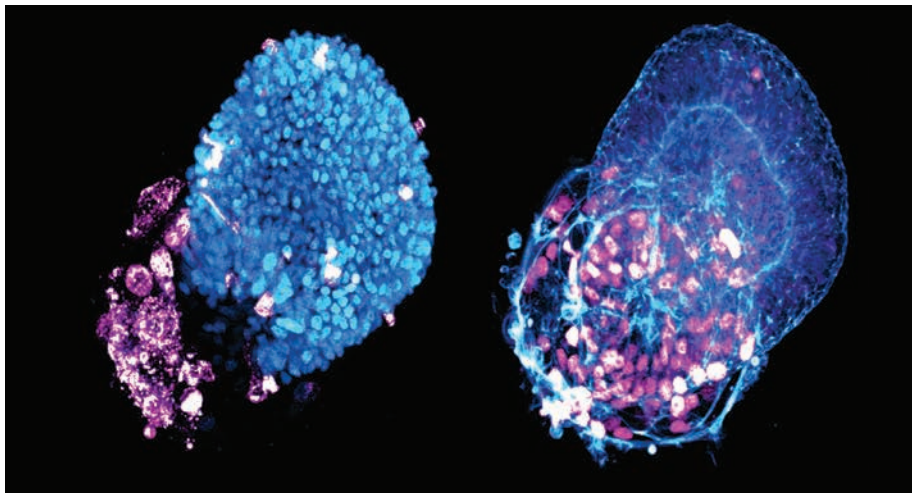
LABORATORY OF SYNTHETIC EMBRYOLOGY

TRANSFORMATIVE TECH

Brain cell biographies

OUR BRAINS continually produce new cells, a generative process that slows down as we reach our golden years. With new technology, scientists are now able to capture the ebb and flow of various brain cell types during normal development and aging and investigate what happens to decaying cells in neurodegenerative diseases like Alzheimer's.

Geneticist Junyue Cao and his colleagues zeroed in on progenitor cells, descendants of adult stem cells that differentiate into specialized cell types. By attaching unique ID tags to more than 10,000 newborn progenitor cells in the brains of mice, they were able to track these traditionally elusive cells and study their fates throughout the animals' life span. "It's like an ID card and GPS tracker combined," Cao says of the new technique, called TrackerSci, which offers wide-ranging applications. "If we can systematically characterize the different cells and their dynamics, we may get a panoramic view of the mechanisms of many diseases and the enigma of aging."



MODEL SYSTEMS

Tiny lung buds for big experiments

IT'S BEEN HARD to figure out how SARS-CoV-2 wreaks havoc in the lungs, partly because many studies have been done in samples from patients who respond differently to the virus. If you're trying to pinpoint a disease mechanism, you want to run many comparable trials side by side, not deal with a hodgepodge of genetically diverse situations.

That's why the labs of Ali H. Brivanlou, the *Robert and Harriet Heilbrunn Professor,* and Charles M. Rice, the *Maurice R. and Corinne P. Greenberg Professor*, developed a cell-culture platform capable of growing and infecting millions of lung buds, minuscule structures resembling those that give rise to our breathing organs. With this technology, they hope to light up COVID's attack route like an airport runway. In experiments described in *Stem Cell Reports*, for example, the researchers found that alveoli, the tiny sacs at the end of lung branches, are more susceptible to infection than cells in the airway, which are the first line of defense against inhaled pathogens. If the virus gets past the airway, the alveoli are sitting ducks.

"This technology is ready to confront all kinds of threats," Brivanlou says, from respiratory infections to noninfectious diseases like lung cancer. "It can be used to screen drugs, vaccines, monoclonal antibodies, and more, directly in human tissue."

IN MEMORIAM

His creative thinking transformed our understanding of gene regulation

C. DAVID ALLIS was never afraid to buck conventional wisdom.

In the 1980s, most scientists thought that histones, the proteins around which DNA is wrapped, were little more than packaging material for the molecule of life. Together, histones and DNA form a substance called chromatin, and researchers believed the histone part was basically the bubble wrap. Allis enthusiastically threw himself into studying these seemingly uninteresting proteins using *Tetrahymena*, an obscure single-celled organism. Many of his colleagues thought he was wasting his time.

"But of course, what he discovered in that little organism would turn out to be relevant to all of us," says Robert G. Roeder, the *Arnold and Mabel Beckman Professor*.

Allis found that histones play a critical role in turning genes on and off and in fine-tuning their effects—a breakthrough that revolutionized our understanding of how the basic instructions encoded in DNA are expressed in our tissues. Moreover, his research offered fresh insight into diseases as disparate as cancer and dementia and paved the way for new treatments.

He was also known for his humor, gentle demeanor, and dedication to his students and postdocs. "For someone so accomplished, he was the kindest, most humble, and relentlessly positive person you could imagine," says Richard P. Lifton, the *Carson Family Professor* and president of The Rockefeller University.

Allis, the *Joy and Jack Fishman Professor*, died in January last year. He was 71. ◎

DATA

3–12%

The typical fraction of total body weight lost after a year of treatment with GLP-1-based drugs.

WEIGHT-LOSS DRUGS

Where credit is overdue

OZEMPIC, WEGOVY, SAXENDA, VICTOZA—these blockbuster drugs for treating diabetes and obesity have all but become household names. But what about the name Svetlana Mojsov?

A research associate professor at Rockefeller, Mojsov laid the scientific groundwork that made these treatments possible (learn more about her work in "A drug's discovery," on page 41). While working at Massachusetts General Hospital in the 1980s, she discovered glucagon-like peptide 1, or GLP-1, a hormone secreted by the gut that triggers insulin production and lowers blood sugar. The new drugs—which began as diabetes treatments and were later found to induce weight loss as well—mimic the hormone's effects. The first to come on the market, Victoza for type 2 diabetes and Saxenda for weight loss, were based on the GLP-1 sequence that Mojsov discovered.

Her work was a godsend to drug developers. Safe, effective weight-loss treatments have long eluded researchers. Many drugs have had to be pulled from the market after causing life-threatening side effects or hard-to-kick addictions. The new class of GLP-1 agonists operate in a fundamentally different way, however, and are now being reliably used by millions across the world to lose weight and manage diabetes.

But while other researchers have received major awards for contributing to the development of these drugs, Mojsov remained unrecognized. Over the years, she has had to fight to have her name included on GLP-1 patents as a coinventor, and correct papers in high-profile journals that didn't acknowledge her work.

Now, that long overdue credit is finally rolling in. And while Mojsov is glad that she is no longer faced with the prospect of being erased from scientific history, what matters most to her is that these drugs are helping to improve the health and well-being of millions of people. "That makes me feel professionally and personally fulfilled," she says. ◎

Illustration by HelloVon

CELLULAR NUTRITION

Hunger games for tumors

CANCER SCIENTISTS MAY have hit on a way of forcing tumors to self-sabotage. It emerged from experiments in which a team led by Sohail Tavazoie, the *Leon Hess Professor*, examined cancer cells that were running low on arginine, an amino acid present in protein-rich foods.

These malnourished cancer cells pursued several coping strategies during their lifespan, including accruing a DNA error that made their offspring less arginine-hungry. Experiments described in *Science Advances* showed that the longer the cells grew without arginine, the more these mutations piled up. And in theory, the more mutations a tumor has, the more likely it is to be detected and destroyed by the body's immune cells.

Dennis Hsu, a former member of Tavazoie's Elizabeth and Vincent Meyer Laboratory of Systems Cancer Biology who is a physician-scientist at UPMC Hillman Cancer Center in Pittsburgh, suspects that cancer cells' dependence on arginine could thus be leveraged to make tumors more vulnerable to immunotherapy drugs that rally the immune system to destroy weird-looking cells. Withdrawing arginine might make it possible to trigger a rash of tumor mutations, essentially painting an immunological bull's-eye on the cancer cells and obviating the need to attack them with toxic chemicals or radiation.

"We haven't tested this yet," Hsu says, "but it would be a really cool thing to try."

OLFACTION

Smelling danger

WHETHER FORAGING, FIGHTING, or parenting, ants are continuously sending and receiving smell signals called pheromones. Recently, scientists used a glowing protein derived from a bioluminescent jellyfish to ask which parts of an ant's brain responds when it catches a whiff of an alarming scent.

Daniel Kronauer, the *Stanley S. and Sydney R. Shuman Associate Professor*, and his team injected the eggs of clonal raider ants with genetic material encoding the protein GCaMP, which glows neon green when calcium levels change due to cellular activity. They aimed these proteins at specialized brain structures called glomeruli, which are essential to scent processing. They then used a custom imaging technique to monitor GCaMP levels in the glomeruli as the ants took in a range of odors. Alarm pheromones caused six glomeruli in one particular region to light up, suggesting that this area may act as the brain's panic button.

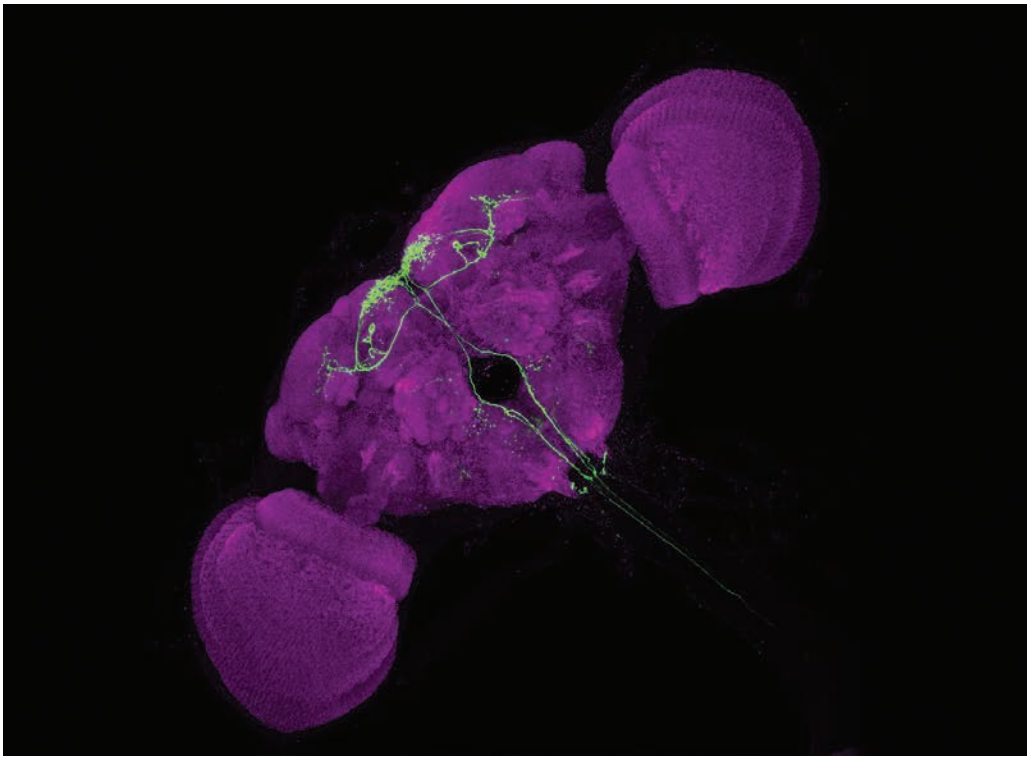
A transgenic ant pupa flashes neon in the presence of alarm pheromones.

The technique, described in *Cell*, could potentially be used to reveal what hundreds of odorant receptors are up to, says first author Taylor Hart, a postdoctoral associate in Kronauer's lab who has bred hundreds of such gleaming ants.

"This opens up a big range of questions that were inaccessible to us until now," adds Kronauer.

CLAIRE HOLT; LABORATORY OF SOCIAL EVOLUTION AND BEHAVIOR

Egg-laying neurons (in green) light up as a fruit fly makes a decision.



COGNITION

Decision time

IF A ROCK comes whizzing at your face, you'll duck without a thought. But if you're scanning the menu at a well-rated restaurant, you might spend several minutes weighing your gustatory options.

Both represent decisions, but while one occurs over a few seconds or less, the other takes much longer. And while neuroscientists have learned a lot about the mechanisms governing reflexive decisions, they know far less about those that operate over longer timescales.

Recently, Gaby Maimon's Laboratory of Integrative Brain Function gained insight into how these slower decision-making processes unfold in the fruit fly brain as female flies choose a good spot to lay their eggs. In work published in *Nature*, the researchers homed in on a set of cells known as oviposition descending neurons, which play a key role in the process.

They identified a calcium signal in these neurons that fluctuated as flies inspected different egg-laying options. The signal peaked at exactly the moment a fly began laying eggs, indicating it had crossed some kind of decision-making threshold.

Choosing where to lay eggs is not a reflexive decision but a considered one. In experiments where female flies were placed on a rotatable treadmill and allowed to walk across different surfaces mimicking fruits they might encounter in the real world, the little critters often took up to a minute to choose just the right spot for their eggs.

And when first author Vikram Vijayan, a research associate in Maimon's lab, inhibited the insects' oviposition descending neurons, prolonging the time it took for the calcium signal to reach threshold, the flies took even longer to decide on a spot. The extra time benefited them: They laid more eggs on the surface that matched their expected preference in the wild.

"The more time the flies spent exploring," Vijayan says, "the more they tended to pick an option that presumably ensured better survival of their offspring."

The team's findings could build a foundation for understanding how humans make educated and strategic decisions, says Maimon: "This work allows us to imagine that a similar rise-to-threshold process might exist in our own brain as we select what clothes to wear in the morning." ◎

226.7

DATA

Estimated number of food-related decisions a person makes in a given day.

Pregnant flies spent up to a minute pondering where to lay their eggs.

JANELIA FLYLIGHT TEAM

OBSERVATIONS

Nightmare scenario

ONE DAY, DURING his late morning nap, Costello the octopus had a nightmare.

Video footage captured by cameras in biophysicist Marcelo O. Magnasco's Laboratory of Integrative Neuroscience showed that while Costello began his snooze peacefully hanging from the side of his glass tank, the little octopus suddenly flushed with color and fell, thrashing and twisting, to the pebbled floor. He flexed his mantle into a cone shape, a defensive posture against predators; wrapped himself around a PVC pipe (one of his many toys) as if subduing a nemesis; and ended the fracas with a dramatic squirt of black ink. Eight minutes later, he was milky-hued and calm again.

The researchers were amazed when they reviewed the footage the next day. A Brazilian reef octopus, Costello had been captured off the coast of Florida with a missing arm—evidence of a battle he'd at least partially lost. Had he been dreaming about that altercation or a similar one? If so, what did that mean for octopus cognition, the focus of their research?

Just like human beings, octopuses have active sleep states during which they ignore external stimuli. But scientists don't yet know whether they dream as we do, melding memory and invention into a full-blown narrative.

Costello had three more such episodes over the next month before dying of natural causes. Since publishing an article exploring the implications of this unusual behavior as a preprint on bioRxiv, Magnasco has continued to study octopus cognition (read more about his work in "The octopus examination room," on page 48).

Sleep occurs in virtually all animals, while dreaming has long been thought to be confined to neurologically complex vertebrates. But Costello's episodes are an intriguing suggestion that our distant kin—and perhaps other spineless animals—may in fact be capable of complex sleep.

"If invertebrates dream," Magnasco says, "then perhaps dreaming exists throughout the tree of life." ◎

BIOMARKERS

An early flag for Parkinson's

A WOEFUL CATCH-22 plagues efforts to treat Parkinson's disease: Early diagnosis can stave off the most severe symptoms for years; but without symptoms, doctors have no way of detecting the illness.

This is especially unfortunate because by the time symptoms do appear, sufferers have already lost 50–80 percent of the brain cells that produce dopamine, a neurotransmitter that plays a crucial role in various functions, from movement to memory.

Researchers are now looking for disease biomarkers that could be detected before symptoms surface. Thus far, their quest has been complicated by the challenges involved in examining brain tissue from living Parkinson's patients.

So when Krithi Irmady, an instructor in clinical investigation, found overlapping RNA changes in the blood of living patients and the brains of deceased ones, she and her colleagues knew that they'd found something significant. The team's findings, published in *Nature Communications*, could potentially be used to develop tools for predicting the course of the disease and creating treatment options tailored to a patient's symptoms and disease stage.

The group discovered a bevy of RNA-driven gene-expression changes in one region of the brain associated with cognitive impairment and another region linked to motor control problems. Each bore distinct molecular signatures that could be linked to a patient's symptoms—the first such markers to be found in Parkinson's.

