ISSUE
08
SPRING
2022

Seek

THE ROCKEFELLER UNIVERSITY

Endless possibilities

Stem cells help our bodies form, develop, and heal—and they're powering a new surge in science.

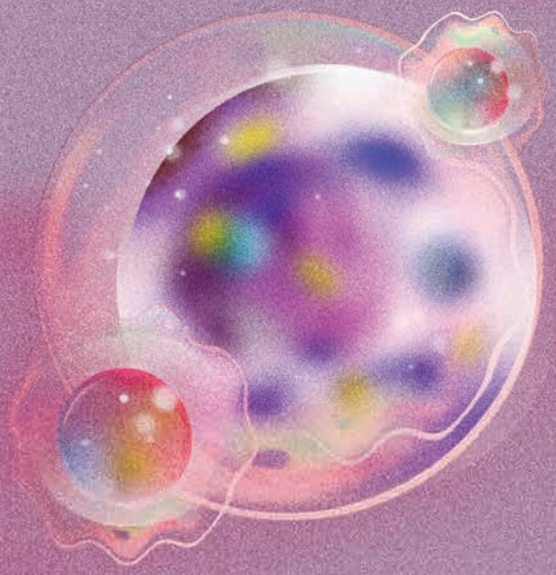
ALSO

How we started speaking

Instrumental instruments

Funding and fairness

“Claims were made about stem cells that would clearly not be easy to fulfill.”



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Less hyped, still sensational

Twenty years ago, stem cell science was the talk of the town, supposedly poised to yield lab-grown kidneys, hearts, and livers for organ replacement. But it mostly developed in other directions—and while the headlines may have faded, the promise of stem cells remains bright as ever.

Illustration by Mark Pernice

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How can it be that our strongest competitors in vocal learning are, of all things, a bunch of feathery dinosaur descendents with brains no bigger than a grape?

Photograph by Matthew Septimus

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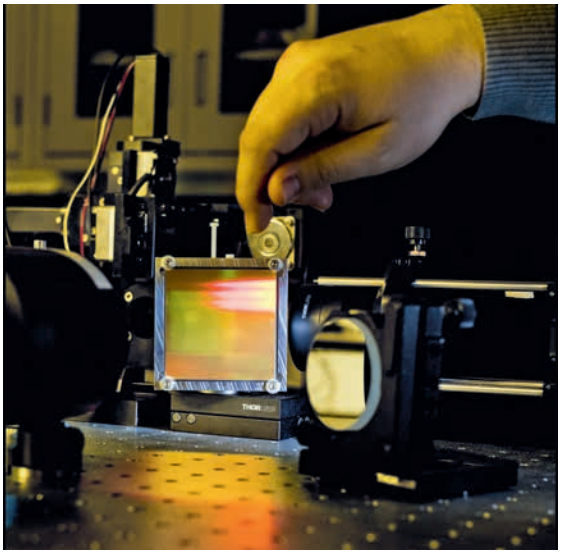
What if the tool needed to move science forward doesn't yet exist? Here are gadgets and techniques born from improvisation that made impossible experiments possible.



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"If you wanted to ridicule someone's argument, you would dismiss it as just another grandmother neuron."

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Kaleidoscopic colors. For two decades, Rockefeller's Pearl Meister Greengard Prize has been shining a light on the accomplishments of extraordinary women scientists. Now, light shines through a translucent display of the winners, installed recently in the Abby Aldrich Rockefeller Lounge. Passersby can experience a play of shifting colors as the iridescent panels catch the light at different angles—an effect inspired by natural phenomena like butterfly wings and oyster shells. So far, 23 panels have been etched with past winners' portraits, and blank ones stand ready to celebrate future awardees.

PHOTO BY C&G PARTNERS, DESIGNERS

Reported by Lori Chertoff, Bahar Gholipour, Eva Kiesler, Joshua Krisch, and Zachary Veilleux.

FOREFRONT

RISK FACTORS

The brain on quarantine

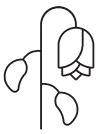
LONELINESS IS TOXIC. Not only can it make you feel sad or unfulfilled, but it has been linked to various health issues, from increased blood pressure to depression, cognitive decline, and cancer. The year 2020 was a case in point: Under shelter-in-place orders, Americans tended to be more anxious and depressed, had shoddier sleep quality, and, according to some estimates, put on more than half a pound per person every 10 days—a recipe for medical problems.

On the other hand, scientists have found that when people experience a sense of belonging—in a romantic partner, a pet, or at the local quilting club—they tend to live longer. So, what changes in the brain when we're cut off from society?

Recently, scientists went looking for answers in a somewhat unexpected model, the seemingly primitive *Drosophila melanogaster.* Despite its tiny brain, the fruit fly is in fact a gregarious little creature with a highly complex social life. It forages, feeds, and explores its environment in the company of peers. It engages in elaborate mating rituals that, like human wedding traditions, are passed down through generations by social learning. And according to the new research, it suffers under lockdown.

Wanhe Li, a research associate in the lab of Michael W. Young, let some flies abide on their own while congregating others in groups of various sizes. Seven days into the

Illustration by Katherine Lam



DATA

In a recent study, 36 percent of Americans reported feeling lonely "frequently" or "most of the time." Young adults aged 18–25 and mothers with young children were among the most affected.

experiment, the solitary flies were sleeping less and eating more, just like isolated *Homo sapiens*. The control flies carried on sleeping and eating normally, however, as long as they had one or more companions.

"It may well be that our little flies are mimicking the behaviors of humans living under pandemic conditions," says Young, who is Rockefeller's *Richard and Jeanne Fisher Professor* and a 2017 Nobel laureate.

The scientists identified a small group of neurons that might be driving a fly's loneliness response. When they shut down these neurons in genetically manipulated flies, the animals maintained normal sleep and feeding patterns, even after a week in exile. The findings, published in *Nature*, might inform research into the biology of social isolation in mammals, which is currently a black box.

"When we have no road map, the fruit fly becomes our road map," Li says. ◎

EYE OF SCIENCE / SCIENCE SOURCE

LOCOMOTION

How petite pedestrians evolved

TARDIGRADES: YOU CAN freeze them or burn them. You can shoot them out of a gun at 18,000 miles per hour and even expose them to the cold vacuum of space. These dumpy micro-animals, also known as water bears, will shake it off and live to plod another day. Water bears are virtually indestructible, but that isn't the most remarkable thing about them if you ask Jasmine Nirody, a visiting fellow in Rockefeller's Center for Studies in Physics and Biology, whose fascination with the hardy tardigrade stems from the way it gets from point A to point B.

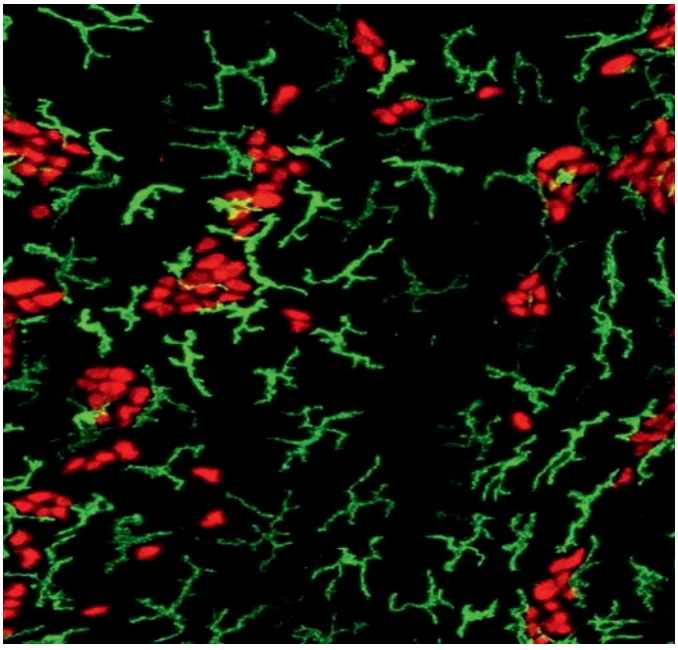
"No other animal of that size can walk like a tardigrade," Nirody says.

At less than a millimeter in length, one would expect tardigrades to wiggle or thrash about, like similarly appointed roundworms or insect larvae. Instead, the tardigrade trudges through soil and water upon stubby legs, in the ponderous, bear-like gait that earned it its nickname.

"The similarities between this locomotive strategy and that of much larger arthropods like beetles and scorpions have very interesting evolutionary implications," says Nirody, whose research appeared in PNAS last year. Among her most impactful discoveries is that water bears, like scurrying insects, can switch from a leisurely stroll to a mad dash by simply increasing the speed of a single stepping pattern. This transition is different from that of a horse raring into a gallop, for example, because it doesn't involve swapping one movement pattern for another. And the findings could mean that a wide range of panarthropod species, from insects to water bears, share a common ancestor.