These and other recent findings by the team, which is headed by Robert B. Darnell, the *Robert and Harriet Heilbrunn Professor*, raise hope for the development of better drugs and prediction tools for the disease.

"I think our findings will generate excitement about the promise of blood-focused studies for Parkinson's disease," Irmady says. ◎

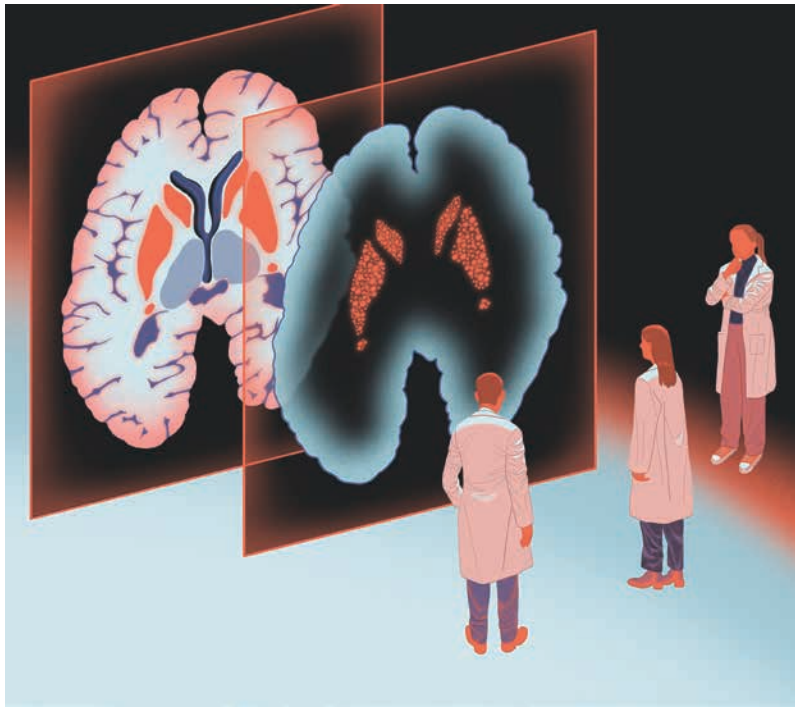
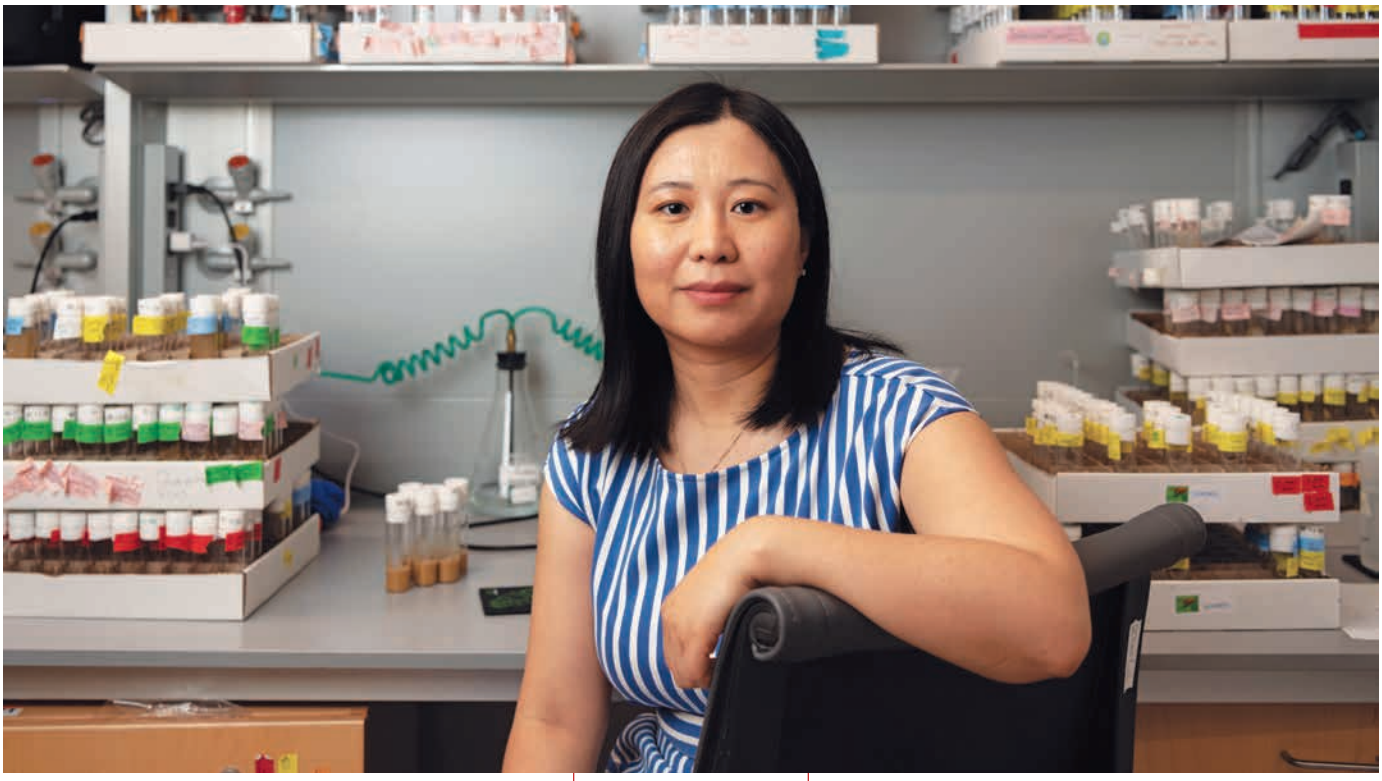


Illustration by Jun Cen

Q&A

The rapid evolution of de novo genes

With Li Zhao



IN 2006, JUST a few years after the fruit fly genome had been sequenced, geneticists at the University of California, Davis, made a startling discovery: Several new genes had cropped up, seemingly out of nowhere.

These "de novo genes" weren't simply new variants of existing ones; they had sprung forth from the supposedly inert spaces in between the coding sections of DNA—regions long dismissed as the junkyards of the double helix. Since the days of Darwin, such sprightly biological change agents had never before been seen.

A young graduate student at the time, Li Zhao was so intrigued that upon graduating in 2011, she set out to join the lab of David Begun, where the discovery was first made. She soon revealed that these little genetic big bangs happen all the time—over the past decade, she and her team have identified more than 500 de novo genes in the *Drosophila* lineage alone.

But de novo genes are just one of several types of rapidly evolving genes that enable organisms to quickly adapt under intense environmental pressures. Such genes have now been discovered in virtually every organism so studied, including humans.

Since founding Rockefeller's Laboratory of Evolutionary Genetics and Genomics in 2017, Zhao, an associate professor, has probed deeper into questions about how genes arise, spread, and evolve, shaping individuals and entire species. We asked her to explain what rapid evolution looks like, and how research into its basic mechanisms might expand our understanding of how new life-forms come to be.

Zhao and her team have already discovered more than 500 de novo genes in flies alone.

What made you jump at the opportunity to study de novo genes?

That genes could build themselves from scratch was just a fascinating idea. When the first ones were found, there had already been hints that genes can evolve very quickly. In the early 2000s, for example, scientists discovered several so-called orphan genes which lacked similarity to genes in other species or lineages. People assumed that these orphans did in fact come from a parent gene, although they had changed so much in a relatively short time span that they looked unique. And in some cases, newly found orphan genes were chalked up to mere sequencing errors.

But I wondered if some orphan genes had in fact emerged in a de novo manner, without a parent. And as I kept learning more and more about de novo genes, I realized that our next challenge would be to understand

CLAIRE HOLT

how they are born—how a supposedly useless piece of DNA turns into an evolutionary asset. It's a tremendously exciting puzzle to solve.

Can de novo genes show up anywhere in the genome at any time?
Not exactly. We know their origin is mainly related to functions that kick in under very strong evolutionary pressures, like immune functions that get activated during an infection. Organisms are in constant battle with pathogens that lurk in their environment, so genes that can help them keep up with the arms race tend to evolve very quickly.

The same is true for genes that support reproduction. Sexual selection is an incredibly strong pressure, making the male sex organs a hotbed for evolutionary innovation. Any advantage an aspiring father might get from a new gene, such as brighter plumage or hardier sperm, can make all the difference for his reproductive fitness. That's why my team focused on the testes when we first started looking for rapidly evolving genes in *Drosophila*.

When you say "rapidly," in the context of evolutionary change, how rapid is that?
It depends on what genes and what species we're talking about—the speed of change varies greatly in evolution. On the one hand, many genes governing the most basic biological functions have barely changed since the first life-forms originated on Earth. For example, about a thousand human genes have similar counterparts in bacteria, fungi, and other very distantly related species, which means they come from a common ancestor that lived billions of years ago.

On the other hand, you have rapidly evolving genes whose DNA sequences change much more quickly. In *Drosophila*, we study rapid-evolution events that occurred over up to a few million years. The timescale can be much shorter for humans, however—tens of thousands of years. Many Tibetans, for example, can tolerate high altitudes thanks to mutations in genes playing a role in oxygen use and UV light exposure, an adaptation to the local environment that probably happened only 30,000 years ago.

98%

DATA

Portion of the human genome that doesn't code for amino acids, the building blocks of proteins.

How does a supposedly useless bit of DNA become an evolutionary asset?

What does a rapidly evolving gene look like in action?
Let's stick with UV light exposure, and I'll give you an example that we've recently seen in *Drosophila*. A senior research associate in my lab, Nicolas Svetec, studied how fruit fly populations in two sites with different exposure, one in Florida and the other in Maine, respond to UV light. When he collected eggs from each location and incubated them under the same UV light, he found that the Florida population survived the exposure at a higher rate. What really grabbed our attention was that the Florida flies also carried unique genetic signatures related to genes that help repair UV-induced DNA damage, suggesting that this population adapted to a sunnier environment by quickly acquiring the ability to counter the effects of UV rays.

What about behavioral adaptations? Do fruit fly mothers in Florida lay their eggs in shadier areas than the Maine mothers do?
That's a great question, but we have no idea! *Drosophila* is a wonderful model for studying many genetics-related questions, but we know relatively little about their life outside of the lab.

Have you found any rapidly evolving genes in humans?
Yes, we've discovered evolutionarily young genes coding for small proteins of unknown function. Most of these genes are expressed in only a few specialized cell types, such as in heart tissue, and we hope to learn more about them soon.

We've also found what look like new genes that are present in cancer cells but absent from normal tissues. We have yet to confirm that these sequences are actually genes, and we still don't know what proteins they code for. Are they just spurious creations, or are they specifically doing something related to tumor growth or metastasis? We don't know that either, but it will be fascinating to find out.

What's next in the field?
Human de novo genes are a very, very hot topic right now. For example, recent work from a group in China found 74 such genes related to brain development, around half of which arose after our lineage branched off from chimpanzees. Although there are controversies around this study, these findings created a lot of excitement as they suggest de novo gene origination might have enabled humans to evolve bigger and more complex brains.

We need to be careful not to draw premature conclusions from such studies, however. This is a brand-new area of research, and it's very tricky to answer questions about the evolution of human-specific traits. For one thing, the type of experiments we did with the *Drosophila* eggs under UV light cannot be done in humans—it would involve putting human populations in a controlled environment and following them over decades or centuries! So we need to rely on model species such as *Drosophila* to delve into the functions and fitness effects of de novo genes.

And while it's easy to think that humans gained dominance over other primates because our brains are bigger, we actually know very little about what kinds of selective pressure drove the origin and evolution of our species. Are we really smarter and stronger than our primate cousins? And if we are, how exactly did our cleverness give us a leg up in surviving the flu or having children? ◎

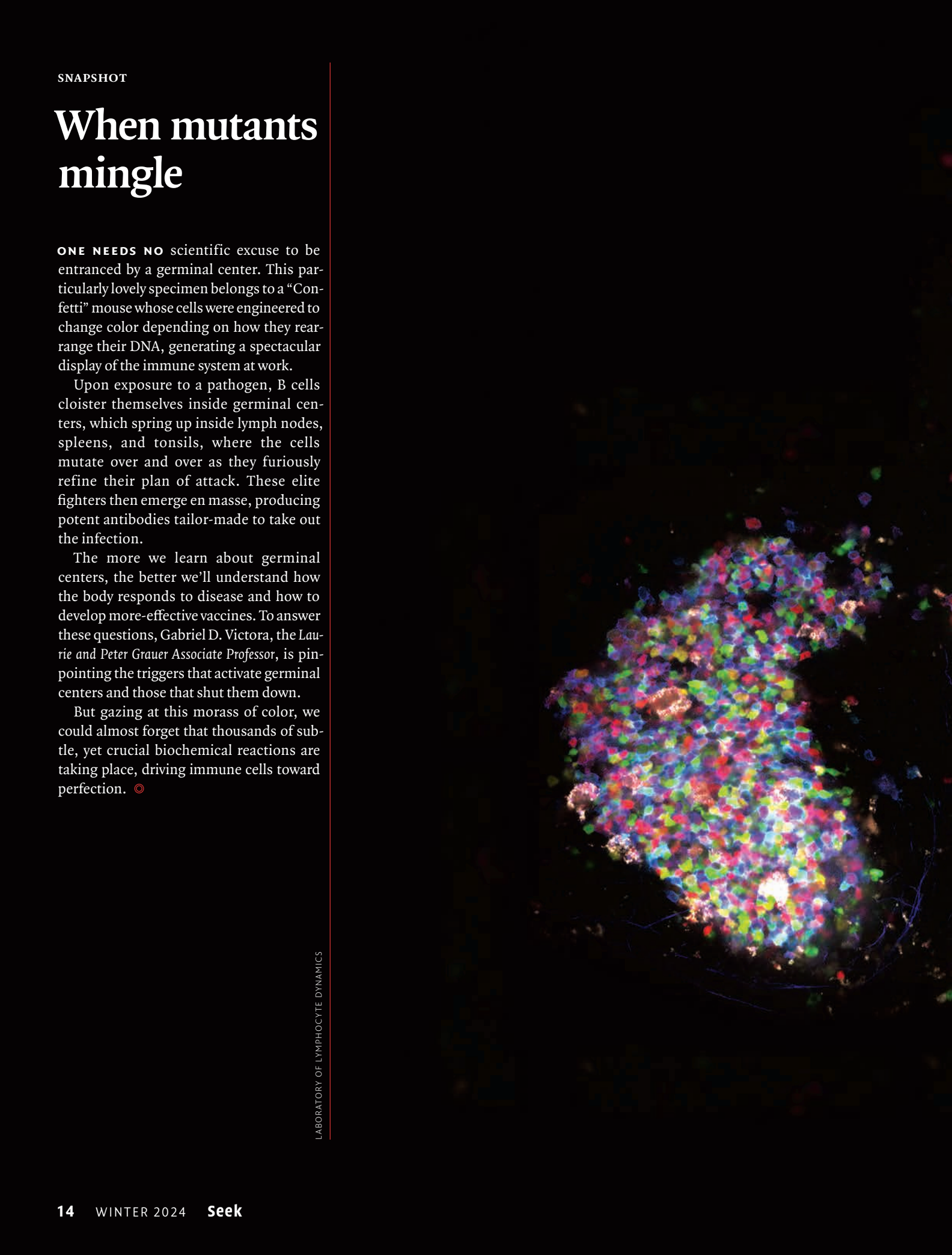
When mutants mingle

ONE NEEDS NO scientific excuse to be entranced by a germinal center. This particularly lovely specimen belongs to a "Confetti" mouse whose cells were engineered to change color depending on how they rearrange their DNA, generating a spectacular display of the immune system at work.

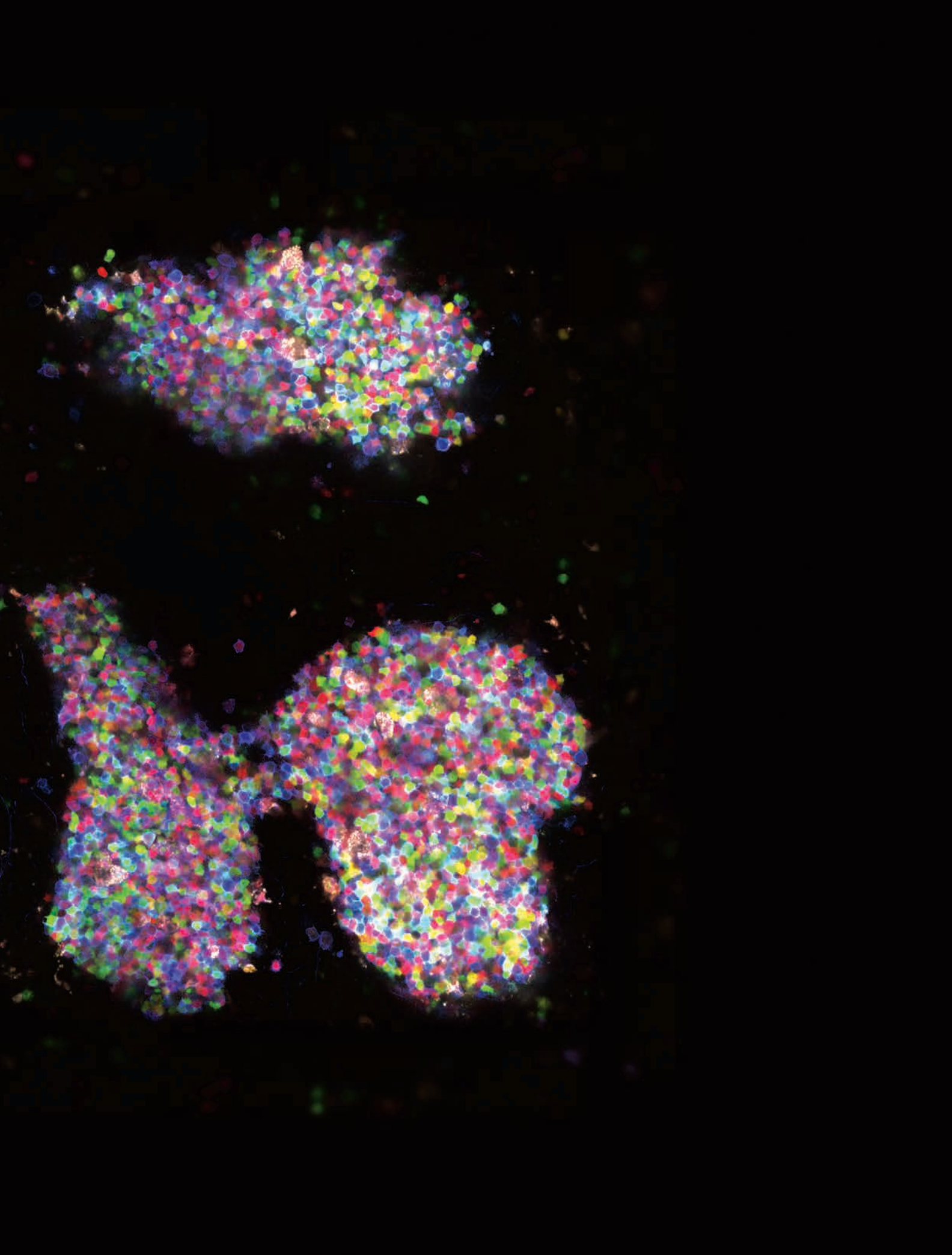
Upon exposure to a pathogen, B cells cloister themselves inside germinal centers, which spring up inside lymph nodes, spleens, and tonsils, where the cells mutate over and over as they furiously refine their plan of attack. These elite fighters then emerge en masse, producing potent antibodies tailor-made to take out the infection.

The more we learn about germinal centers, the better we'll understand how the body responds to disease and how to develop more-effective vaccines. To answer these questions, Gabriel D. Victora, the *Laurie and Peter Grauer Associate Professor*, is pinpointing the triggers that activate germinal centers and those that shut them down.

But gazing at this morass of color, we could almost forget that thousands of subtle, yet crucial biochemical reactions are taking place, driving immune cells toward perfection.



LABORATORY OF LYMPHOCYTE DYNAMICS



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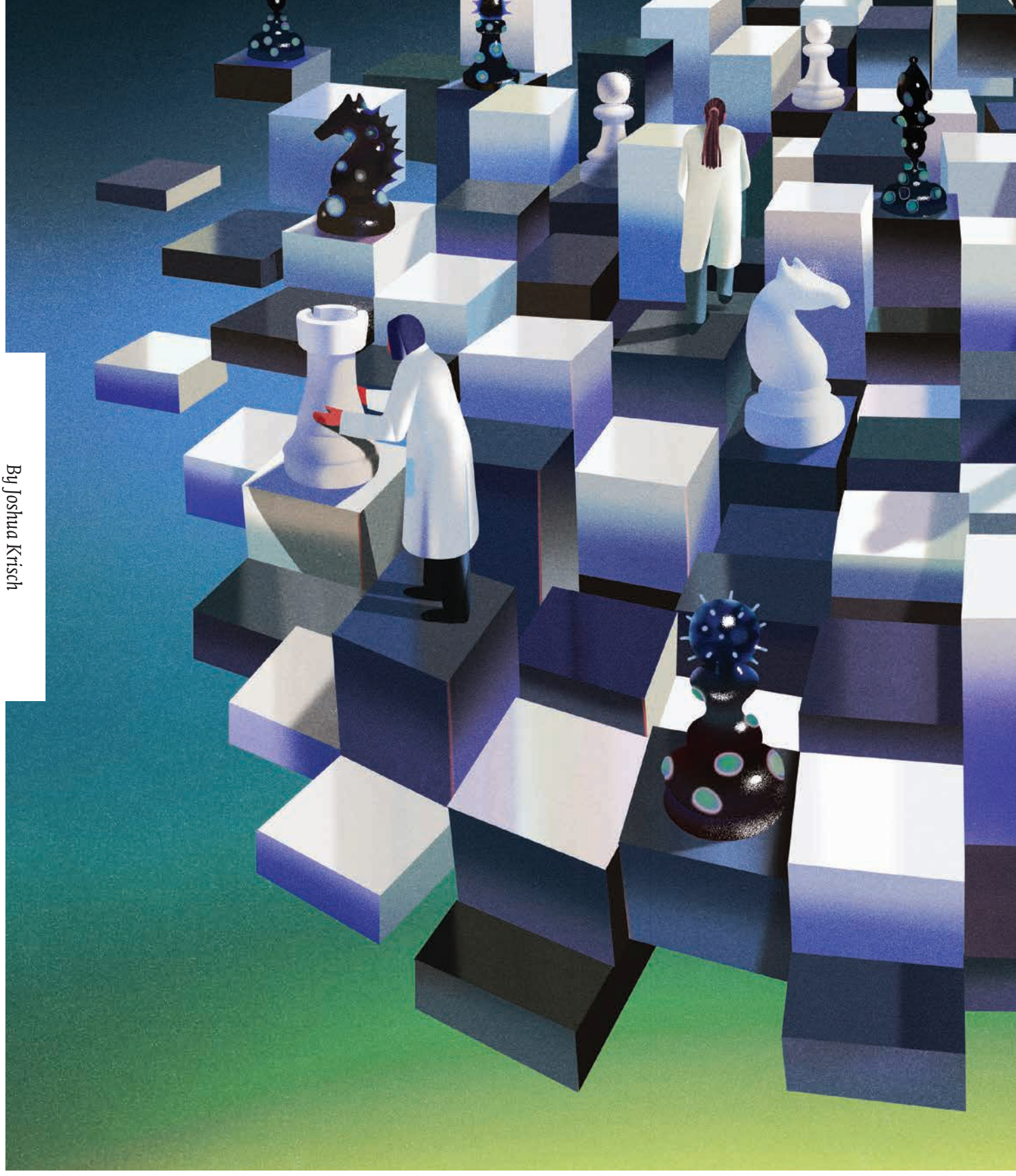


UPPING OUR GA

Illustration by Ibrahim Rayintakath

ME

Multidrug-resistant infections already kill five million people each year. Will new discoveries put science back on top?

One day, Elizabeth Campbell got a call from a group of frustrated researchers at the University of Zurich. The Swiss scientists were trying to coax the antibiotic fidaxomicin—commonly used to treat infections from *C. difficile* (a germ that typically attacks the large intestine, causing colitis and other problems)—to beat back drug-resistant tuberculosis. Fidaxomicin had been shown to do a great job of killing *Mycobacterium tuberculosis* cells in a petri dish; the researchers couldn't figure out how to move the drug from the gastrointestinal tract into the bloodstream and on to the lungs, where TB lives.

Colleagues often turn to Campbell for help with such problems. Maybe they've seen promising experimental results for a potential new drug but can't explain the mechanism of action. Or maybe they've unexpectedly hit an impasse in a once promising line of experiments. In the eyes of her peers, Campbell, a structural biologist in Rockefeller's Laboratory of Molecular Biophysics, is something of an antibiotics whisperer.

"We analyze, step-by-step, how potential drugs behave inside the cell—how an antibiotic attacks a bacterium, for instance," says Campbell, who is also a research associate professor. "In the case of fidaxomicin, we were able to suggest a few specific adjustments that could potentially nudge the drug into the bloodstream."

It's hard to overstate how precious this type of information could be. TB is no longer the disease it was in the latter half of the 20th century, following the 1943 discovery of streptomycin, a potent antibiotic. Virtually overnight, a disease that had terrified humanity for millennia—responsible by the 1800s for killing one in seven of all those who had ever lived, and more lethal than the Black Death, leprosy, or AIDS—was finally being tamed.

Today, more than 80 years later, multidrug-resistant TB strains kill hundreds of thousands of people each year. And what keeps researchers like Campbell up at night is the knowledge that this emerging resistance could someday render the disease impossible to treat once again—making diagnosis akin to a death sentence.

What's more, TB is just one potential tidal wave in the hurricane of antibiotic resistance headed our way.

"We're in a state of constant warfare with all infectious microbes," Campbell says. "Every time we innovate, they immediately respond by mutating."

At Rockefeller and beyond, scientists like Campbell are tenaciously working to keep medicine one step ahead. They're leveraging the latest advances in AI and CRISPR to reveal new genetic targets, learning new techniques from the age-old battle among microbes, and opening up entirely new approaches by unpacking the basic biology of their microscopic foes.

The COVID pandemic accelerated what had already been a crisis, creating a petri dish for breeding drug-resistant bacteria.

Elizabeth Campbell

The stakes could not be higher. Whereas the golden age of antibiotic discovery—a period that stretched from the 1930s through the 1950s—miraculously reduced the lethality of infections ranging from TB to rheumatic fever, so-called superbugs that can shrug off any drug we throw at them now kill someone in the United States every 15 minutes. Globally, five million people die from drug-resistant bacterial infections every year, a number expected to double by 2050. The World Health Organization has declared that if left unchecked, antibiotic resistance will pose one of the greatest threats to us as a species.

So how did a signature advance of the 20th century become an existential crisis of the 21st?

By 1924, four years before Alexander Fleming discovered penicillin, doctors had already documented resistance to the first antibiotic (arsphenamine, a cure for syphilis). Even

then, scientists saw the basic outline of the problem: The bacteria most susceptible to an antibiotic are killed, leaving only those that are resistant to proliferate. What's more, because bacteria multiply quickly, they quickly acquire random mutations—and the quicker they reproduce, the higher are their odds for picking up resistance-conferring mutations. Those variants can then quickly spread as bacteria readily exchange DNA with one another. The COVID pandemic accelerated what had already been a crisis of growing resistance, as millions of patients were admitted to hospitals where they often received treatment for secondary infections while already immunocompromised—creating a global petri dish for breeding drug-resistant bacteria.

What's at stake isn't just our ability to treat infections but also a staggering number of other advances in modern medicine that depend on easy access to powerful antibiotics. When these drugs are no longer effective, routine surgeries—appendectomies, hip replacements, and nearly all elective procedures—will often be ruled too risky. Cancer treatment will be set back decades (chemotherapy ravages the immune system), and childbirth will end more often in tragedy than in celebration.

Barbara Bosch, an instructor in Campbell's lab, researches new treatments for TB.

Countering resistance has always meant devising new treatments. But when it comes to antibiotics, precious few are being discovered. Between 1909 and 1990, 110 antibiotics came to market. From 2017 to 2021, just a single drug capable of treating the most critical class of resistant bacteria had been approved, and today only two more such drugs are in clinical trials. Currently, 27 antibiotics for the most threatening infections are undergoing trials. The pipeline has essentially run dry.

The reasons are complicated. For starters, as is the case with many modern medicines, it can cost hundreds of millions of dollars to develop and test new antibiotics—there are new targets to identify, refinements to add, and clinical trials to run. But unlike other desperately needed drugs, new antibiotics are designed not to be used until absolutely necessary, and even then as sparingly as possible, significantly slowing the return on investment. This has led many pharmaceutical companies to deprioritize such research.

Because approved drugs don't have to revisit that lengthy process, advances in the world of antibiotics have recently centered around improving existing compounds—a strategy in which Campbell's ability to pinpoint a new receptor or other molecular subtlety can mean the difference between life and death. But this is just one piece of the puzzle.

When scientists talk about new antibiotics, they mean something truly novel: a compound that looks like nothing present-day bacteria have seen before. That is exactly what Sean F. Brady, Rockefeller's *Evnin Professor* and head of the Laboratory of Genetically Encoded Small Molecules, hopes to mine from dirt.

Brady began this work in graduate school, where he coaxed fungi into producing the small molecules that serve as the basis for most pharmaceuticals. Now, he is trying to convince soil bacteria to manufacture the very antibiotics that could be their undoing.

It's not an entirely original idea. Streptomycin was derived from soil bacteria, and today most antibiotics on the market are similarly taken directly from

Photography by Kholood Eid

Researchers in Brady's lab extract DNA found in dirt microbes sourced from locations around the globe.

microbes or synthesized in the lab to resemble natural bacterial products. Bacteria have been in a mutual arms race for eons, evolving ways to kill off microscopic neighbors that encroach on their territory. There is nothing better at killing bacteria than other bacteria, and the heyday of antibiotic drug discovery more or less revolved around scientists collecting disparate samples from near and far, growing *Streptomyces* or *Bacilli* in the lab, and bottling their secrets.

Today scientists know that bacteria store the genes that produce their chemical weapons in biosynthetic gene clusters—families of about 40 genes that encode natural antibiotics—and that most of these clusters cannot be expressed in the lab. "There's all this hidden information in gene clusters that we never see," Brady says, "books we can't read that would tell us how to make new antibiotics from scratch."

His lab focuses on unlocking these mysterious gene clusters in hopes of spawning an entirely new cache of drugs that bacteria have not yet learned to resist. One of Brady's methods involves using detailed bioinformatic analyses to tease apart the genetic instructions within a DNA sequence in order to predict the structure of any antibiotic-like compounds that a bacterium with those sequences might produce. Synthetic chemists can then take that data and synthesize a new drug candidate.

"We felt the field had gotten to the point where informatics tools were powerful enough to predict a molecule from a gene cluster," says Brady, who refers to the technique as bioinformatic prospecting. "We were right, and that has led to a growing number of antibiotics being produced in a biology-independent way. Computers predict it, and a synthetic chemist produces what the computers predicted."

Several synthetic antibiotics produced by Brady in this manner are now in preclinical development, including cilagicin, which the lab discovered in 2022. Brady and his team fed the relevant bacterial DNA sequences they had identified into an algorithm and synthetically produced the compound, which is effective against several drug-resistant bacteria in mice. Because cilagicin seems to disable two molecules key to keeping bacterial cell walls together, it may prove more potent than existing antibiotics. Resistant bacteria can adapt to life without one of those two molecules but have not yet learned to cobble together a cell wall when both molecules are offline.

One day, though, they probably will. But with enough alternatives on hand, countering resistance could come down to a numbers game. For instance, if we were to place penicillin on the back burner for 30 years while a drug like cilagicin took center stage, penicillin resistance might naturally fade away. And with a sufficient number of options to cycle through, humanity may be able to keep bacteria on their toes indefinitely.

The world's largest collection of strep bacteria sits in Vincent A. Fischetti's lab. Fischetti runs Rockefeller's oldest infectious disease lab, where an ongoing battle against strep, or *Streptococcus pyogenes*, has been waged for nearly 100 years. To control this pathogen and other instances of antibiotic resistance, Fischetti's Laboratory of Bacterial Pathogenesis and Immunology focuses on developing alternative treatments like lysins, enzymes produced by bacteriophages, viruses that infect bacteria. Once phages reproduce inside a cell, they use lysins to break down the bacterial cell wall and release their progeny.