But it is also possible, Nirody notes, that the soft tardigrade lacks ancestral links to insects and other hard-shelled creatures. It may have evolved its little legs independently. That would suggest that a water bear's tread and an insect's scuttle are practical solutions when it comes to navigating unpredictable terrain with a small body—and that such solutions are repeatedly favored by natural selection. ◎

The tardigrade walks on eight stubby legs, with a gait resembling that of insects 500,000 times its size.



STOMACHACHE

Intestinal memories

THE GASTROINTESTINAL TRACT boasts the largest cache of neurons outside of the brain itself—acting like a "second brain" that, among other things, controls the body's flow of nutrients and waste. A mighty army of T cells and macrophages protects these neurons, defending them from stomach bugs and other stressors.

Daniel Mucida, who heads the Laboratory of Mucosal Immunology, has shown that these immune cells learn to rally around GI neurons exposed to foodborne diseases. His lab recently demonstrated that mice suffer neuronal assault mainly the first time they are infected with salmonella or certain parasites; with a second infection, their gut neurons remain unscathed.

"We're describing a sort of innate memory that persists after the primary infection is gone," Mucida says about the findings, published in *Cell*. He suspects that some GI conditions may occur when the body fails to develop this tolerance, leaving its second brain out on a limb. ◎

DATA

100M

Number of neurons in the human small intestine. The brain has at least 800 times as many.

RECOGNITION

The butt of a joke, redeemed

THE THEORY OF the grandmother neuron—a single brain cell purportedly responsible for remembering specific faces, like that of your grandmother—was dismissed just a few years after it was first put forth, in the 1960s. Decades later, when Winrich Freiwald trained as a neuroscientist, people would mostly refer to it in jest.

"If you wanted to ridicule someone's argument, you would dismiss it as just another grandmother neuron, a hypothetical that could not exist," Freiwald says.

But the central question that had prompted the grandmother neuron hypothesis endured: What happens in the brain when we spot a familiar face, or any recognizable object for that matter? According to modern neuroscience, neurons collaborate rather than act on their own. They don't operate like buttons on a control panel—no single neuron can produce a complex brain function all by itself. If anything, neurons are more like piano keys whose coordinated activities create endlessly intricate harmonies.

Facial recognition apparently works the same way. Some neurons process visual face data, for example, while others are tasked

Where are the neurons that ensure we never forget a familiar face?

Illustration by Keith Negley

with storing such information. So imagine Freiwald's surprise when his team recently discovered—well, not the quintessential grandmother neuron, but what he believes might be the closest thing to it.

In findings published in *Science*, the researchers used functional magnetic resonance imaging to monitor neural activity in subjects viewing a selection of photos, including portraits of individuals the subjects had previously encountered in the flesh and those they had seen only virtually, on a screen. An interesting pattern emerged within a small face-recognition area in the brain's temporal pole region.

"The neurons responded three times as strongly to faces that the subjects had seen in real life," says Sofia Landi, a former graduate student in Freiwald's lab now doing postdoctoral work at the University of Washington. This could mean that our brains react differently when we see people we've gotten to know on Zoom, Landi says, compared to those with whom we've had real-life encounters.

Moreover, the experiments showed that the temporal pole neurons are unlike any other cells known to be involved in face recognition. They simultaneously behave both like sensory cells and memory cells and are hence able to connect our visual perception of a face with our remembrance of it. In that sense, the cells seem to play a role similar to that once ascribed to the legendary grandmother neuron.

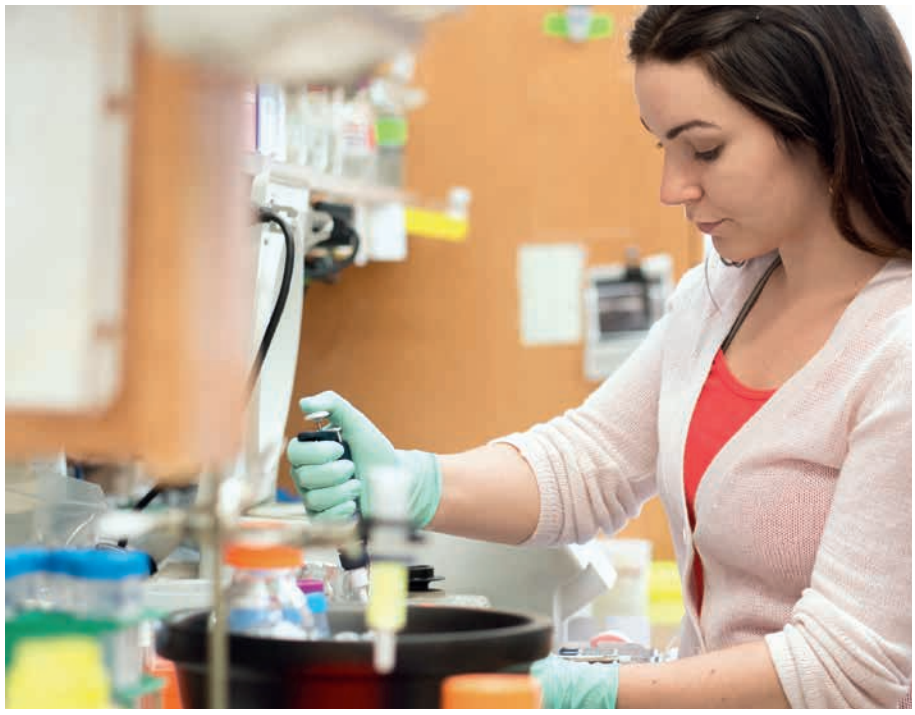
The analogy goes no further, however. A temporal pole neuron doesn't code for a specific familiar face, neither Granny's nor that of one's mother, boss, Nancy Pelosi, or anyone else.

"What we've discovered is more like a grandmother face area of the brain," Freiwald says.

LABORATORY OF MUCOSAL IMMUNOLOGY (FACING PAGE); MARIO MORGADO

PERCEPTION

Wake up and smell the coffee



Josefina del Mármol has discovered how olfaction differs from other forms of sensory perception at the molecular level.

EARTHY, NUTTY, COCOA with a hint of caramel—the aroma of a perfect cup of coffee. More than 200 chemical components coalesce in carafes and demitasse cups around the world to produce that familiar scent. And the human nose happily receives the message.

But how the brain then processes this surge of olfactory information is one of the great mysteries of neuroscience. Because while millions of molecules can unite in countless permutations to form any number of unique smells, humans are endowed with only a few hundred odor receptors to sniff through it all. And unlike most sensory receptors, which bind only to specific molecules, those that detect odors must multitask among many different ones.

Theories about how odor receptors pull this off have long wafted through the neuroscientific community. Some suspected that the receptors are glad to bind to any molecule that possesses a few basic features. Others proposed that odor receptors are as selective as any receptor but are pockmarked with numerous binding sites, allowing a single receptor to interact with many different molecules at once.

Yet when Josefina del Mármol, a postdoctoral associate in Vanessa Ruta's lab, inspected the odor receptors of an insect known as the jumping bristletail, visualizing them at atomic resolution, she found evidence for neither approach.

Instead, del Mármol and her colleagues reported in *Nature* that each odor receptor contains a single pocket that can form weak bonds with several different odorants. "The receptor is not selective to a specific chemical feature," Ruta says. "Rather, it's recognizing the more general chemical nature of the odorant." Computational modeling revealed that one particularly hardworking receptor sports a pocket that is at once selective and accommodating—rejecting unwanted odorants while weakly binding to many others.

Ruta suspects that the findings can be extrapolated to humans. "They point to key principles in odorant recognition, not only in insects' receptors but also in receptors within our own noses that must detect and discriminate the rich chemical world," says Ruta, the *Gabrielle H. Reem and Herbert J. Kayden Associate Professor.*

HUMAN SUBJECTS

Coveted, COVID-proof genotypes

AS COVID CONTINUES to find new victims, one of its biggest mysteries remains unresolved: why certain people appear to keep dodging it.

We all know of individuals who quickly lost their lives to the disease. Others had infections that triggered long COVID, a concoction of debilitating symptoms that may linger for years. Still others, including fully vaccinated people with limited exposure to the virus, caught the disease several times.

And then there are the curious cases of people with ample exposure who never got sick. Among those who shared a bed with an infected partner or those who spent months with COVID patients in the ICU, some never tested positive. Are these individuals impervious to the disease, or did they just escape it by chance?

"We want to know if there are gene variants that protect people from SARS-CoV-2 infection," says Jean-Laurent Casanova, Rockefeller's *Levy Family Professor*, who is leading a major effort to answer that question. "If there are, and we could find them, that would be huge."

If mutations that prevent infection indeed exist, the researchers want to learn precisely how they stop the virus from replicating. Knowledge of those mechanisms could make it possible to develop antiviral drugs that make people less prone to catching COVID, and less likely to spread it to others.