The idea of leveraging whole phages rather than just their lysins for medicinal purposes has been around for a while. At about the same time that Fleming was puzzling over penicillin, other scientists were exploring the possibility of therapies that pitted phages against germs. It was an intriguing premise that lost

Sean F. Brady

With enough alternatives on hand, countering resistance could come down to a numbers game.

Kevin Moti, a research assistant in Fischetti's lab, uses an anaerobic chamber to study bacteria sensitive to oxygen.

momentum due to bad timing. Once antibiotics proved so stunningly successful, Fischetti says, "whole-phage therapy was largely scrapped."

The rise of antimicrobial resistance has changed that. There is now an entire center dedicated to phage applications at the University of California, San Diego, and the broader movement has seen some tangible success: Currently a boutique treatment with cocktails customized for individual patients, phage therapy has proven itself effective against multidrug-resistant bacteria on a limited basis.

Fischetti notes that phage therapy has been difficult to scale and that bacteria become resistant to phages very rapidly—even more rapidly than to antibiotics—because just as phages evolved to target bacteria, bacteria have evolved to evade phages.

The lysin therapy approach he uses, by contrast, can be scaled like current antibiotics. More importantly, no resistance has been seen for lysins during the 20 years of their development.

Initially, Fischetti used lysins exclusively as a laboratory tool. He purified the enzymes for his Ph.D. thesis and used them to extract material from *Streptococcus* bacteria. Then in 2001, as the extent of the threat posed by antibiotic resistance began to emerge, Fischetti gave lysins to mice infected with strep. When the mice were tested shortly thereafter, "Lo and behold," he says, "the *Streptococci* were gone!" His experiment became the first in vivo application of phage lysins.

Almost immediately, Fischetti received grants from the Department of Defense to develop lysins against anthrax—a significant national security concern at the time—and other major resistant pathogens. "In those days, every paper we submitted on the topic was quickly published because therapeutic lysins were a new anti-infective," Fischetti says.

Many laboratories subsequently began pursuing lysins as novel therapeutics, but

Lysin therapy may one day prove to be one of the most durable approaches to killing bacteria.

Vincent A. Fischetti

Fischetti was already a step ahead. One lysin that can fight off a particularly dangerous drug-resistant strain of *Staphylococcus aureus* was licensed and quickly moved to human trials. It's the first and only antibiotic alternative to have successfully completed Phase II clinical trials, and a Phase III trial is now pending. Fischetti thinks that we could see lysins in the clinic within the next five years.

If so, lysin therapy may prove to be one of the most durable approaches to killing bacteria. "We can't say it will never occur, but we have yet to see resistance to lysins," Fischetti says. That's because the way phages use the enzymes self-selects for success. Phages that produce ineffective lysins never get the chance to pass that defect down to their future generations. Because lysin resistance is so difficult to achieve, lysins could be used to prevent secondary infections. That's something that antibiotics—as the cause of secondary infections—can't.

The economic toll of a post-antibiotic age

Unchecked, antimicrobial resistance (AMR) will have a devastating impact on human health—and on the global economy. The costs of treating sicker patients, coupled with factors like lost productivity and significantly less efficient farming, illustrate how AMR quickly adds up to a public health threat with a uniquely high price tag.

$4.6 BILLION

National cost per year to treat infections caused by six multidrug-resistant pathogens.

$1.5 BILLION

Cost of developing an antibiotic in the United States. The average revenue per year for a new antibiotic is estimated at $46 million, one reason why so few are in the pipeline.

$210 TRILLION

Projected loss in global GDP due to AMR by 2050. The U.S. alone already takes a $35 billion hit each year as AMR causes an aging population to work fewer years and die younger—and is projected to lose an additional $28 billion in the next decade due to lost productivity related to AMR.

11%

Projected loss in livestock production by 2050 due to AMR infections. Farms lean heavily on antibiotics—70 percent of the volume of antibiotics in the U.S. is sold for agricultural uses. As these drugs become less effective and livestock sicken more easily, prices of milk, eggs, and meat will skyrocket.

Illustration by Dan Matutina

Kathryn Eckartt, a graduate fellow in Rock's lab, is making predictions about how resistance will develop in the future.

Jeremy M. Rock

In any face-off, the ability to predict your opponent's next move confers an invaluable advantage. "What if we could predict resistance in the clinic before we brought an antibiotic to market and solve that problem before it gets to the patient?" Brady asks. Each year, the WHO leads a massive international effort to forecast flu strains months out so that effective vaccines can be engineered ahead of the winter season. Is there likewise a way to head off antibiotic resistance at the pass by both better managing how we use existing antibiotics and better understanding how bacteria foil them?

Brady's most recent work focuses on teasing out the underlying causes of resistance and predicting how quickly and under what circumstances bacteria will become resistant. One day, that kind of information could translate into a ranking system that helps clinicians keep resistance at bay by allowing them to choose the right antibiotic for each circumstance.

But long-term planning and international coordination are key to any successful strategy for combating resistance. As Brady notes, focusing solely on today's strains in one part of the world doesn't adequately address the problem; without heading off developing resistance around the globe, scientists can only kick the can down the road.

Consider TB, which generally doesn't get the same attention (or funding) as resistant staph, strep, and other widespread drug-resistant bacteria "because it doesn't kill Americans and Europeans nearly as often as it kills people in sub-Saharan Africa and Southeast Asia," Campbell says. But microbes know no borders, as the pandemic so dramatically illustrated, so addressing multidrug resistance in only one part of the world will ultimately benefit no one. And it turns out that the knowledge generated by studying TB is broadly transferable to other infectious diseases.

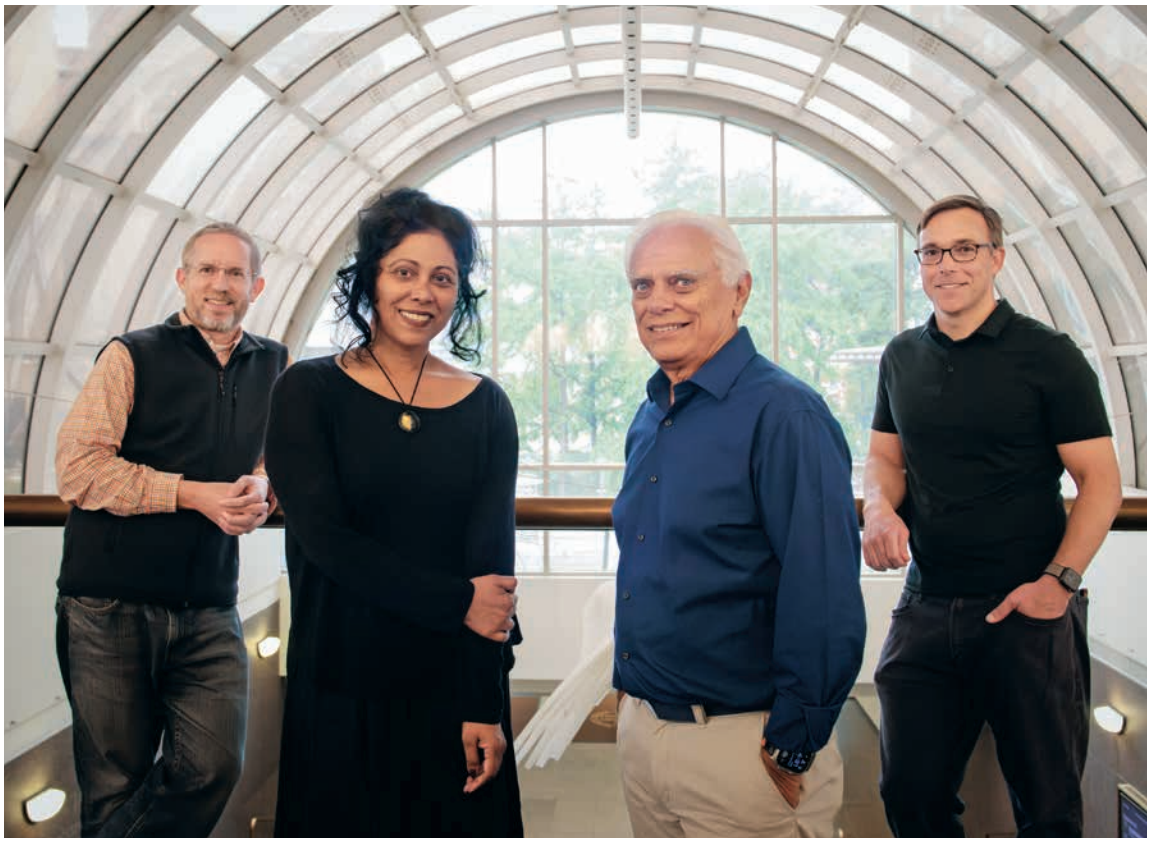
Much of Campbell's work, for instance, centers on unpacking the activities of the TB bacterium's RNA polymerase (RNAP), the enzyme that creates RNA from a DNA template. But go-to antibiotics invariably target bacterial RNAP in general, meaning Campbell's efforts shed a broader light on how a range of resistant bacteria dodge frontline antibiotics.

"There's a lot of unsolved fundamental biology here," says Jeremy M. Rock, the *Penrhyn E. Cook Assistant Professor*, about the mechanisms that breed antibiotic resistance.

Rock, who heads the Laboratory of Host-Pathogen Biology, is trying to solve those biological mysteries with a large-scale method that lets him knock down almost every gene in *Mycobacterium tuberculosis* and observe whether that makes the bacterium more or less vulnerable to any given compound. Rock's lab has used this platform, which employs the revolutionary gene-editing technology known as CRISPR, to discover genes that make TB more sensitive to certain drugs. "You have to wonder," Rock says, "whether we could use this to rationally design combination therapies for drug-resistant bacteria that would be more potent than expected."

What Rock and his colleagues are learning from TB proves the point. Rock and his team have identified 1,373 genes that, when silenced, render the bacteria vulnerable to antibiotics, and another 775 genes that make them more resistant. They found that silencing two genes, *mtrA*

Brady, Campbell, Fischetti, and Rock are pursuing complementary solutions to the antimicrobial-resistance problem.



COVID was a stark reminder of the need to stay ahead of infectious diseases.

and *mtrB*, left even resistant bacteria vulnerable to existing therapies and that mutations in a third gene, *bacA*, may be key to promoting multidrug resistance. They also discovered genes that could allow clinicians to safely use linezolid, an antibiotic that works against drug-resistant TB but only at dangerously high doses. When these genes are inhibited, Rock found that linezolid is effective at far lower—and safer—concentration.

In one of their most promising discoveries thus far, Rock's team found that a strain responsible for half a million tuberculosis cases each year in Southeast Asia appears to have long ago acquired a mutation that renders the bacteria highly vulnerable to the well-tolerated, FDA-approved family of antibiotics known as macrolides. These drugs have not traditionally been used to treat TB, and deploying them against the disease could be a game changer, especially in the developing world. "Macrolides are cheap, generic, and safe," Rock says.

COVID CAST A spotlight on how vulnerable we still are to infectious diseases and reminded both those who practice science and those who fund it of the importance of keeping ahead of them. It also illustrated how scientific mountains can be moved when the right resources are focused in the right way.

"What we're learning in the lab has so much lifesaving potential," Brady says of the work that he and his colleagues are doing to understand, overcome, and circumvent antibiotic resistance. "But this knowledge has to translate beyond the lab so that we can have alternatives ready to go before we need them."

With each potential solution—creating novel antibiotics, improving existing ones, or developing entirely new classes of antimicrobial weapons—these scientists' work is informed by a shared sense of urgency.

Or as Brady puts it, "You don't build a fire truck *after* the fire starts." ◎

At the center of it all

BY MINDY FARABEE

Researchers on a quest to understand how memory functions may have unexpectedly discovered something about the brain's organizing system. Can what they're learning teach us something about the elusive nature of the self?

BUDDHISTS WOULD TELL US THAT THE SELF is an illusion, and neuroscientists would tend to agree: Where we posit a coherent, integrated individual, they see 180 brain regions all constantly chattering over one another. But if personhood is essentially a fiction, memory may be the thing that fools us into thinking it's real. No other cognitive process feels so intimately bound up in our experience of ourselves. Memory underpins the basis of personality, our relationship to the world, and how we make decisions about the future.

Illustration by Veronyka Jelinek


me
ONCE
A DISTANT SUMMER
SMILE

"If we could identify how different types of memory interact, could we identify larger, unifying principles that underpin the entire system?"

But what is this thing upon which our entire notion of continuity rests? How does a moment in time—a unique combination of emotion, sensory experience, and cognitive churning—crystallize in the brain? Where does it go after that, only to reemerge, either on cue or seemingly out of nowhere, decades later? What mechanisms determine which moments we hold on to and which we let go of and allow to be lost to history? What, in the end, holds memory together?

"We've known for a long time where memories are initially formed and stored—in the hippocampus," says neuroscientist Priya Rajasethupathy. "What we don't understand is what happens afterward: how they gradually reorganize across the brain, how they evolve over time so that some are stabilized while others are forgotten. And for those memories that we keep, we don't know what triggers recall."

Rajasethupathy, who heads the Laboratory of Neural Dynamics and Cognition, could have based a career on tackling any one of those questions. Instead, she reframed the whole question.

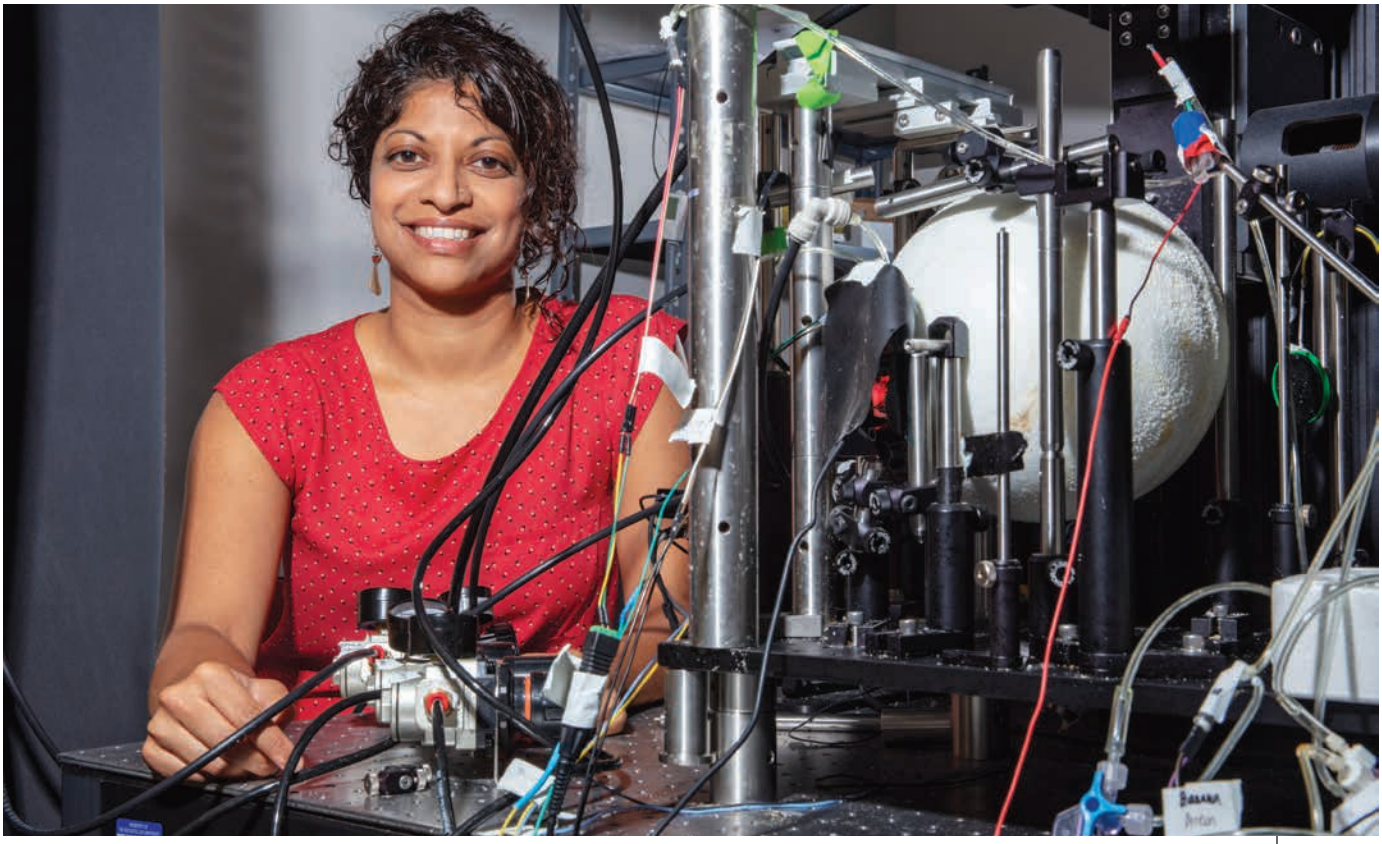
"What if instead of focusing in on disparate links in the chain of memory, we zoom out and ask ourselves, How precisely does a memory change and evolve over time?" she says. "If we could identify mechanisms by which working memory and short- and long-term memory interact, could we identify larger, unifying principles that underpin the entire system?"

It's heady work, and Rajasethupathy, Rockefeller's *Jonathan M. Nelson Family Associate Professor,* knew going into it that she would be sorting through the intricacies of genes and molecules and neural circuits. She also knew that she would be spending a lot of time devising new ways to probe memory's usual suspects—the hippocampus, of course, but also different areas of the cortex now known to play a significant role. But things have taken an intriguing turn. Lately, almost every project in her lab has unexpectedly directed her toward a small, egg-shaped structure situated in the center of your noggin: the thalamus.

Historically, Rajasethupathy says, cognitive neuroscientists didn't focus too much on the thalamus, because it was thought to simply relay sensation from our environment to the brain. "We didn't think it was necessarily involved in complex cognitive functions," she says.

But according to a spate of new studies from her lab, this inconspicuous node has a pivotal role to play at almost every stage, from the moment a memory begins to form to the slow and gradual process of stabilizing it for the duration. These experiments are also raising tantalizing questions about what else scientists might have missed in misunderstanding the thalamus: Might this hub within an endless web of neurons be holding things together in some way—functioning not only as a sort of command center for memory, but potentially also as another kind of linchpin, one that could begin to explain how that unified "me" inexplicably emerges?

Memory is a complicated process further complicated by its multiple dimensions. There's the relational type (like remembering that the library is across from the grocery store), but also the procedural kind (like riding a bike), and then the sensory variety (such as the smell of spring rain). It's a list of birthdays, a timeline of World War II battles, and the ability to follow through on a promise



Rajasethupathy and her team create multisensory labyrinths using novel VR setups.

made. It's knowing the meaning of all the words in this sentence. And when memory fails, that can be either a nuisance easily compensated for by Google or a fundamental unraveling.

Our experiences of forgetting vary in part because each category of memory touches on a different region of the brain with its own nuanced mechanisms for representing the dimension it governs. Years ago, neuroscientists overturned long-held assumptions in the field of memory, most significantly the idea that it functions like a static filing cabinet—a specific site in the brain where past experiences are tucked away. Everyone now knows that memory isn't a pin on a map but a dynamic process driven by recurrent signaling and rewiring, that's widely distributed throughout the brain, including across the prefrontal cortex (PFC), where both our most fleeting and longest-lasting impressions pop up, and the entorhinal cortex, a relatively thick band of gray matter near the back of your ears that's active in initial processing. Unpacking this intricate dance could help reverse the ravages of dementia and other neurodegenerative diseases. It may also open up therapeutic avenues for intractable mental illnesses like schizophrenia that include debilitating memory deficits.

But in pursuit of such a knotty subject, scientists have caught only partial glimpses. In the 1970s, pioneering neuroscientist Patricia Goldman-Rakic and her colleagues first captured evidence that working memory—those few seconds to a minute in which information is actively held and used (say, as we respond to a friend's text)—resided in the PFC. They found the evidence within a numerical anomaly. Neurons are lightning fast, firing on average at millisecond intervals. But when Goldman-Rakic and her team gave a working memory task to a rhesus monkey, they recorded a specific group of cells in the PFC firing continuously for many seconds—a long haul in the world of neurons—as their subjects actively recalled information.

These observations were groundbreaking. But many decades later, it's still not clear how these particular neurons in the PFC stay active for so long and what unique properties allow them to do so.

Three years ago, Rajasethupathy's lab discovered a key piece in this puzzle. The researchers used a traditional approach to tax a mouse's working memory: Put it in a maze, show it a treat, relocate the animal for a brief interval, then release it and track how quickly

Photography by Matthew Septimus

The experiment led them right to the thalamus, a region mostly ignored in the study of cognition.

it finds its way back to the reward. But the scientists added their own twist on this classic setup: Don't form a hypothesis.

Typically, researchers looking for the gene that drives a certain behavior will create a list of possible candidates, then test each one using genetically altered animals. Instead, in a process as laborious as it was ingenious, Rajasethupathy's lab obtained hundreds of genetically diverse mice representative of variations found in nature. They let these diverse mice run through the maze, segregated out the high performers, mapped their genomes, and identified one gene expressed at elevated levels in those brains.

That gene produces a receptor known as GPR12, and mice who excelled at working memory possessed more than twice the number of these receptors than did low performers. Moreover, when the forgetful comrades had their GPR12 levels artificially increased, their accuracy suddenly nearly doubled.

"It's rare to find a single gene with such a large effect on a complex cognitive process," says Alessandra Bonito-Oliva, a senior research associate in Rajasethupathy's lab who is developing therapeutic approaches to target GPR12. Things got even more interesting when she and her colleagues mapped GPR12's distribution in the mouse brain. "This receptor has one prominent function: to bring two regions into sync with each other to amplify and sustain brain activity during working memory," she says. "And it functions predominantly in just one part of the brain."

To everyone's surprise, that place turned out to be the thalamus (in particular, its middle portion), a region mostly ignored in the study of cognitive functions such as working memory.

It proved to be the beginning of an exhilarating run of experiments.

We love the thalamus here," says James Brandt, a fourth-year Ph.D. student in Rajasethupathy's lab who has been working to unpack

In putting a unique twist on a classic maze experiment, the team discovered a key protein active in working memory.

the molecular characteristics of GPR12 and how it interacts with other proteins to synchronize the brain during working memory. (Ultimately, the lab wants to connect the dots between how GPR12 functions in single cells of the thalamus, how that sustains long-range coordinated brain activity between the thalamus and frontal cortex, and how that maintains working memories as animals go about their various activities.) "After our initial findings," Brandt notes, "Priya was able to conceptualize all kinds of ways the thalamus could be involved in memory."

The Greek physician-philosopher Galen gave the thalamus its name in the second century CE, using the Greek word for a private storehouse or living quarters because he believed it to be a hollow vessel for channeling the "vital spirit" that governed our vision. Experiments done in the 18th century introduced the idea of the thalamus as a sensory relay station; by the 1980s, scientists knew its connections extended to the amygdala, the hippocampus, and the entire cerebral cortex. They subsequently learned that the thalamus wasn't actually one thing, but more of a neuronal sausage packed with different clusters related to sensory information, physical movement, and arousal states. In the 2000s, researchers discovered its role in regulating sleep-wake cycles and that it also steers our directed attention in any given moment.

Meanwhile, the cortex was stealing all the cognitive thunder—particularly the prefrontal cortex, where executive function was officially mapped in the early 1970s. Up to that point, much of what scientists knew about the human brain had been learned as a consequence of nonfatal injuries, which are more likely to occur in the peripheral, or cortical, regions. In one notorious industrial accident, the 19th-century railroad engineer Phineas Gage received a spike to the frontal lobe; the resulting personality and cognitive shifts helped give birth to the idea of a "control mechanism" residing in the PFC.

Letting mice run wild

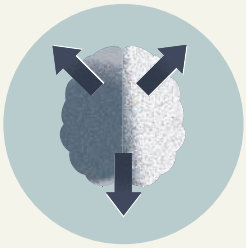
Most lab mice are bred to be mutants, with one or more of their genes engineered out of existence. This allows scientists to pinpoint the functions of individual genes in a clearcut way—by asking if a given gene promotes tumor growth, for example, or whether it makes a mouse hungrier.

But memory is a complex brain function often requiring that many genes work in concert. That makes it difficult to ferret out those that are most important.

So Rajasethupathy's team takes an approach that lets them learn from natural variations in behavior. Just as people can have good memory, or bad memory, or everything in between, so can mice. Her lab has thus launched new lines of inquiry studying genetically diverse mice (DO for short). Their method first determines how memory performance varies among their mice and then links those differences to genetic variations within the group. That makes it possible to identify, in an unbiased way, the most important genes, and particularly those that can improve memory. It's an approach nearly unique among neuroscientists, and it's paid dividends.

When you don't know what you don't know

Like us, mice in the wild are genetically unique individuals with different abilities, preferences, and temperaments. Capturing the commonalities when such diverse creatures perform a task leads the researchers down paths they may never have been able to predict—and to stronger confidence in their findings. "Tracking where in the brain all these variations converge gives us the power to say, these are the cells and the circuits most central to this cognitive task," Rajasethupathy says.



Genes-to-behavior

Historically, neuroscience has kept a useful divide, with researchers mostly working either on the molecular level or with circuits and systems—and with lab animals genetically tailored for either focus. But Rajasethupathy's lab wants to connect all the dots, and that requires a holistic picture of brain activity. Using DO mice, researchers can trace every step in a system, from molecular interactions through neural networks to behavior.

The hidden gems

Sometimes, a gene's significance gets overlooked simply because no one's yet thought to study it (as with GPR12). Sometimes it's not that simple. In one project, Rajasethupathy's team uncovered that within a widely studied gene lurked a version (Homer1a) affected not just by its protein's prevalence in a particular brain region but also by the animal's developmental stage. "Because previous studies had knocked out the gene in the whole animal from birth, they had obscured this gene's prominent contribution to attentional processing," says lab member Zach Gershon.

Lab members Alessandra Bonito-Oliva and James Brandt are investigating therapeutic approaches to targeting GPR12.

With the development of MRI, the thalamus again got short shrift; it was considered too "noisy" to image effectively due to its proximity to the brain's fluid-filled ventricles. But there was an additional technical challenge stemming from its sausage-like quality. Higher-order brains still outcompute computers, so capturing and analyzing the broad swath of raw information needed to follow a thought as it moves around the brain are daunting feats of engineering. One strategy for dealing with those limitations is bulk resolution, in which all the outputs from neurons in a given region are bundled and averaged together. The technology needed to capture a more informative level of detail is largely lacking; Rajasethupathy, whose background is in biology and engineering, works with her group to invent it.

For instance, her lab was the first to generate 3D images at neuron-level resolution in three different brain regions simultaneously, something they accomplished even as the mouse moved about the maze. "That kind of detailed resolution provides so much more understanding in trying to make sense of a heterogeneous brain region such as the thalamus," says Brandt. "There are so many different kinds of neurons in there. If you bulk that data together, quiet motor neurons and firing dopamine neurons could just cancel each other out—your data looks like nothing is happening in a spot where, in fact, something really important and very specific is happening."

Big insights come from tiny treadmills. In one darkened corner of her sprawling lab, Rajasethupathy's team has built a very 21st-century miniature labyrinth, where mice navigate in VR environments to form and retrieve longer-term memories. Here, mice perched atop a Styrofoam ball enter an endless number of "rooms" as sights, sounds, and scents are combined around them in unique ways. Here is where Rajasethupathy's team learned something unexpected about what happens in the mysterious interval between when memories form and when they are tucked away for the duration.

As researchers came to understand the distributed nature of memory, the prefrontal cortex emerged as the warehouse—the long-term destination for short-term memories first formed in the hippocampus. But that's about as far as our knowledge went. How that happens, and how the brain decides which memories to archive, was largely uncharted territory. Attempts to explore further have

been limited by current constraints on imaging technology, which typically has the capability to record high levels of localized detail in short bursts. Long-term memories, meanwhile, take weeks to consolidate in rodents and months to years in humans.

By devising a method to record multiple brain areas in a behaving animal continuously for up to a month, Rajasethupathy and her team were the first to see that there is a distinct brain highway shuttling memories from short-term into longer-term storage. Unexpectedly, this circuit again involved the thalamus—this time its anterior portion—meaning that the thalamus is not just the primary link coordinating working memory. It's also holding things together for the long term: an intermediary acting as archivist in chief, stamping only the most salient events "Highly significant. Please send to the PFC."

How does your thalamus decide what's important to you? "The question of saliency is such a subjective question," says Bonito-Oliva, who worked on the experiment. "It fluctuates based on numerous factors, including your current physiological state, personal preferences, past experiences, or in-the-moment understanding of the importance of the information."

Repetition also plays a defining role in determining what you come to value: Is this the seventh time your classical music–loving father took you to the symphony or the 10th time your science-loving mother took you to the natural history museum? Add that to all the above and it's easier to see how, as memories accrue over a lifetime, the labile neurochemistry of saliency and the malleable structure of personality may be shaping each other.

The team discovered that for the brain, the process of determining saliency doesn't stop once a memory has been consolidated: It goes on and on, perhaps indefinitely. Using optogenetics, a technique that employs light to alternately inhibit and boost regional function, they determined that the anterior thalamus plays referee, continuously evaluating emotional quality and intensity over time to decide whether a given memory still hits the threshold.

"We validated what was driving this activity four different ways," says former lab member Josue Regalado, one of the experiment's codesigners. "By the time we got to the third, I was like, this is really something."

Regalado recalls the moment from his desk in the group's break room, where worn copies of 19th- and early 20th-century thinkers like Eccles, Russell, Whitehead, and Wittgenstein sit stacked on the shelves above. Descartes ("I think, therefore I am") he carries everywhere, tattooed on his left arm as an illustration of the philosopher's conception of mind-body dualism.

Rajasethupathy is known for creatively combining different fields in her work, and her wide-ranging interests are reflected in the staffing of her lab. Regalado is a bit of yin to Brandt and Bonito-Oliva's yang—while they are probing deep into the molecular heart of GPR12, he's pondering how the whole system wires itself together.

"One way to think of it is that your hippocampus learns very quickly, but your cortex learns very slowly," he says. "The thalamus may be the thing that's linking the two and teaching the cortex."

This much we now know: "The thalamus is not just capturing what's out there in the world in order to pass it along to the higher brain," Rajasethupathy says. "We can see it leveraging its own data collection and actively sculpting and routing information to higher-order brain areas. It can help curate what's really important to the organism. This is an evolving anatomical understanding."

Where this understanding may evolve next could resonate far beyond memory studies. Even in its simplest moments, it's a comic understatement to say that your brain is a complicated machine. In a single day, it outputs around 74 gigabytes of data, navigating through 180 internal regions, two hemispheres, and both the cortical and subcortical layers. Which begs a question that scientists cannot yet answer: How do all the various and sundry pulses and signals zooming around your head somehow add up to recognizable feelings, logical actions, and brilliant ideas instead of disintegrating into noise?

The answer may lie in some kind of organizing structures—mini–command centers that shuttle information around while simultaneously monitoring and interpreting it. For Rajasethupathy, the existence of such a system would help explain the lingering scientific mystery of coherence. "It makes sense that the thalamus could be actually orchestrating a lot of things," she says. "All of a sudden, the complexity seems manageable."

If that's true, then the thalamus, sitting right in the middle of it all, could be pointing the way there, as a structure both simple and complex, operating on short and long timescales, connected to everything—a conductor of sorts for the symphony of thought. ◎

Will science end obesity?

By Alexander Gelfand

First, it was a status symbol. Then, a flaw of character. Now we know it's a biologically complex disorder whose mechanisms are just beginning to emerge.

THE FIRST PHARMACOLOGICAL approach to weight loss was a total failure. Introduced in 1933, the metabolic stimulant DNP was pulled from the market in 1938 after it was shown to cause sudden death. Perhaps its toxicity shouldn't have been too surprising: DNP had previously been mass-produced by the French as an ingredient for WWI munitions.

DNP wasn't the last questionable weight-loss treatment, nor the first in a general sense. As ancient artworks attest, obesity has likely been around for as long as human culture itself. The word, which derives from the Latin *obesus*, meaning "having eaten until fat," was coined in the early 1600s by a British physician, who recommended bathing in warm natural springs. If not terribly effective, at least his prescription sounded pleasant. In ancient Greece, Hippocrates advised patients to ingest herbal emetics and cathartics, "eat only once a day, … and walk naked as long as possible."