András N. Spaan, a postdoctoral fellow in Casanova's lab, adds that human genetics studies have traditionally focused on the type of mutations you don't want—those that are linked to poor outcomes. "But we can learn a lot about the pathophysiology of an infectious disease by studying beneficial mutations that make the immune system better equipped to deal with it," he says.

With an international consortium of scientists, Spaan and Casanova are recruiting participants for a clinical study aimed at discovering the genetic factors of COVID resistance. They have already heard from hundreds of people from around the world who've demonstrably been exposed to the virus without being infected.

To learn more about the trial, visit the COVID Human Genetic Effort at www.covidhge.com. ◎

DATA

480

Number of inherited mutations shown to make the immune system vulnerable to specific pathogens. So far only four mutations have been shown to protect from infections, but many more may be out there.

PRIORITY PROJECTS

Our dicey future

LET'S FACE IT: Public health is in a tight spot. Not only is the world still plagued by the viral pandemic; experts have long warned that, unless novel antibiotics are developed, multidrug-resistant bacteria will soon render current ones inefficient. Already, at least 700,000 people die each year from infections with strains like XDR *Acinetobacter baumannii* and *Neisseria gonorrhoeae* that don't respond to existing antibiotics. Even colistin, long used as a crucial last option when other drugs fail, is becoming obsolete.

But hardworking scientists might be able to forestall the impending apocalypse. In January, a team led by Sean F. Brady, Rockefeller's *Evnin Professor*, reported in *Nature* their discovery of macolacin, a natural compound that might make it possible to vanquish pathogens that don't respond to colistin or other antibiotics. A chemical cousin of colistin, macolacin is produced by soil bacteria that live in conflict with other microbes.

Bacteria found in soil produce an antibiotic effective against multidrug-resistant pathogens.

When the researchers synthesized and tested macolacin, they were impressed by its potency. In cell assays, the agent killed several types of colistin-resistant bacteria, including intrinsically resistant *N. gonorrhoeae*; and in mice, it completely cleared away infections with colistin-resistant *A. baumannii*.

Both strains are common causes of infections in health-care settings, and both are classified as public-health threats of the highest level by the Centers for Disease Control and Prevention. A novel drug to defeat them would be a promising milestone on the road away from a superbug dystopia. ◎

JACOB ARTHUR PRITCHARD

PROKARYOTES

When bacteria self-vaccinate

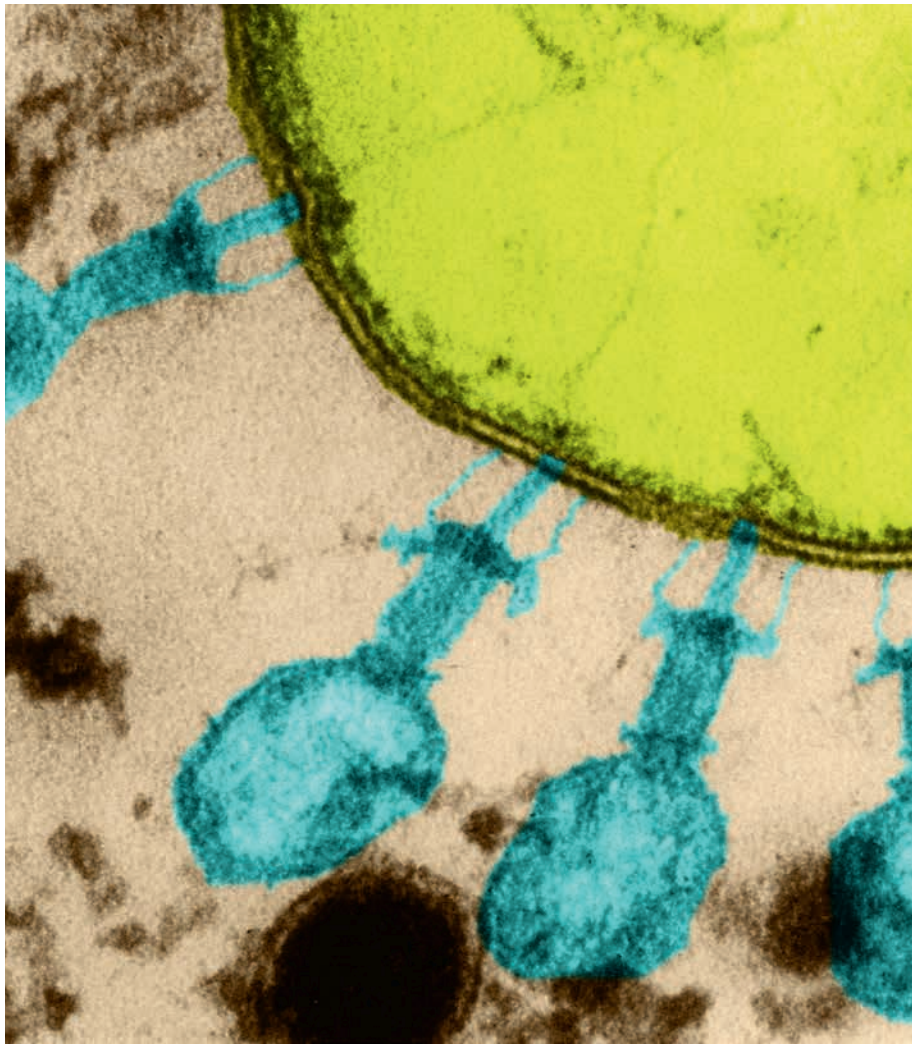
All organisms, including bacteria, have enemies.

LIKE MOST ORGANISMS, bacteria are prey to viruses—and their go-to approach for destroying the invaders is to simply chop them up. When it sees a virus, a bacterium may employ a host of immune strategies to slice up its genome using a molecular cutter called CRISPR-Cas, also the name of a popular laboratory tool.

But before engaging CRISPR-Cas, the bacterium will usually launch a first line of defense: its so-called restriction enzyme, a molecule capable of cleaving short DNA sequences. If the restriction enzyme fails to cut the virus and stop it in its tracks, CRISPR- Cas, a more sophisticated weapon, comes online. The CRISPR cutter slits the viral intruder with immaculate precision by neatly aligning it to a molecular guide targeting a specific genetic sequence. Whereas the restriction enzyme approach chops up viral DNA with the crudeness of a lawn mower, CRISPR-Cas is more akin to razor-sharp gardening shears.

Both types of bacterial defense are commonly exploited by biologists whose day-to-day chores involve manipulating DNA for various purposes—like sequencing genes, making molecules fluoresce, or creating animals with modified genomes. Restriction enzymes came into vogue in the 1970s, making it possible to cut up pieces of DNA swimming in a test tube; and a decade ago, technology based on CRISPR-Cas revolutionized bioscience by giving scientists the means to edit with high precision genomes within living cells and organisms.

But Pascal Maguin, a graduate fellow in the lab of Luciano Marraffini, remains committed to exploring the bacterial basics—and, in the process, he recently clarified how one facet of bacterial immunity operates. Working with *Staphylococcus aureus*, Maguin and his colleagues were able to explain why the virus-chopping strategies of this bacterium work better together than on their own. When staphylococci are protected only by restriction enzymes, their defenses are short-lived; and after a while, the research shows, the bacteria growing in the dish will start to dwindle. Maguin discovered how the two systems work in concert—segments previously clipped by restriction enzymes help the CRISPR-Cas machinery gain a foothold in the viral DNA, which it then uses to generate the molecular guide needed to put an end to the infection.



SCIENCE PHOTO LIBRARY

Viruses (blue) latch on to a bacterial cell. The bacterium may use a combination of primordial defense strategies to destroy the invaders.

"It's a bit like vaccination," Marraffini says. "The restriction enzyme cuts little pieces of the virus that CRISPR will then use to mount an adaptive response."

The findings, reported in *Molecular Cell* earlier this year, might not only help us understand how staphylococci defend themselves from viruses but also could make us better equipped to defend ourselves from staph—a species notorious for its ability to become resistant to antibiotics and a common cause of outbreaks in hospital settings. Last year, Marraffini's lab published other findings showing that the bacterium uses its CRISPR-Cas system not only to fend off viruses but also to develop multidrug resistance. Understanding the system better could one day allow scientists to manipulate it with drugs to fight staph infections that respond to no other treatments, says Marraffini, who is Rockefeller's *Kayden Family Professor*.

Replicons provide a safe system to study pathogens that normally require strict biosafety measures.

COVID

Rise of the replicons

IT'S BIOSAFETY 101: Whatever you do, don't let the dangerous pathogen escape the lab. It's why much of the research on SARS-CoV-2 has been done in sophisticated and massively expensive negative-pressure laboratories, complete with air locks and HEPA filtration.

For some experiments, however, there's a more creative approach: replicons, lab-made self-replicating viral genomes that are not infectious but otherwise identical to the real pathogen. Replicons have proved instrumental in the development of drugs for other viruses. For instance, hepatitis C replicons developed by Nobel laureate Charles M. Rice, the *Maurice R. and Corinne P. Greenberg Professor in Virology*, led to the creation of powerful new drugs to effectively cure that disease.

Now, given the urgency for more effective COVID drugs, Rice and his team have created SARS-CoV-2 replicons that can be used to investigate how the virus hijacks the cell's own machinery and how it generates new copies of itself. And, as the researchers point out in a paper in *Science*, replicons might make it easier to develop new drugs.

The new replicons mimic nearly every aspect of the coronavirus life cycle. Their genetic content has all the information the virus needs to mass-produce copies of itself and pack them into new virus particles, but it lacks instructions for making spikes, the proteins that enable the particles to enter and infect human cells. Once introduced to cells in a dish, a replicon makes progeny that are unable to contaminate neighboring cells.

"If the virus were a race car, we made a version without wheels. It has the engine and all the parts that would allow the car to move, but it can't actually go anywhere," says Joseph Luna, a postdoc in the Rice lab who worked on the project along with research associate Inna Ricardo-Lax.

Replicons are typically created by cloning DNA sequences that can be used to make replicon RNA artificially. But the researchers realized that standard cloning methods wouldn't work for the coronavirus, whose RNA is exceptionally long. So instead, they used a platform developed by collaborators at the University of Bern and the Institute of Virology and Immunology, in Germany, which involved assembling coronavirus genomes from smaller fragments in yeast instead of synthesizing whole genomes directly in the test tube.

Luna says scientists will be able to use the replicons to test drugs against SARS-CoV-2 and evaluate its response to neutralizing antibodies. It's a way to speed up the science without sacrificing safety.

30,000

DATA

Approximate size of the SARS-CoV-2 genome measured in nucleotides, the basic components of RNA. The genomes of hepatitis C, HIV, and rhinovirus are more than three times smaller.

"If the virus were a race car, we made a version without wheels."

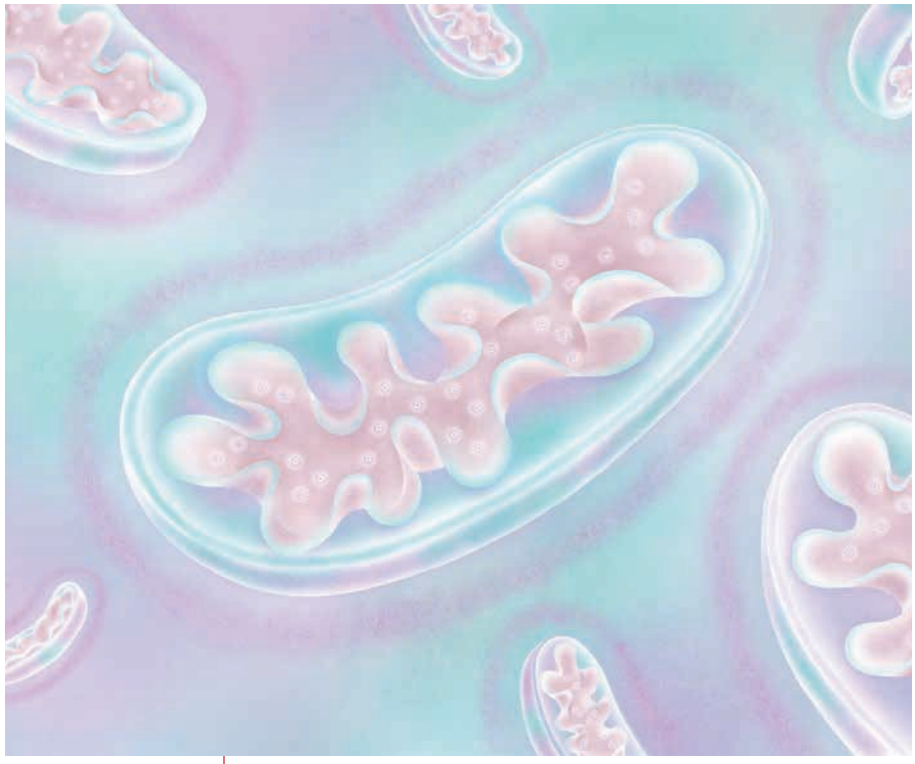
FRANK VERONSKY

ORGANELLES

Metabolic mishaps

MITOCHONDRIA KEEP OUR cellular lights on. Floating in a cell's gelatinous interior, these bean-shaped bubbles act like its nuclear power reactors, churning out energy that drives everything we do, from replicating DNA to running marathons. Moreover, failures in mitochondrial maneuvers can be Fukushima-like, leading to the accumulation of chemically reactive free radicals inside mitochondria that may trigger cancer, neurodegeneration, or other problems.

Recently, Rockefeller scientists took a leap forward in studying such havoc, known as oxidative stress. A team led by Kivanç Birsoy, the *Chapman Perelman Assistant Professor*, discovered how glutathione, an antioxidant produced outside of mitochondria, enters these powerhouses to neutralize free radicals. Their experiments show that glutathione transport relies on a protein in the mitochondrial membrane whose function was hitherto unknown.



Many diseases can be traced to the cell's mitochondria.

The findings might inspire further research on aging and the various diseases linked to oxidative stress. "These conditions could potentially be treated or prevented by stimulating antioxidant transport into mitochondria," Birsoy says. ◎

DATA 10,000 Number of cases of tick-borne encephalitis reported worldwide each year.

JAN ERHART, BIOLOGY CENTRE CAS

PARASITISM

Viral venom

OF THE MILLIONS of spineless creatures crawling about planet Earth, ticks may be the least loved. These minuscule, hard-bodied beasts spread many dangerous infections, including tick-borne encephalitis, endemic in Russia, China, Mongolia, and many European countries. A cousin of the viruses causing dengue, yellow fever, and Zika, tick-borne encephalitis is just as nasty as it sounds—a rampant neurological disease that inflames the brain and thwarts cognition.

In analyzing blood samples from 800 infected people, Marianna Agudelo, a graduate student in the lab of Michel C. Nussenzweig, found that some samples contained unusual antibodies capable of neutralizing the virus. As reported in the *Journal of Experimental Medicine*, Agudelo and her colleagues cloned these antibodies and successfully used them to curb the sickness in infected mice. They are now working to translate their findings to humans with the goal of developing new treatment and prevention methods.

For example, a vaccine that coaxes the immune system to produce the rare antibodies on its own "would be more elegant and more focused than existing vaccines," says Nussenzweig, the *Zanvil A. Cohn and Ralph M. Steinman Professor*. ◎

Illustration by Hoi Chan

Q&A

The machine that built all living things—and itself

With Sebastian Klinge

BEHOLD THE RIBOSOME, enabler of all life, fossil of our primordial past. Colossal among molecules, it has been observed from every angle using a host of elaborate techniques—suffused with electrons, bombarded with X-rays, deep-frozen in liquid ethane. But after decades of dogged research, its myriad nooks and knobs remain blurry.

Until scientists arrive at a perfect picture of the ribosome, their understanding of living things will be incomplete—and so will their ability to heal diseased cells or thwart pathogens.

Discovered at Rockefeller almost 70 years ago, ribosomes have long been known to execute a cell's most fundamental function: translating its genetic code into protein. But the precise mechanics of that process have been harder to pinpoint. Today, biologists keep adding to the list of "solved structures," molecules whose 3D shapes have been fully mapped, but the ribosome still holds many secrets. A majestic mountain, it seems too tall to climb.

After 70 years of research, we still don't know what makes the ribosome tick.

Sebastian Klinge is not intimidated. He has dedicated his career to understanding how ribosomes string together amino acids, the building blocks of protein, using RNA as a template. Like a watchmaker disassembling an antique timepiece to figure out what makes it tick, he seeks to learn how the ribosome functions by figuring out how one forms. Using ultramodern technology, his lab recently revealed the earliest steps by which numerous ribosome components come together, creating never-before-seen footage of a nonbacterial ribosome folding into shape.

We spoke with Klinge, who heads the Laboratory of Protein and Nucleic Acid Chemistry, about his investigative trek and where it's headed.

Mapping molecular structures is hard work. Why focus on one as complex as the ribosome?

Ribosomes make the world exist! From an evolutionary standpoint, they have become so indispensable in

ZACHARY VEILLEUX

nature that all cells need ribosomes for everything they do—including making more ribosomes.

Ribosomes are, in fact, so crucial for life that they don't neatly fit into the boxes in which we generally sort molecules. In most areas of biomedical research, we can tell that a molecule is important by the fact that diseases occur when a mutation arises that prevents it from doing its job. This logic doesn't apply to ribosomes. If your protein factories aren't functioning, you'd be unable to even exist. For this reason, we rarely see cells or organisms with serious mutations in the ribosome. And the minor ribosomal mutations that don't immediately kill the organism tend to have major consequences, causing cancer predisposition or craniofacial malformations.

It's a chicken-and-egg problem: You need ribsomes to make ribosomes.