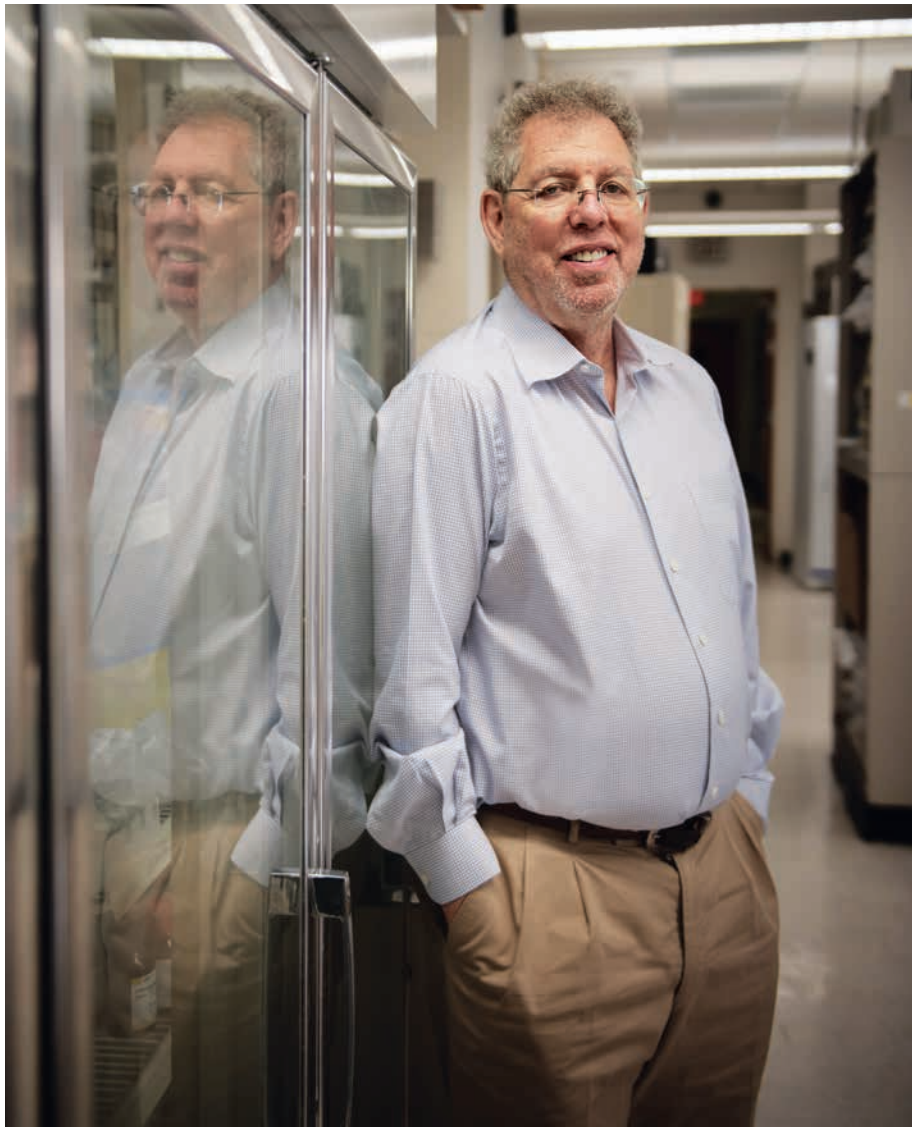
Like so many who followed, the Greek physician was trying to help. It has long been known that, beyond a certain point, carrying excess weight can become a serious health concern. And for just as long, the history of obesity treatment had been a litany of failures. Curiously, even once the era of modern science arrived and physicians began to understand the outsize role that genes and other biological factors play in human health, the overweight were still stubbornly admonished to just eat less or burn more calories.

"Unfortunately, that remedy is more a symptom of a common misconception and no more effective today than when Hippocrates proposed it more than 2,000 years ago," says Rockefeller neuroscientist Jeffrey M. Friedman, who in the 1990s essentially launched the field of molecular obesity research.

The simple act of eating is an extremely complex behavior: the execution of a fundamental biological need that's influenced by culture, demographics, and economics. Having once been so rare it served as a sign of wealth and status, obesity has become significantly more prevalent since the 1980s, especially in developed countries: Here in the United States, some 100 million people are now considered to be clinically obese. At the same time, however, an equivalent number of Americans remain lean. How could that be? Why—even in the presence of a similar environment—do some people pack on the pounds while others don't? And more importantly, why does excess weight lead to serious health complications in some people and not in others?

Science is beginning to find answers. Over the last four decades, Friedman and a network of researchers—some working together, others in parallel—have begun to unearth the genetic and molecular roots of obesity and eating-related behaviors, as well as the nature and function of fat and the crucial role played by various hormones in regulating appetite. Besides overturning conventional wisdom about the disorder, their work has led to the creation of a new class of blockbuster weight-loss drugs and the promise of an even wider array of safe, effective treatments to come (read about Svetlana Mosjov's groundbreaking contributions in "A drug's discovery," on page 41).

Illustration by Helen Musselwhite



Friedman's discovery of the hormone leptin launched molecular research on obesity.

Leptin, a key hormone

IN 1994, FRIEDMAN discovered leptin, a hormone secreted by fat cells that plays a crucial role in regulating appetite and body weight. Because animals need leptin to stay lean, he named the hormone after the Greek word for thin, *leptos*. As head of the Laboratory of Molecular Genetics and Rockefeller's *Marilyn M. Simpson Professor*, Friedman has since been tracing the neural circuits that govern food intake and leptin's role in that process, work that has ultimately shed light on the mechanisms driving overconsumption and obesity. "I got into this," Friedman says, "because it's not understood how behavioral decisions are made in the brain, or even where they are made."

To investigate such decisions, Christin Kosse, a postdoctoral fellow working in Friedman's laboratory, initiated a series of experiments designed to define the nature of the neuronal circuit that triggers feeding-related activity, like biting, in response to sensory stimuli such as hunger or leptin. Kosse identified just such a group of neurons in the mouse hypothalamus, a brain region controlling feeding and other innate drives. Moreover, she devised a way of alternately activating and deactivating the cells in a mouse as it wandered freely about its cage. And that's when things got strange: As Kosse silenced the neurons, the mouse began to nibble on the metal lick spout of its water bottle. "It looked like something in its brain was directing it to eat the lick spout," she says.

Indeed, something was. Kosse and her teammates eventually found that by modulating the neurons in question, they could cause their furry subjects to chew on anything that happened to be in front of them, even a block of wood, aptly demonstrating how involuntary the supposedly voluntary act of eating can be. The neurons Kosse identified receive direct inputs from cells that are regulated by leptin and in turn send signals to key brain stem neurons that control chewing and other movements associated with food consumption.

New research is revealing just how involuntary the supposedly voluntary act of eating can be.

The simple feeding circuit Kosse discovered is composed of only three neurons, and its architecture resembles that of an involuntary reflex. Friedman was astonished by the finding, not because he didn't think that such primitive, reflex-like feeding-related circuits existed (he suspects that many do), but because of the technical challenges involved in mapping one. "What's shocking to me is that we were able to directly connect inputs to outputs," he says. Kosse and Friedman believe that simple circuits centered in more primitive brain regions such as the brain stem and hypothalamus evolved early in vertebrate evolution and that their activity is in turn

Photography by Kholood Eid

modulated by cortical circuits in mammals that process complex sensory information.

Drawing on the work of two Nobel laureates, the pioneering neurophysiologist Sir Charles Sherrington and the ethologist Nikolaas Tinbergen, Friedman has come to think that feeding and other innate behaviors are controlled by a large ensemble of simple reflex-like circuits. While these circuits are subject to a degree of top-down control, including our conscious efforts to curb our appetites, they are exceedingly hard to resist in the long run. Friedman gravitates to an analogy presented by Sherrington—the urge to cough. A cough is controlled by a simple reflex arc, and it can be stifled for a while but probably not forever, especially if the stimulus (an irritant in the lung) is strong.

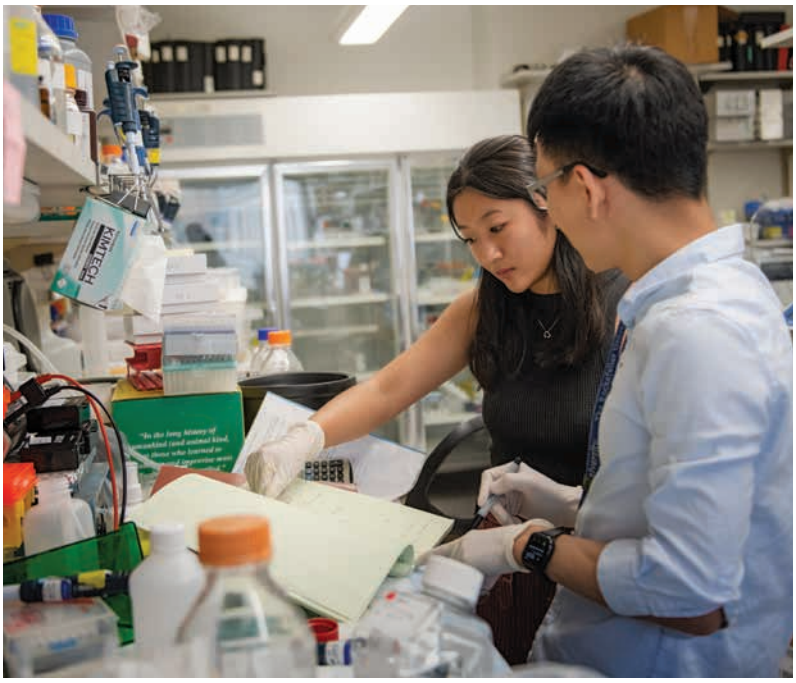
The circuit Kosse uncovered is one of the first instances in which a complete circuit connecting inputs to outputs in a mammal has been uncovered. But it is only one in a long chain of discoveries by Friedman and his associates that have defined the biological basis of obesity. Among other things, the discovery of leptin changed the way scientists thought about fat. For example, it allowed Stephen O'Rahilly, a researcher at Cambridge University who found the first genetic causes of obesity in humans, to establish that the congenital inability to produce leptin also resulted in severe obesity in humans. What's more, O'Rahilly cured his patients nearly instantly by injecting them with the missing hormone.

The leptin levels in your blood regulate a neural system that balances the number of calories you consume against the number that you burn over the course of weeks, months, and years. This long-term system for regulating body weight keeps your fat reserves within a narrow range that is largely dictated by your genetic makeup. As you gain fat, you produce more leptin; the hormone signals to the hypothalamus to decrease appetite and increase energy use, causing you to lose weight. Conversely, when you lose fat, you produce less leptin, causing your appetite to increase and your energy expenditure to dip. This is why obese people who shed weight struggle mightily to keep it off: Because their leptin levels fall after weight loss, their bodies are constantly fighting to regain those lost pounds.

Kristina Hedbacker, a research associate in Friedman's lab, is studying leptin's metabolic effects.

Studies have shown that body fat mass is the most heritable trait after height, and scientists have discovered dozens of genes that help regulate body weight as part of the overall system governed by leptin. Mutations in one or more of these single genes cause obesity in humans and in aggregate account for as many as 10–15 percent of cases of morbid obesity (defined as a body mass index, or BMI, greater than 40). Among the remaining population, many other genetic variants are likely to influence whether an individual in the general population is heavy or lean. This, coupled with environmental factors, explains why obesity rates are rising. As high-calorie diets and sedentary lifestyles become increasingly common, more and more people who are genetically predisposed toward obesity end up consuming or retaining the calories necessary to become obese.

People with defective leptin genes, like the ones that O'Rahilly first identified, don't make the hormone at all. As a consequence, they are ravenously hungry all the time and become morbidly obese even as infants. In fact, some of the children O'Rahilly treated were so heavy that they needed a wheelchair. Giving them leptin, he says, produced nothing short of a miracle, as they shed their excess weight and rose from their confinement.



Summer student Samantha Le with Han Tan, a research associate in Friedman's lab.

Science versus stigma

The new generation of weight-loss drugs target specific neurons in the brain stem and hypothalamus that process information conveying feelings of fullness, which can be experienced as satiety or, when extreme, nausea. But even before these new drugs took off, the scientific and medical communities were waking up to a new understanding of obesity. Last year, the American Society of Pediatrics (ASP) called for a new and aggressive approach to curbing the condition in childhood using drugs like Wegovy along with more invasive treatments like bariatric surgery.

This move toward medicalizing obesity at such a young age came as a shock to many, but it was based on alarming evidence. Numerous studies show that obesity has profoundly negative effects on people's emotional and psychological well-being, often due to the societal stigma they experience (in some cases, these emotional challenges can have as deleterious an impact on health as the weight itself). It can also be devastating to physical health. The nearly 42 percent of American adults who are obese—defined as a BMI of 30 or above (BMI remains the clinical standard, though recently some researchers have challenged its validity). They are also more susceptible to ailments including cardiovascular disease, stroke, diabetes, and sleep apnea. Moreover, obesity changes the body structurally, upping the number of fat cells, so that losing weight becomes increasingly difficult.

For children, obesity locks in a lifetime of problems. But for the ASP, the most concerning evidence had to do with the limits of diet and exercise: Study after study has shown that while people can temporarily shed pounds by eating less and moving more, they almost invariably regain them over time, presumably due to the tight grip exerted by leptin over fat mass and metabolism.

By establishing that obesity is seldom remediated by lifestyle choices alone, Friedman's research offers a revolutionary alternative to fighting it. His findings prove that food intake and metabolism are tightly regulated by a physiological system, reframing excessive weight as a problem of biology rather than willpower, of genes and molecules rather than gluttony and sloth.

And yet the myth of willpower endures, even among health care professionals. "Some of my colleagues in the medical profession tell me, 'Well, I do still think people should just show more discipline and get themselves together,'" says Matthias Tschöp, a German researcher who discovered a new category of diabetes and weight-loss drugs that combines multiple therapeutic agents in a single medication. These included the approved diabetes and weight-loss drug tirzepatide (sold under the brand names Mounjaro and Zepbound) and other novel drugs that promise to be even more effective than the first breakthrough medications based on the drug semaglutide (sold under the brand names Ozempic, Wegovy, and Rybelsus).

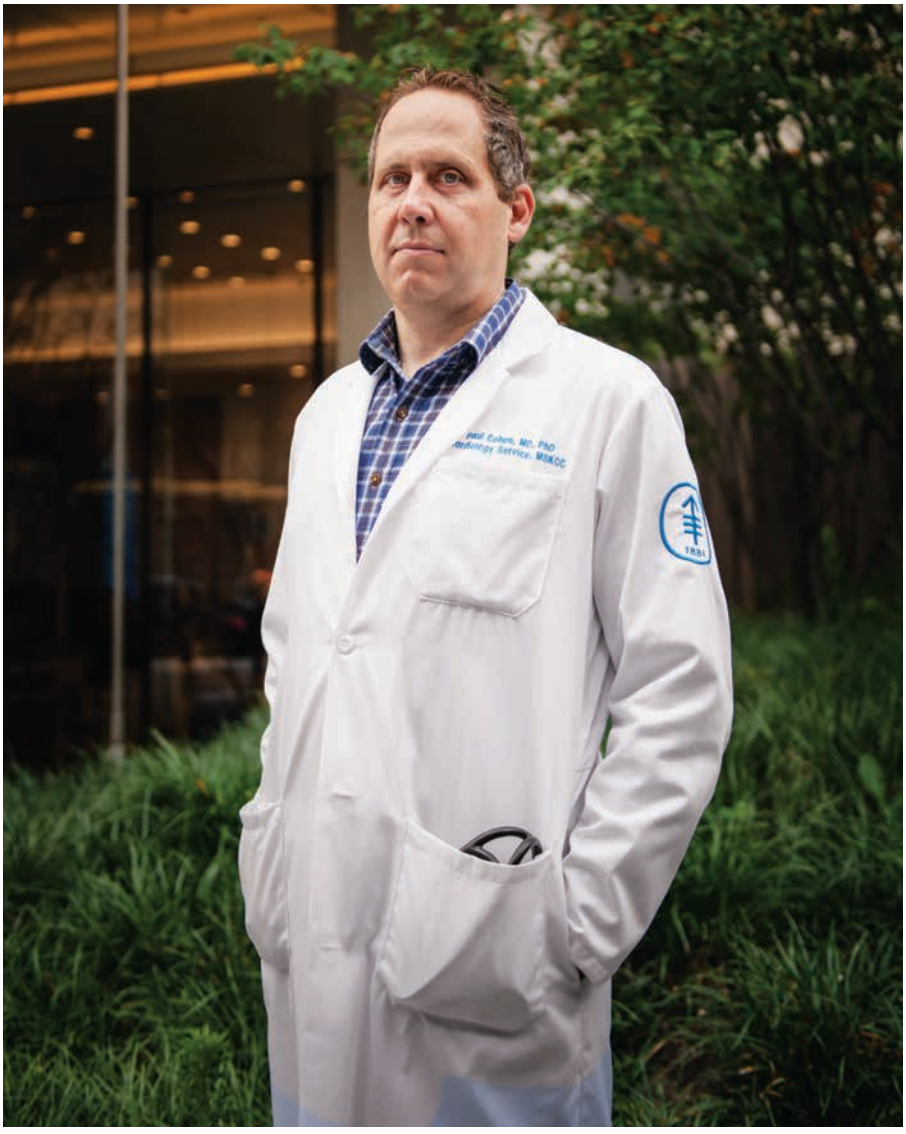
Friedman and his colleagues remain undeterred. By tracing the purely biological pathways that regulate appetite and body weight, they continue to chip away at the idea that obesity can be countered simply by making the right lifestyle choices. Their work not only has opened up fresh avenues to treating and preventing obesity but also has led researchers to rethink the nature of fat itself.