If cells need ribosomes to make ribosomes, where did the first ribosome come from?

It's almost a chicken-and-egg problem, because that's right—to make a ribosome you need RNA as well as ribosomal proteins, which would have been made by another ribosome.

There must have been a point in evolutionary history when the ribosome was much simpler, a machine consisting only of RNA that manufactured polypeptides. In all likelihood, some of those polypeptides ended up interacting with the RNA and so thoroughly stabilized it that they eventually became part of the machine itself. Over time, these proteins evolved to become more efficient and sophisticated—and better at avoiding errors—eventually becoming ribosomes as we know them today.

How much do we know about how ribosomes are put together?

For a long time, we didn't even know what ribosomes looked like. When Rockefeller scientist George E. Palade first discovered them in 1955, he called them "particulate components of the cytoplasm" because they appeared as dark dots under the electron microscope. Decades later, in the late 1990s, X-ray crystallography started to reveal the many different components of ribosomes, yet we still didn't know how these structures were assembled.

Imagine archaeologists unearthing an ancient structure but having no idea what materials it was made of or how it had been put together. This was our problem. We had detailed 3D images of the ribosome, but still no idea how it was built. Only in recent years has the development of cryo-electron microscopy technology enabled my lab and others to gain insight into the ribosome assembly process.

Even cryo-EM only yields a snapshot, though. We're still trying to string snapshots of ribosomal formation and activity together into the right order to understand how the ribosome gets from one point in its development to the next—no easy task with molecules at this level of complexity. We've only recently accumulated enough molecular snapshots of these pathways that we can begin to tackle this task, putting together movies that allow us to really understand how ribosomes form and operate.

DATA

Each of the body's cells may contain up to 10 million ribosomes.

How might this work inform our understanding of human disease?

Although the vast majority of ribosomal mutations are so deleterious that they cannot sustain life, we know of some that are subtle enough to cause disease without killing the organism. Specifically, a handful of blood diseases have been linked to mutations in ribosomal proteins.

Research has shown that mutations in ribosomal proteins can decrease the amount of ribosome assembly taking place or the quantity of ribosomal proteins available to synthesize a transcription factor critical to the development of red blood cells. The upshot is that the body is unable to produce sufficient red blood cells and their overall count drops, leading to anemia and related problems. Once we have a better grasp of how ribosome assembly happens, and how it fails, we might have opportunities to develop new therapies to manage these conditions.

A better understanding of ribosome assembly may also lead to novel therapies for microbial disease. Many existing drugs kill bacteria or fungi by targeting their ribosomes. By studying ribosomal structures, we have been able to pinpoint the precise mechanisms by which some of these drugs work. For example, we now know where the antifungal cycloheximide binds in eukaryotic cells and where the antibiotic azithromycin binds in bacterial cells.

Ultimately, this research might aid the discovery of new antimicrobial treatments with better specificity and fewer side effects—drugs capable of attacking the pathogen without destroying beneficial bacteria or human cells.

What else about ribosomes do we still need to study?

Now that we are beginning to understand the principles of the ribosome, the basic machinery, and its parts, we can move on to study how ribosomes are controlled and regulated. For instance, how does a cell decide which messenger RNAs should get translated into a protein? How does the ribosome make new ribosomes? And how does cellular metabolism influence all of this? One crucial question that we're looking into right now is how the control and integration of ribosomes works. We still don't know how misassembled ribosomes are recognized and what controls ensure that ribosomes are assembled correctly.

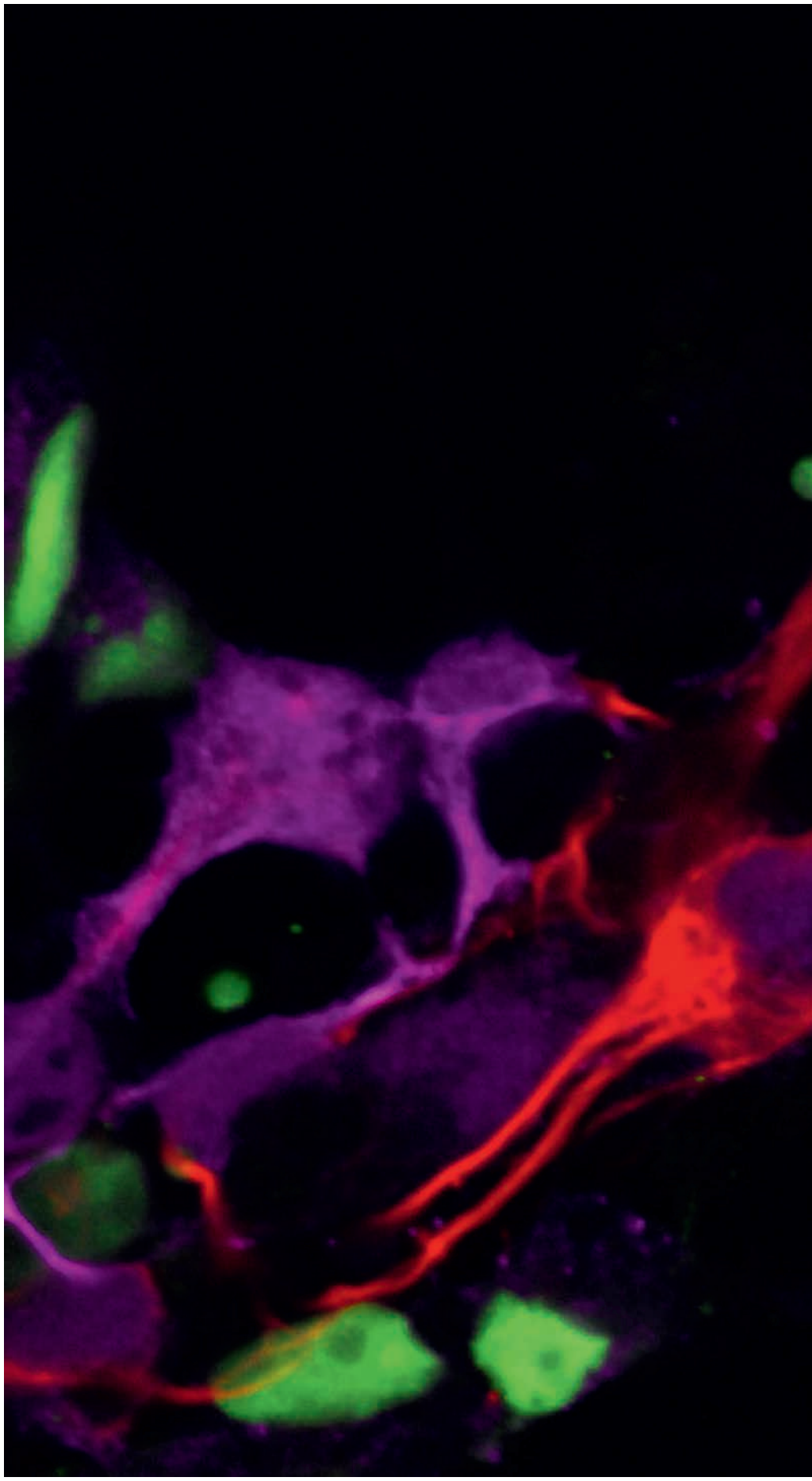
These questions are the future of ribosome research. At Rockefeller, where we have a rich history of ribosome discovery and investigation, I am just the latest in a long line of people working on these problems. ◎

Journey to the cerebellar cortex

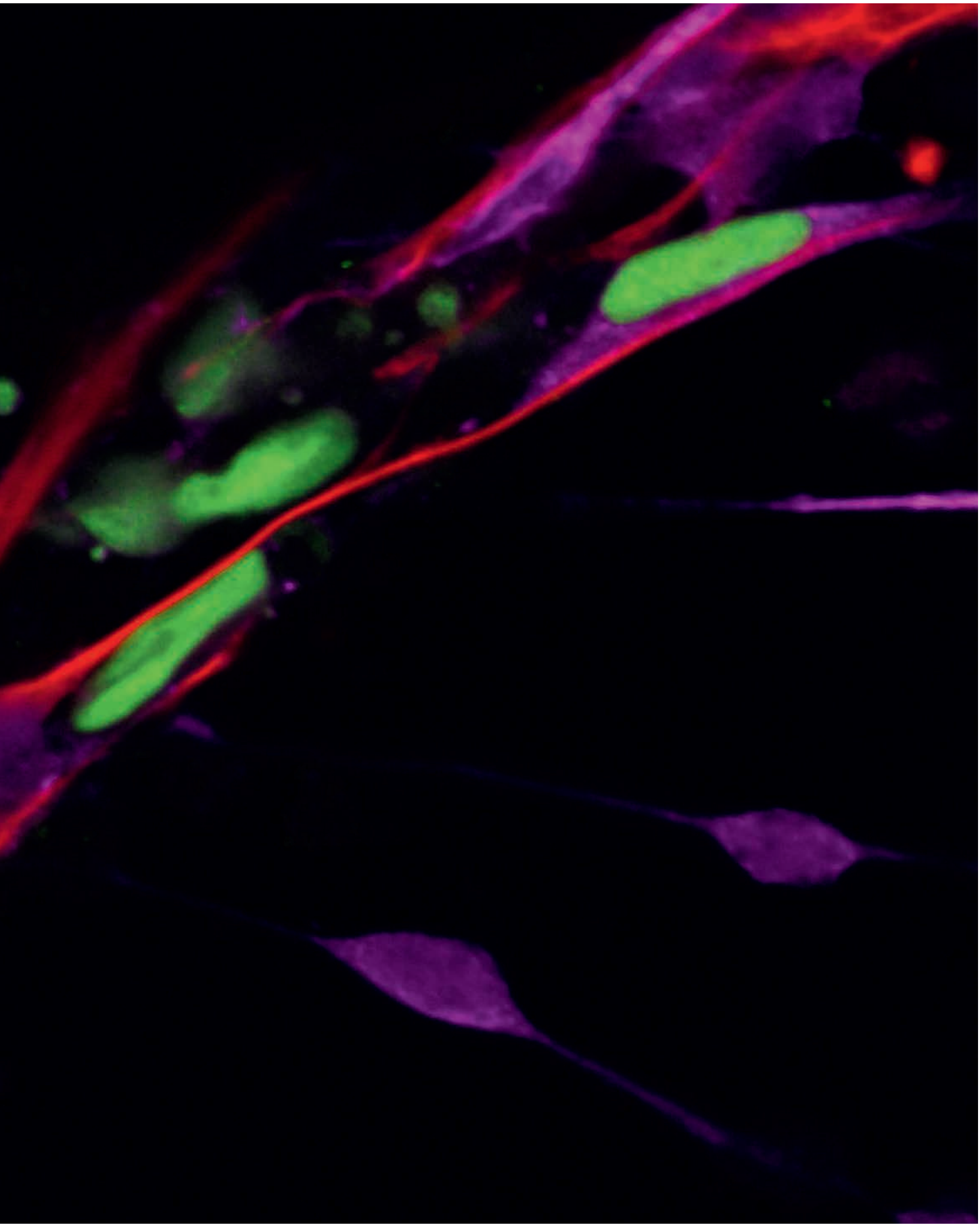
ABOUT 80 PERCENT of the brain's neurons are packed into what is sometimes called our "little brain," the cerebellum. During development, some reach their proper places by climbing along delicate fibers extended by glia, another type of brain cell.

To recapitulate the process, Hourinaz Behesti, a research associate in the lab of Mary E. Hatten, the *Frederick P. Rose Professor*, grew cerebellar neurons from human stem cells. These engineered neurons (green) knew precisely what to do when Behesti inserted them into a young mouse brain. They dutifully found their glial tracks (red) and followed them toward their next developmental destination—the compact cellular network that will eventually become the cerebellum.

Read more about stem-cell science in "Stem cells are growing up," on page 22.



LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY



Biologically speaking, the human gut is terra incognita. One clinician-scientist is leaving no stone unturned in exploring it.

Rachel Niec

By Rachel Nuwer

Rachel Niec is at her microscope, looking at a jellylike clump of cells from the lining of a mouse intestine. The view is chaotic: a jumble of immune cells, neurons, stem cells, and their surrounding vasculature, each component lit up in its own fluorescent color. Somewhere within the blob is a clue that Niec believes will lead her to a meaningful discovery, and perhaps suggest new ways to effectively treat inflammatory bowel disease (IBD).

Immunologists and gastroenterologists—Niec is both—typically think of IBD as a malfunction of the immune system. In the lawless landscape of the gut, immune cells must bravely sort the good bacteria from the bad—a daunting task. Any errors in their judgment can lead to runaway inflammation that irritates the delicate lining of the intestine and causes debilitating disease.

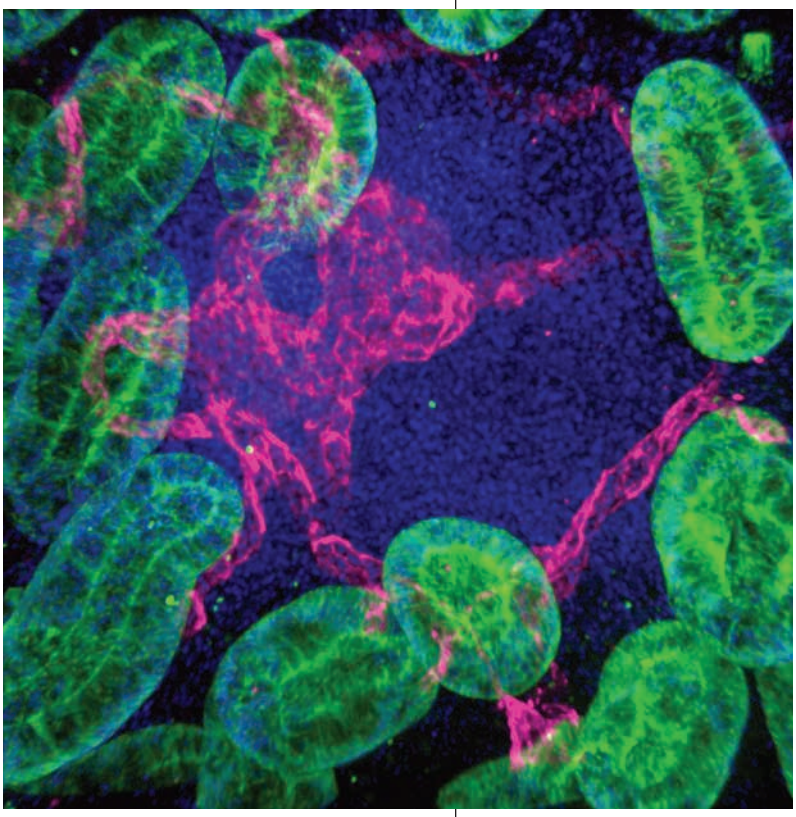
Yet the exact drivers of these conditions, likely a complex mix of genetics, biology, and environment, remain a mystery. For Niec, that means taking in the whole picture. It means looking at the epithelium—the gut's version of skin—and considering everything that occurs there, from the role of microbial communities to the influence of the immune system to the processes of nutrient absorption.

"It's not only overexuberant immunity or runaway microbiota; it's more complicated," Niec says. "To understand what's going on, it's helpful to study the entire tissue with all its constituents."

Niec grew up in the Bay Area of California, the daughter of a middle school science teacher and a sound engineer. She spent weekends backpacking in Yosemite and exploring Northern California, where she learned to canoe and kayak in whitewater and wore out a dozen pairs of hiking boots. Niec's budding interest in science was encouraged by her mother, who taught her to identify trees and birds and enthusiastically facilitated science experiments at home and in the woods. Niec attended Mills College, a small liberal arts school in Oakland known for its support of women's leadership. She planned to become a science teacher.

But a summer internship in an immunology lab at the Center for Infectious Disease Research in Seattle shifted her

Photograph by Matthew Septimus



A Crohn's disease biopsy showing intestinal crypts (green) and lymphatics (pink).

"Being able to make a difference in patients' lives is a constant reminder of what my work in the lab is ultimately about."

thinking. Even as a volunteer intern, Niec was conducting important experiments, screening antibodies for their ability to recognize cells infected with HIV.

"This is when I started thinking about medicine," Niec says. "It was my first experience generating scientific results that were going to impact the course of other people's research, and eventually affect how we treat patients. It was satisfying to iterate experiments with a team, and it felt important because it related directly to human health." She ended up spending three summers at the lab.

After graduating, she worked for a year as a Fulbright scholar at an HIV clinic for sex workers in Bali. Located in bustling Denpasar, the Kerti Praja Foundation—a three-story concrete building where Niec both worked and lived—saw up to 40 women per day. They came for reproductive health services but also for sewing classes and assistance securing microgrants. Niec had joined the team to help upgrade the clinic's diagnostic capabilities and to support the doctors and join outreach workers in the field. She worked on programs to deliver clean water to the towns where patients lived, and she taught English.

Upon her return to the United States, Niec enrolled in a graduate program at the University of Washington in Seattle, and transferred to the Tri-Institutional M.D.-Ph.D. Program in Manhattan two years later when her adviser, Alexander Rudensky, moved to Memorial Sloan Kettering Cancer Center. She finished her medical training at Weill Cornell Medicine, including a residency in internal medicine and a gastroenterology fellowship, and did her thesis work in Rudensky's immunology lab.

Seeking a research project that would dovetail with her interest in gastroenterology, Niec embarked on postdoc training in the lab of Elaine Fuchs, a renowned stem-cell biologist. "Elaine is among the best in dissecting stem-cell mechanisms, and merging our respective interests has really led to some fun questions," Niec says.