The good grams

Part of what makes obesity such a complicated disease is that fat isn't just one thing. In fact, it isn't even the one thing it was thought to be.

"Many people didn't accept that fat is a tissue in the same way that the lung or the heart or the liver is a tissue; they just thought of it as a simple bag of cells," says Paul Cohen, head of Rockefeller's Weslie R. and William H. Janeway Laboratory of Molecular Metabolism and a former student of Friedman's. By demonstrating that white fat—scientifically known as adipose tissue and the main storage depot for calories—wasn't just a sack for storing excess calories but rather a dynamic organ that sent hormones through the blood into the brain, Friedman inspired others to wonder what else it might do.

Cohen is one of them. As a cardiologist who regularly sees patients at Memorial Sloan Kettering Cancer Center (MSK), he is all too aware that cardiovascular disease



Cohen's research is informed by his clinical practice.

is a leading cause of death and that obesity is a major risk factor for it. More broadly, obesity is a component of metabolic syndrome, a cluster of conditions that greatly increase the risk of diabetes, stroke, and heart disease. "If you take care of patients," says Cohen, who is also Rockefeller's *Albert Resnick, M.D. Associate Professor*, "you see how common excess body weight and obesity are and how many different health outcomes they influence in patients."

Precisely how excess fat influences metabolic and cardiovascular health on a cellular and molecular level remains something of a mystery, however. To solve it, Cohen investigates the fundamental biology of adipose tissue itself.

His clinical work both fuels and informs his research. Some of the patients Cohen sees at MSK, for example, are adult survivors of childhood cancer—a group that tends to develop metabolic disorders such as hypertension and high cholesterol relatively early (i.e., in their 20s and 30s, as opposed to their 50s and 60s) and to die at unusually high rates from cardiovascular disease even when they aren't obese. Cohen and colleagues at MSK wondered if radiation therapy early in life might have caused these health problems by damaging the patients' fat tissue, and a study they conducted at The Rockefeller University Hospital revealed that childhood cancer survivors who had been treated with radiation displayed an inflammatory gene signature in their adipose tissue, indicating that their fat cells had in fact been permanently injured.

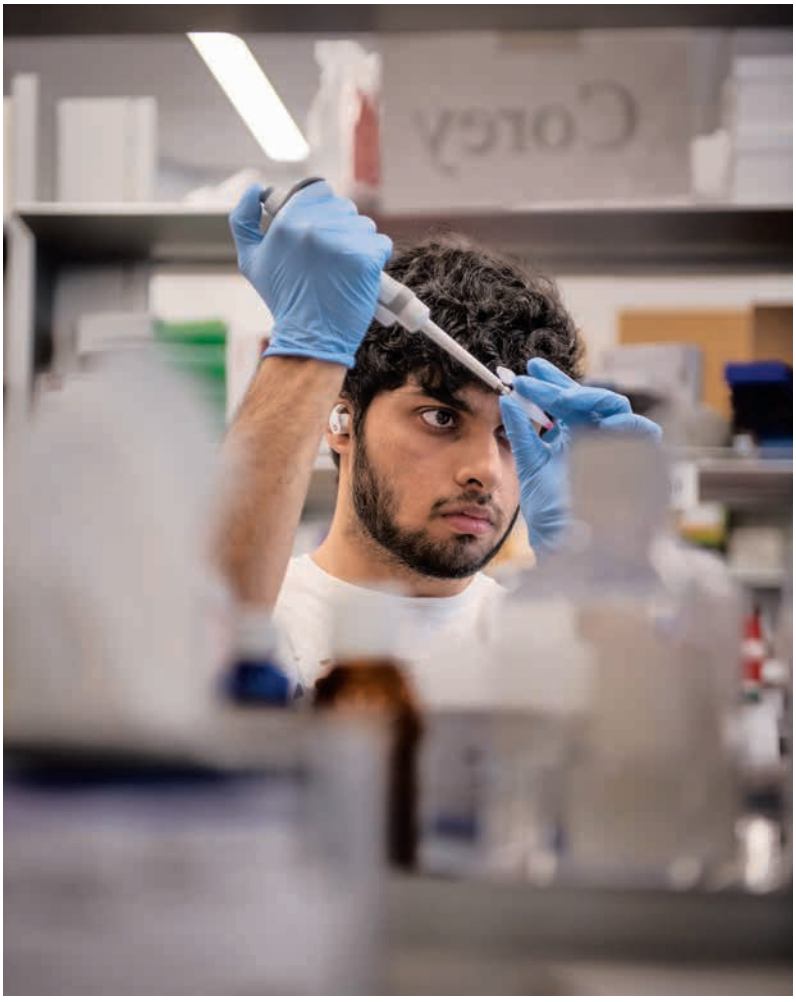
> "Many people didn't accept that fat is a tissue. They just thought of it as a simple bag of cells."

Cohen's work as a clinician has also led to groundbreaking work on a special form of adipose tissue known as brown fat.

Unlike the white fat that tends to pool around our bellies and thighs, brown fat collects in far smaller amounts above the diaphragm (some people have modest deposits, while others have almost none). Moreover, it does not produce leptin. Brown fat has other important functions, however: When the temperature plummets, it burns energy to generate heat. And as Cohen demonstrated in a landmark 2021 study, people with more brown fat activity are at lower risk of developing the various illnesses associated with obesity.

That work also emerged directly from Cohen's clinical experience. More than a decade ago, physicians discovered that brown fat shows up on a particular kind of PET scan that is routinely used to detect cancerous tumors. Soon after he began seeing patients at MSK in 2016, Cohen realized that the hospital must perform thousands of such scans every year. Better yet, he learned that radiologists there routinely recorded the presence of brown fat deposits.

Cohen and his colleagues analyzed over 140,000 PET scans from more than 50,000 patients, linking the presence or absence of brown fat in their bodies to all of the other



Cohen's team investigates the interplay between various metabolic disorders.

information in their electronic health records. Patients with detectable brown fat deposits were at lower risk of everything from high cholesterol to heart disease and type 2 diabetes. Somehow, brown fat protected them from a whole host of metabolic and cardiovascular ills. And the more obese they were, the more it helped.

Cohen and his MSK partners are now sifting through the same trove of patient data to determine if particular genetic variants might account for how much brown fat a person has and to see if particular medications can influence brown fat activity. What they learn could eventually help boost the protective effects of brown fat.

At the same time, he and his lab mates are trying to figure out exactly how brown fat confers its various benefits in hopes of replicating them.

Research by Mascha Koenen, a postdoctoral associate in the Cohen lab, suggests that brown fat may prevent the small arteries that regulate blood pressure from stiffening, a finding that could lead to novel therapies for hypertension—yet another constituent of metabolic syndrome and one that contributes to millions of deaths each year from heart disease, heart attack, and stroke. "You don't die from obesity; you die from the comorbidities," says Koenen. "So this protection from cardiovascular disease is super important."

Research associate Kaja Plucinska, meanwhile, is investigating whether brown fat works its metabolic magic by secreting its own unique repertoire of hormones. Toward that end, she is analyzing the blood of people who have been exposed to cold to see what molecules brown fat might be pumping into their bodies. Such molecules could be used to measure brown fat activity, predict a person's metabolic status, and treat diabetes and cardiovascular disease.

As part of a human study, Plucinska took blood from a small group of young, healthy individuals, had them wear so-called cold vests chilled with circulating cold water, and then took their blood again, screening both sets of samples for thousands of different substances. This past winter, she worked with researchers in Minnesota to collect blood samples from several hundred "ice dippers," who regularly hop into frozen lakes, and she plans to compare those samples with ones drawn over the summer to see if any potentially useful molecules stand out. "It's a fishing expedition," Plucinska says, "hopefully, one that leads to a good catch."

Through these and other projects, Cohen hopes to identify molecules that could be used to develop drugs that mimic the effects of brown fat. Such drugs could help protect anyone, regardless of how much weight they carry, from metabolic diseases that lead to illness and death.

Overcoming resistance

Friedman's early work encouraged other scientists to explore the molecular underpinnings of the body's weight-control system. Tschöp, for instance, recalls thinking that the discovery of leptin represented "the end of obesity." Inspired by Friedman's example, he eventually discovered that another hormone, ghrelin, acts as leptin's nemesis, driving hunger and food consumption. Now history appears to be proving his initial hunch right, albeit at its own measured pace.

As it turns out, only a tiny fraction of obese individuals do not manufacture leptin, while perhaps 10 percent don't produce quite enough and would benefit from receiving more. The other 90 percent, meanwhile, manufacture plenty of the hormone

but don't respond to it in the way that they should, a condition known as leptin resistance. This resistance renders leptin treatment ineffective for most obese people.

Nonetheless, the discovery of leptin and the astonishing effect it produced in a small number of genetic outliers offered scientists a powerful tool for tracing the biochemical, genetic, and neural pathways that regulate body weight in general, and established a road map for finding ways to prevent and treat obesity in the broader population.

Semaglutide and tirzepatide, for instance, circumvent leptin resistance by targeting a physiological system that regulates food intake over the short term. This system is controlled by hormones and neural signals originating in the gut, and instead of regulating body weight over months and years the way leptin does, it determines how much food you are likely to consume over the course of a single day. Semaglutide creates feelings of satiety by mimicking the gut hormone GLP-1, whose active form was first identified by Rockefeller scientist Svetlana Mojsov. Tirzepatide, meanwhile, mimics both GLP-1 and a second gut hormone called GIP.

Unlike earlier weight-loss drugs, which were ineffective at safe doses and unsafe at effective ones, these new therapies can safely help obese individuals lose enough weight (more than 15 percent of their body weight with semaglutide or more than 20 percent with tirzepatide) to significantly improve their health. They are not for everyone, however. The new drugs are very expensive and carry side effects (e.g., nausea, vomiting, and stomach pain) that some people cannot tolerate. Moreover, they must be injected rather than taken orally (although pharmaceutical companies are working on making them in pill form). And while they are widely effective at decreasing and even normalizing body weight, the genetic complexity of obesity virtually guarantees that they will not work for everybody. Nor, as Tschöp points out, do they actually cure anything: When the treatment stops, the weight comes back. As a result, the need for additional therapies remains acute.

A war on multiple fronts

CHRIS TAGGART

Since his original discovery, Friedman has been exploring two separate sets of questions. One focuses on intervening in the long-term system for regulating body weight at a point where leptin

A drug's discovery

It was the mid-1980s, and Svetlana Mojsov was trying to find new treatments for type 2 diabetes at Massachusetts General Hospital in Boston. Mojsov had already succeeded in synthesizing glucagon-like peptide 1 (GLP-1), a molecule with therapeutic potential, and experiments with rats had been encouraging. So she and her colleagues began clinical trials with humans, hoping to ramp up insulin levels and drive down blood sugar. The results were astonishing. "Every single patient responded," says Mojsov, noting that this almost never happens in clinical trials. "It was at that point I was sure it was going to be a drug."

Indeed, her work laid the foundation for the recent blockbuster treatments for diabetes and weight loss: an entirely new class of drugs based on GLP-1 agonists such as semaglutide and liraglutide, marketed under the names Ozempic, Wegovy, and Rybelsus.

Mojsov had come to Mass General from Rockefeller, where she had worked in the laboratory of R. Bruce Merrifield, a biochemist who won the 1984 Nobel Prize in Chemistry for developing a new way to synthesize protein fragments known as peptides. Her work with Merrifield had focused on glucagon, which scientists considered promising for diabetes treatment. At Mass General, Mojsov discovered that the gene from which glucagon is made also produces GLP-1, a related molecule. She suspected that GLP-1 might be a gut hormone regulating blood glucose levels—the very characteristics necessary to combat diabetes.

No one, however, had managed to determine the active structure of GLP-1 until Mojsov. In her experiments, she and her colleagues established that GLP-1 indeed reduced blood glucose by stimulating insulin production. It took another 20 years, but "when the first drug [Victoza] went on the market, I felt professionally and personally fulfilled," says Mojsov, who had by then returned to Rockefeller as a research associate professor.

Mojsov hadn't imagined that GLP-1 could also be used to treat obesity. Neither had anyone else; its slimming side effect didn't surface until the drug underwent testing as a potential diabetes treatment. But what is certain is that without Mojsov's pioneering work, these revolutionary drugs would not exist.

Research associate Kaja Plucinska is studying the molecules secreted by brown fat.

isn't directly involved, so that leptin resistance isn't an issue. The other involves reversing leptin resistance itself.

Several years ago, Friedman and his colleagues identified a group of neurons in the brain stem that, when activated, caused mice to lose weight. While these neurons are connected to the leptin circuitry in the brain, they are nonetheless capable of promoting weight loss even in leptin-resistant and leptin-deficient animals. In research recently posted on bioRxiv, Friedman and his team identified an oral drug that causes weight loss in obese, leptin-resistant mice by targeting these specific cells, a finding that could someday lead to an oral therapy for leptin-resistant human beings.

Furthermore, the neurons that caused Christin Kosse's mouse to gnaw on a metal lick spout can also regulate feeding behavior in the absence of leptin, suggesting they might potentially serve as targets for novel weight-loss treatments. In addition, there are likely to be additional populations of cells operating other basic feeding circuits that could be targeted in a similar manner by finding drugs that modulate their activity.

But the key objective has been to decipher the cause of leptin resistance. In recent studies, Friedman lab members Kristina Hedbacker, a research associate, and graduate fellow Bowen Tan found a previously unknown source of leptin resistance, as well as a way to overcome it. They initially set out to find biomarkers for leptin sensitivity. In the process, the pair discovered a distinct molecular signature in mice that were fed a high-fat diet known to cause obesity and leptin resistance. The molecules comprising that signature were associated with a specific biochemical pathway centered on a key signaling molecule known as mTOR. So Hedbacker and Tan decided to see if they could induce weight loss in their obese, leptin-resistant mice by giving them leptin in conjunction with rapamycin, an immunosuppressive drug that inhibits mTOR signaling. "By the third day of the experiment, we started to see that the animals were losing weight," Hedbacker says. "The fourth day, it still held up, and we were so excited that we were screaming."

Over the next 10 weeks, the obese, leptin-resistant mice that received leptin with rapamycin lost over 20 percent of their body weight and had their responses to exogenous leptin restored. Further investigation pinpointed the specific leptin target neurons and biochemical changes that had caused leptin resistance in the animals, and revealed how rapamycin reversed it.

The beginning of the end

THESE DISCOVERIES AND others are helping to unravel the neural and molecular factors that shape our feeding behaviors and prevent or promote obesity and the comorbidities that can result from it. They also point to a portfolio of future drugs tailored to patients with different genetic profiles.

Drugs based on gut hormones, like Wegovy and Zepbound, might be combined with leptin for even greater efficacy. People who lose weight with gut-hormone-based drugs might be given a leptin-resistance inhibitor to prevent them from regaining it, while people who can't tolerate such drugs might be given an entirely different medication that targets a specific feeding-related circuit. And anyone afflicted by the metabolic disorders associated with obesity, whether obese or not, might be prescribed drugs that mimic the effects of brown fat.

There is even reason to hope that as this future unfolds, the stigma associated with obesity will begin to wane, both at an individual level, by helping obese people achieve lasting weight loss, and at a societal one, by making it clear that obesity is not a sign of moral weakness but rather a biological condition amenable to medical intervention.

"There are lots of stigmatized disorders that over time have been destigmatized," Friedman says. "To some extent, they get destigmatized when there's a treatment."

While the end of obesity still lies at some remove, science may have finally brought the beginning of the end into sight.

INTERVIEW

It's time to rethink the societal cost of paywalls and hefty subscription fees that have long sustained academic journals.

Scientific publishing needs fixing. But how?

By Jen Pinkowski

In 2022, when the Biden administration announced that all scientific papers based on federally funded research must be made freely available to the public, many questioned how to roll out such a dramatic overhaul of scientific publishing in time for the new rules to take effect in 2026. But few questioned the need to rethink the industry.

The decision came after the the World Health Organization declared an "urgent need" underlined by the COVID pandemic to transition away from existing article paywalls. Advocates around the globe had been pushing for similar changes since the early 1990s.

For decades, this growing movement has been raising questions about the traditional business model sustaining scientific journals. While paid subscriptions have long kept the lights on at publishing houses, those high fees come at the cost of scientists in low-resource settings, patients, physicians, and the general public, who are all too often priced out of accessing vital information—even if their tax dollars made the

Vosshall (left) serves on the board of the preprint server bioRxiv.

research possible in the first place. At the same time, it's hard to ignore how the economics of publishing can shape the practice of science itself: Lacking the financial resources to participate in the system, researchers unable to place their findings in prestigious journals get caught in a vicious circle, less able to gain the necessary visibility needed to win large grants, attract big donors, and reach colleagues and potential collaborators. Yet, even as the scope of these problems have become clearer, no simple solutions have emerged.

How might new policies coming out of the White House impact scientists around the world and, equally important, the public? Will overturning old business models lead to new challenges? And what might a future look like in which scientific publishing is truly open, transparent, accessible, and sustainable?

We explored these questions in a roundtable conversation with three advocates of change: Leslie B. Vosshall, Susan King, and Nicolas Vabret, all of whom are looking for ways to drive a more open exchange of scientific findings.

Vosshall is vice president and chief scientific officer at the Howard Hughes Medical Institute (HHMI), where she directs HHMI's wide-ranging portfolio of biomedical research programs; she is also Rockefeller's *Robin Chemers Neustein Professor* and head of the Laboratory of Neurogenetics and Behavior, researching the disease-spreading *Aedes aegypti* mosquito. King is executive director of Rockefeller University Press, where she oversees a small but influential quartet of peer-reviewed publications: *Journal of Cell Biology*, *Journal of Experimental Medicine*, *Journal of General Physiology*, and *Life Science Alliance*. And Vabret, an immunologist at the Icahn School of Medicine at Mount Sinai, is a founder of a group called the Preprint Club that reviews papers on the bioRxiv server.

Scientists have been talking for decades about establishing more open systems for academic publishing. How has this conversation evolved?

LBV: It goes back to the birth of the internet, which was incredibly disruptive for traditional print journals. They had long controlled the sharing of new data, charging people both to publish papers and to read them. Suddenly, that monopoly came under threat when almost anyone could make a PDF and share it for free. As all sorts of information became available online, many people realized that science is often a closed world that most don't have access to. Instead, they would encounter paper after paper locked behind a journal's paywall.

NV: It's a system that never made sense: The public makes huge investments in science, while publishers, not citizens, reap the rewards.

SK: For years, many journals have been experimenting with open-access publishing, making either some or all articles available for free. Then came preprint servers like bioRxiv, giving scientists the opportunity to shortcut the peer-review process and share new research findings more quickly.

Today, we have a mixed bag of publishing models—traditional closed journals; hybrids with a mix of open and closed papers; and fully open-access journals, where authors typically pay a fee to publish—and preprints. The pandemic showed us just how critical immediate access to new information can be—for scientists as well as for policymakers, health-care providers, educators, and the rest of us.

Can you talk more about how the pandemic changed the publishing landscape?

NV: Almost overnight, preprint servers became the breaking-news repositories for COVID research. As scientists around the world scrambled to respond to the emergency, preprints served a great function, enabling us to share raw findings online for free so that colleagues could immediately access and review our data.

Additionally, many journals lifted their paywalls around COVID papers. This served as a proof of principle of sorts: Previously, many publishers had argued that the open-access model would be too hard to implement. The pandemic showed us it was possible.

Photography by Chris Taggart

Vabret (left) co-founded an international group of peer reviewers for preprint manuscripts. King has led the Rockefeller University Press since 2015.

LBV: In fact, COVID wasn't the first such lesson. Back in 2016 when Zika became a public health threat, journals like *Cell* said they'd publish research on the virus within two weeks. That's when we realized that it really shouldn't take three years to publish new findings, as often happens under normal circumstances.

New White House regulations will largely end journals' ability to embargo articles based on federally funded research for up to a year after publication. How big an impact do you expect that to have?

SK: These paywalls have essentially meant many publications were out of reach for those without the means to subscribe or who didn't have access to a university library.

I see the new policy as a catalyst for wide change, and it didn't come as a big surprise for the industry. Like many other academic publishers, we at Rockefeller University Press had been expecting it for quite some time. The groundwork was laid in 2013 when the Obama administration issued a memorandum to all federal agencies to develop plans to increase public access to federally funded research.

"The pandemic showed us just how critical access to new scientific information can be—to scientists and the rest of us."

We've been transitioning to open access for some time now. All articles published by Rockefeller University Press are openly available to all no later than six months after publication.

LBV: It will immediately have a global impact in terms of what data becomes available. The United States' National Institutes of Health (NIH) absolutely dwarfs other health funders around the world, with an annual budget of $45 billion. Eighty-five percent directly funds research, which may take place in any number of countries. So, in effect, the policy dismantles the traditional publishing system—and that system is really a strange arrangement when you think about it. Embargoing new discoveries is like saying, "I'll sell you this loaf of bread for $10,000, or you can wait 12 months and get it for free when it's stale."

If you have a child with a rare disease, you'll now have immediate access to the latest research on that disorder free of charge. If you're a clinician at a rural hospital in a low-resource country, you'll be able to keep up with any clinical research funded by the U.S. government—knowledge that may

King (left) says nearly 40 percent of the papers published in Rockefeller University Press journals last year were immediately open.

have previously been out of reach because your hospital couldn't afford the subscription fees.

What role do you see for preprint servers going forward?

SK: Scientists have found utility in preprints, so it's hard to imagine them going away. They won't necessarily replace traditional publishing—many papers posted on a preprint server will eventually wind up in a journal. But they offset some of the delays of the current system by providing immediate and free access to new findings.

Interestingly, not all scientists want to post preprints. Many who publish in our journals, for example, choose not to do it. Anecdotally, I think some see a risk of being scooped—that a rival research group will take their idea and run with it, and potentially beat them to getting a paper accepted for publication in a peer-reviewed journal.

A concern often expressed about preprints is that bad research is easy to publish and more easily disseminated.

NV: That's certainly important to consider, but in my experience, preprints have the opposite effect: When everyone can see everything, that actually shines a light on bad research. Errors or poorly done experiments become easier to spot and easier to call out. At the same time, the preprint system can end up shining a light on good research.

Something to keep in mind about the current system, in which journals conduct peer review behind closed doors, is that a lot of information gets lost. When a manuscript undergoes revision, new data may be obtained and added, existing data points may be taken out, or whole experiments may be omitted—and only the authors, reviewers, and editor get to see that back-and-forth.

With preprints, it's a very different situation: Experts in a field work together in full transparency to ensure quality and accuracy. My lab and others found this model to be especially helpful during the pandemic, when postings on bioRxiv were skyrocketing.

Like many of my immunology colleagues at Mount Sinai, I was working from home during the lockdown and wanted to contribute my expertise. So, we started what became known as the Preprint Club, screening and highlighting bioRxiv manuscripts of note. A group at the University of Oxford was doing something similar, so we began collaborating with them and were soon joined by labs at the University of Toronto and Karolinska Institutet, in Sweden.

Do you think preprints offer additional benefits beyond open access? Do they push the whole industry toward more transparency?

LBV: That's right, and some journals are in fact trying out this "everyone sees everything" approach as well. For example, the nonprofit journal *eLife* has a new model that gives authors a choice in how they respond to feedback from reviewers. They can go back and revise the manuscript, or they can simply say, "Thank you for your opinion," and publish the original version along with the reviewers' comments and questions. It's very exciting.

SK: Readers have been able to check a preprint post against the version-of-record article, but we've also been inspired to experiment with the visibility of reviewer reports. In two of our journals, authors have the option to publish all formal correspondence for their accepted manuscript, including editorial decision letters, peer reviewer

> "Embargoing discoveries is like saying, I'll sell you this loaf of bread for $10,000, or you can wait 12 months and get it free when it's stale."

comments to the authors, and the authors' responses. We provide this correspondence as a supplementary file online with the published article. The idea of this initiative is that the correspondence will provide further context to readers and also serve as an educational tool for authors and reviewers alike.

Let's talk about the economics of the new federal policy. What happens when journals can no longer charge for access?

NV: It's the big question that everyone's asking: Who's going to pay?

The policy doesn't address the loss of revenue that journals face when they can no longer use paywalls. They typically compensate by raising article processing charges, or APCs, that scientists and institutions have to pay to publish with open access—and APCs are a huge problem in and of themselves. They vary from journal to journal, and it's crazy how different they are.

LBV: For instance, every time you publish an open-access paper in a Springer Nature journal, it costs around $11,000. For *one* paper. Elsevier charges similarly, around $10,000 per paper, while several nonprofit publishers charge as little as $2,000. It begs the question of whether these expensive for-profit journals are setting a fair price for the services they provide.

SK: This is a great question that the industry is grappling with: How do we maintain sustainability while ensuring that we are maintaining equity and not placing the burden on the authors? Institutions, publishers, and funders are having these conversations now to determine the best way forward. But while this is happening, our policy remains that the inability to pay does not affect the publication of an author's manuscript if they are experiencing financial hardship or complications with requirements by their funders.

NV: How, for instance, is the NIH going to handle surging APCs? It's an urgent problem to solve because federal money that's now being funneled to publishers could instead be used to fund more research.

One idea is that the NIH could cap the part of a research grant that's earmarked for publishing expenses. A more radical solution would be for the agency to start a publishing service of its own—it has the scientific networks and the expertise to make it happen.

The new mandate applies only to government-sponsored research, which generates about half of the papers published by U.S. scientists. Do you foresee a future in which all scientific publishing becomes widely accessible, both here and in other countries?

SK: It will largely depend on how different governments approach open-access mandates, and the portion of a journal's articles that are published in regions with such policies. Rockefeller University Press journals are already in the process of becoming fully open. It's a complicated transition, but we're making progress. About 36 percent of the articles published this year in our portfolio are now immediately open, and it's growing fast. We expect it will be 70 percent by 2026 with funder and institutional support. I can only speak for smaller operations like ours—journals that come from research institutions or academic societies—but I'm fairly certain we all have the same mission as scientists: to disseminate research as widely as we can.

LBV: HHMI has had an open-access requirement since January 1, 2022. All HHMI lab heads must publish their work on an immediate open-access basis under a CC BY license. They all sign a rights-retention agreement that they submit to journals along with their paper. It says that if the journal ultimately publishes work funded by us, it must be immediately accessible to all.

SK: HHMI is in good company. It's part of Plan S, an international open-access initiative that also includes the WHO and other research-funding organizations around the world. They're on a more ambitious timeline than the White House, aiming to make open access a reality by 2025.

LBV: My dream for the future of scientific publishing is that it will be a marketplace driven by us—the creators of science. ◎

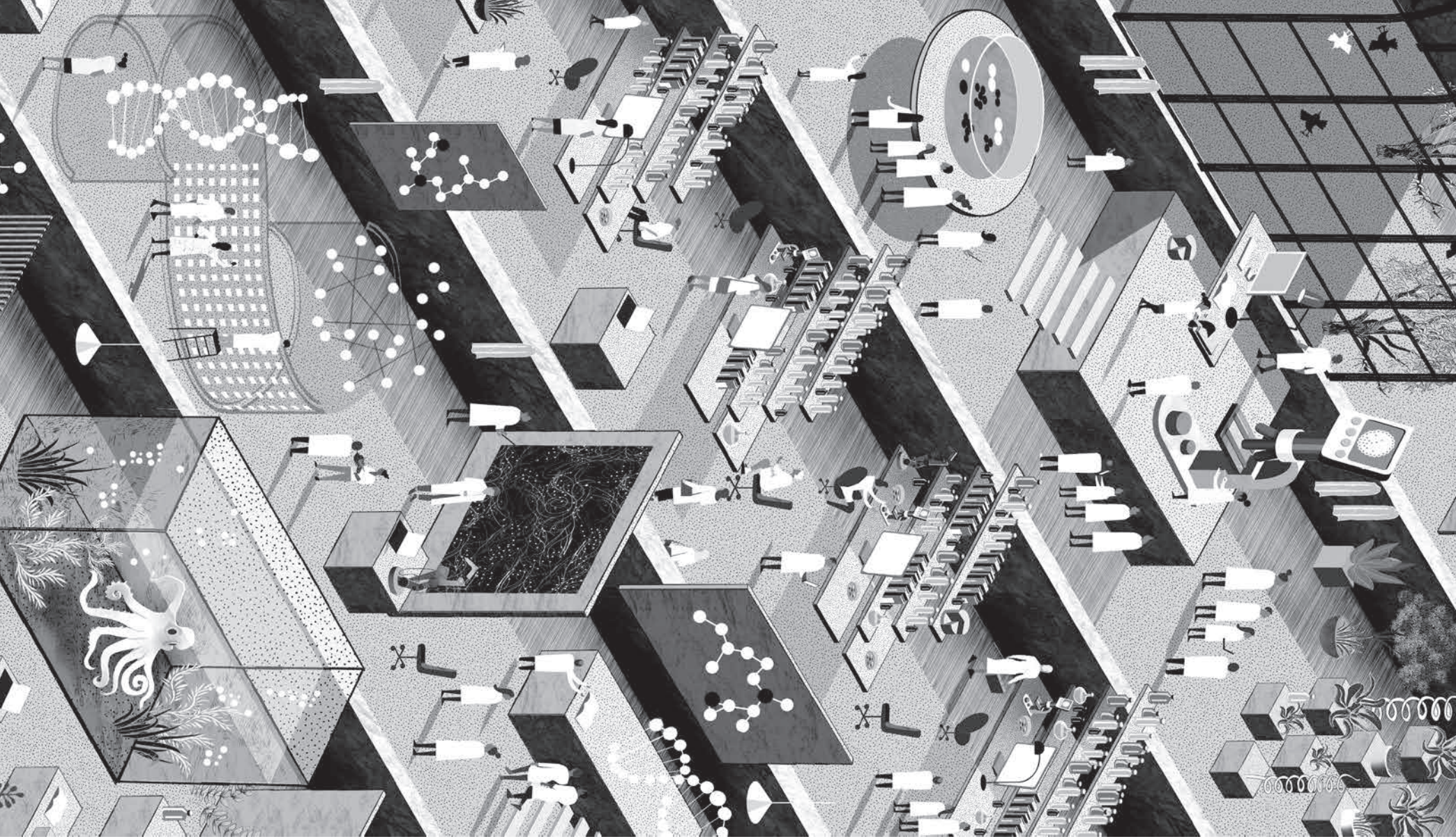
The octopus examination room

OCTOPUSES HAVE SECRETS. In the wild, they're homebodies who spend much of their time curled up inside a seashell, a coconut, or a rocky lair. In the lab, this poses a problem for neuroscientists like Marcelo O. Magnasco, who seeks a clearer picture of how octopuses perceive and interact with their environment.

These camouflaging, shape-shifting, sucker-spotted invertebrates are amazingly intelligent, with neural systems both markedly different from and strangely similar to ours, making them a unique model for studying how any brain engages with the world. Yet because an octopus's behavior is largely hidden from view, the neural processes that drive it remain a mystery—a black box in our understanding of how the animal's nine brains cooperate, and how its cognitive processes ultimately translate into sophisticated problem-solving skills and mischievous personalities.

Ironically, Magnasco's solution is a literal black box, composed of the same black plastic once found in old-school TV remotes. Each of his lab's six cephalopods has its own roomy tank filled with plants, stones, and toys. When a box is introduced, the octopus quickly adopts it as a cozy den. The cube is opaque to visible light but transparent to the infrared camera trained on it 24/7. This allows the inhabitant to feel unobserved while the researchers engage in "a gross invasion of privacy," Magnasco jokes, enabling their exploration of the connection between learning and sleep—and even whether octopuses have nightmares. (Read more about cephalopod dreams in "Nightmare scenarios," on page 11.)

Photograph by Matthew Septimus



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