Stem cells originating in the skin reside in so-called niches beneath the epithelium and can develop into diverse cell types. If you want to understand the complexity of the epithelium, the niche is the place to start. And if you want to understand the niche, Fuchs's lab is the place to be. (Read more about the lab's work on epithelial stem cells in "Stem cells are growing up," on page 22.)

"Elaine and I recognized that there's a real gap in our knowledge at the intersection of immunology and epithelial cells," Niec says. "If we can build the tools to fill that gap, we will be able to ask questions that nobody else is positioned to ask."

With Fuchs as her mentor, Niec enrolled in Rockefeller's Clinical Scholars Program, which trains postdoctoral physician-scientists to integrate translational research and patient care. "This was the right kind of structure for me, because

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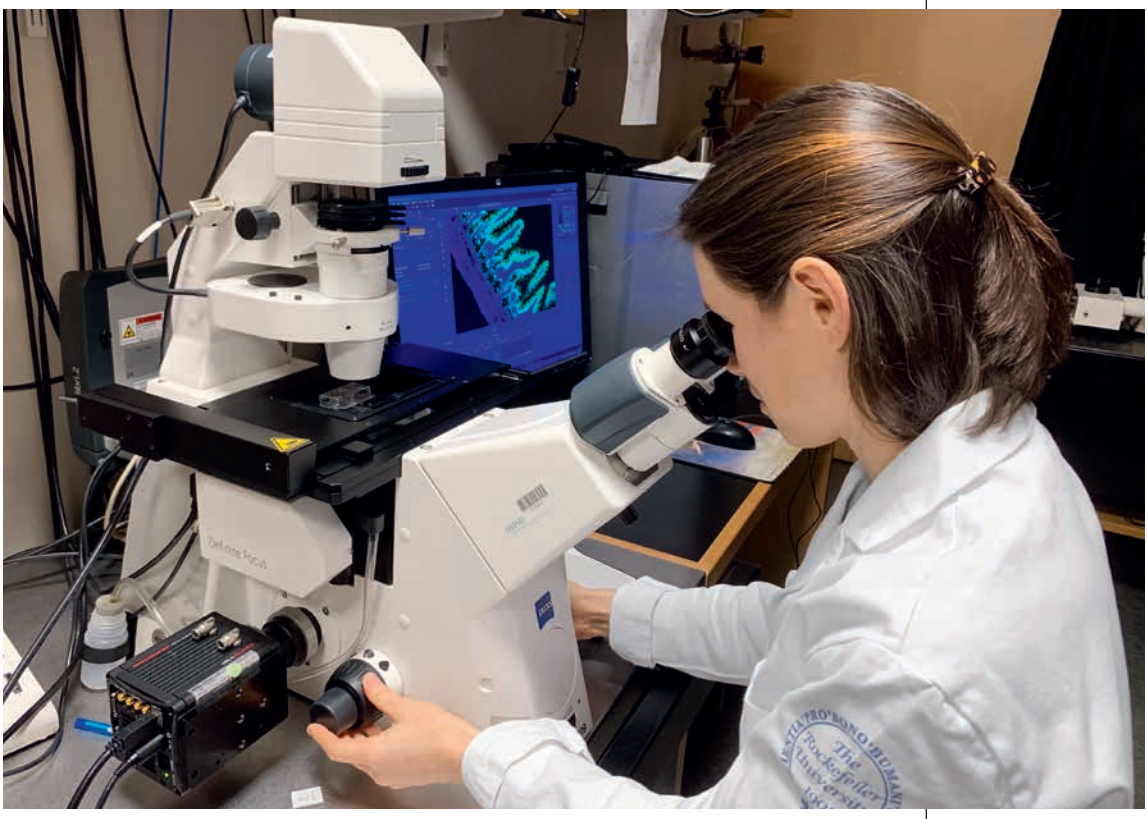
Niec surveys the eclectic population of cells in the gut epithelium.



LORI CHERTOFF

sometimes as a physician-scientist it can seem like you have each foot in a different world—the clinic and the lab. But in reality, these pieces are deeply connected," says Niec. "I recently met with a patient in his later years of life who hadn't responded to any of the conventional therapies for IBD, and had a wonderful response in a clinical trial. Being able to make a difference in this patient's life, and the lives of others like him, is a constant reminder of what my work in the lab is ultimately about."

Inflammatory bowel disease is a cluster of disorders that involve chronic inflammation of the digestive tract, including ulcerative colitis and Crohn's disease. These debilitating and at times life-threatening diseases affect over one percent of American adults, and their prevalence has increased over the past 20 years. No cures exist, and the available treatments are hit-or-miss. Although GI-related disorders can have genetic components and are influenced by the microbiota and other environmental features in a person's gut, it's at the intestinal epithelium—that messy soup of cells in Niec's microscope—where the action happens.

"The key to treating these diseases will be to figure out who talks to whom in this ecosystem and how we can reset the communication networks," Niec says.

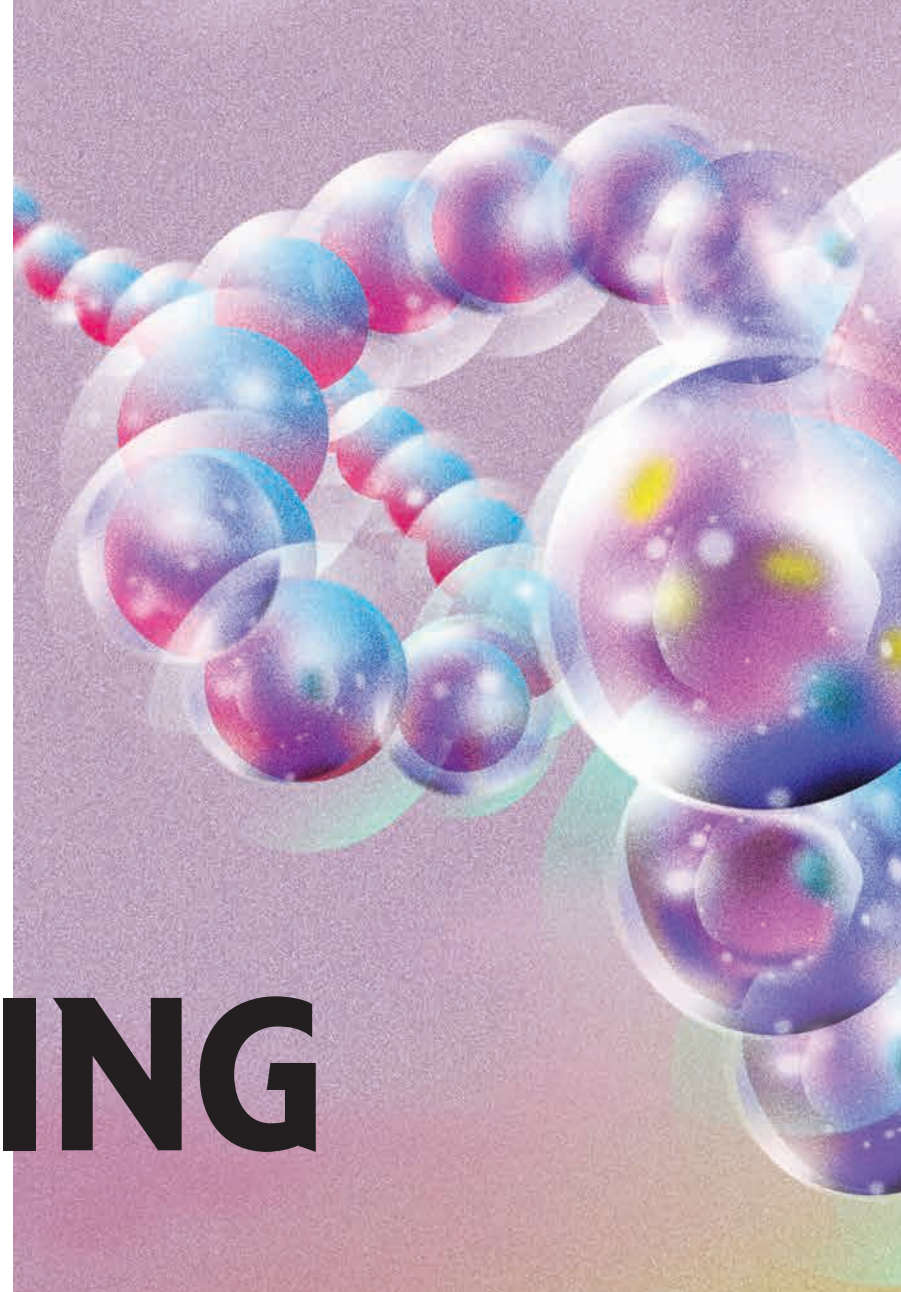
Niec and her team are now working to understand what's happening in the epithelial niche, what cellular actors are involved in immune signaling, and what they are saying. Because the architecture of the niche is so complex, the researchers use 3D imaging technology developed in Fuchs's lab to simplify the picture.

Niec's first significant finding derived directly from these imaging experiments, revealing that lymphatics—vessels that act as drainage canals for tissues—play a central role in communicating with stem cells in the intestine. The discovery is intriguing in part because IBD patients are known to have abnormal lymphatics, hinting that these structures might contribute to disease. Niec uses spatial transcriptomics—a technique to identify genes activated during communication between cells—to look for specific lymphatic signals that might be influencing the behavior of stem cells.

The work is an intellectual challenge, and the latest step on a longer journey that has been equal parts laboratory research and patient care.

"There are many untapped drivers of IBD to be targeted," says Niec, who is a recent recipient of the prestigious Burroughs Wellcome Career Award for Medical Scientists. "Reaching the ultimate goal of translating these discoveries into treatments is what will make all the training worthwhile." ◎

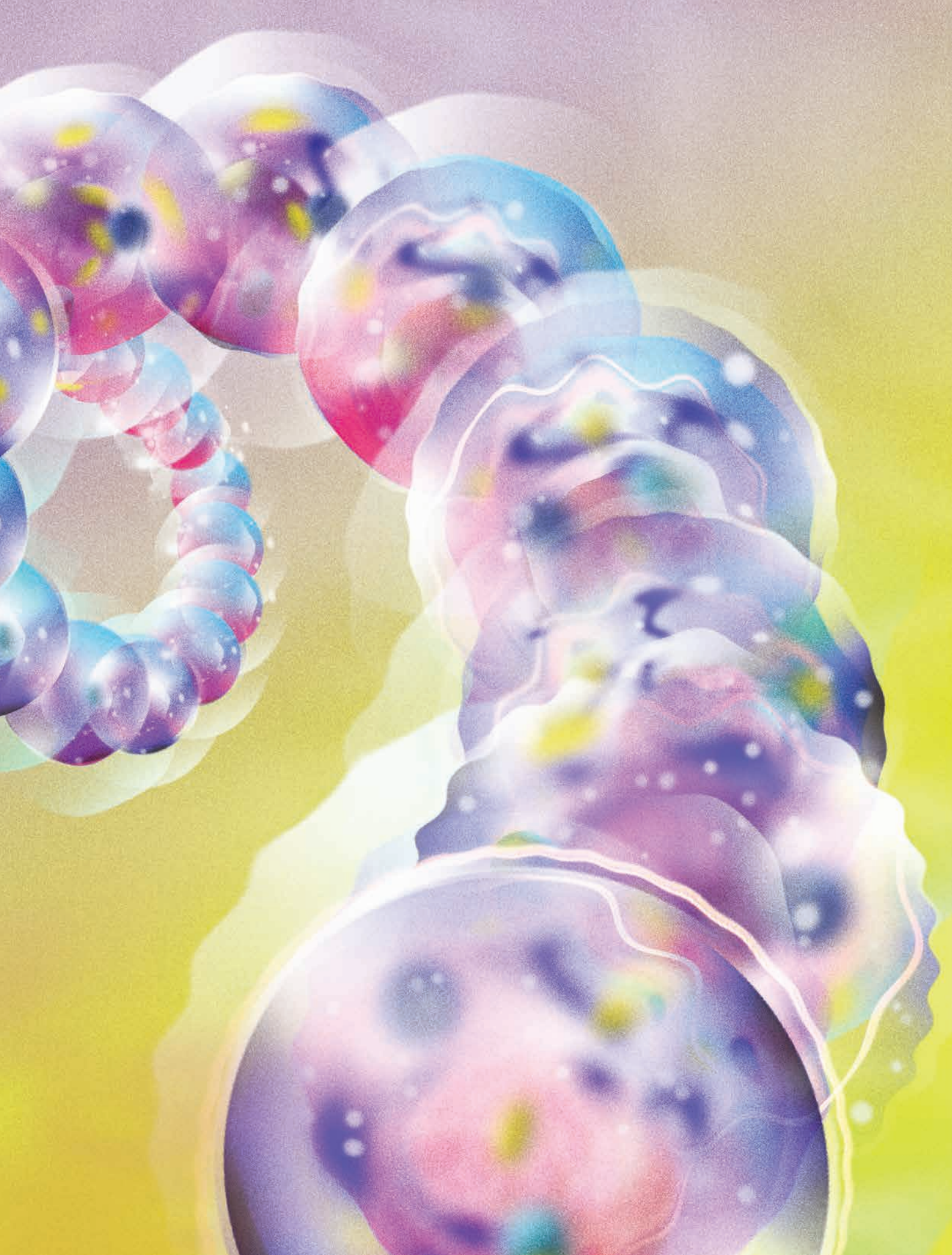
STEM CELLS ARE GROWING UP



BY BAHAR GHOLIPOUR AND ZACHARY VEILLEUX

ILLUSTRATION BY MARK PERNICE

The controversy and hype have died down. The science is very much alive, creating new directions for discovery.



Pinched between his thumb and forefinger, on a round sliver of glass no bigger than a potato chip, Ali H. Brivanlou holds 10,000 human embryos arranged in a neat grid. It has the look of something vaguely electronic, like the inner workings of a smartphone. Yet under more natural circumstances, each of these embryos, a cluster of cells nearly invisible to the naked eye, would have the potential to create something unmistakably organic—a human being.

The lab's incubators contain dozens of these robotically created wafers. The one Brivanlou is holding is just a few hours old, and over the next few days, it will undergo a course of biochemical treatments mimicking signals from the womb. It will then be placed under a specialized microscope, allowing Brivanlou and his colleagues to record the 10,000 embryos' developmental journey.

But these embryos didn't start out as fertilized eggs, and they will never develop into fetuses. The cell clusters, called synthetic or artificial embryos, are the descendants of human embryonic stem cell lines derived in Brivanlou's lab 20 years ago.

"You can see them develop in front of your eyes," he says. "If you watch them as they divide and begin to form into an organism, you will see the beauty inherent in nature, and you may learn some of its secrets. It is something that's difficult to describe in words, but impossible to forget."

Beneath the beauty, there is promise. What makes embryonic stem cells unique is their ability to morph into almost any type of cell in the body, like muscle cell, nerve cell, or blood cell. And because of this spectacular power, known as pluripotency, they've helped scientists forge countless new paths to discovery.

For example, stem cell research has delivered groundbreaking insights into the cryptic first stages of human development, and intriguing ideas about our evolutionary past. It has deepened our knowledge about the root causes of diseases like cancer, chronic inflammatory disorders, and degenerative conditions. Moreover, scientists are using stem cells as tools for a vast scope of work, from basic-science investigations of how the human genome is regulated to translational research seeking to pinpoint disease mechanisms or test novel drugs.

Today it's hard to imagine where 21st-century science would be without stem cells, but their career as research subjects wasn't always smooth.

IT WAS at the start of the new millennium that scientists learned how to grow embryonic stem cells in the lab and nudge the cells to take on new identities. The research attracted headlines partly because of controversy around how the cells were obtained—typically from leftover embryos generated during in vitro fertilization treatment—and partly because they held promise for regenerative medicine, the idea of using stem cells to replace diseased cells or tissues in patients. Some advocates of the science made increasingly optimistic projections: With the right technology, it would soon be possible to grow whole organs in petri dishes. A patient's worn-out liver, kidney, or heart could be swapped for a new one, like a set of tires.

"Claims were made about stem cells, particularly embryonic stem cells, that would clearly not be easy to

MATTHEW SEPTIMUS

Brivanlou uses synthetic embryos to answer longstanding questions about human development.

fulfill," says Brivanlou, who is Rockefeller's *Robert and Harriet Heilbrunn Professor*.

Indeed, working with human embryonic stem cells was challenging from the start, and remained difficult even after 2006, when new technologies allowed scientists to create so-called induced pluripotent stem cells (iPSCs) by reprogramming somatic cells such as skin cells into an embryonic-like state. The iPSCs behaved similarly to stem cells derived from embryos and promised a limitless supply of pluripotent cells for research and medical applications, but they came with their own peculiarities and safety concerns. Scientists also developed methods to guide iPSCs or human embryonic stem cells to differentiate into slightly more mature cells called progenitors, but attempts to push them further, toward full specialization, kept failing. There were multiple challenges, starting with the fact that no developing cell operates in isolation—to function normally, it must exchange signals with nearby cells and sometimes connect with nerves, arteries, and nonliving material in the tissue. It's a puzzle with so many pieces that its solution may still be decades away.

There were other hurdles, too. The creation of the first human embryonic stem cell line caused a cultural uproar so loud that it almost brought the science to a halt. Several European countries banned the creation of the cells, and in the United States, then president George W. Bush cut off the field's access to government funding. Scientists had to secure private funding for work involving embryonic stem cells and, to comply with federal regulations, erect physical barriers in their labs to separate privately funded work on stem cells from government-funded research.

It was in such a cordoned-off corner that Brivanlou's team began studying natural human embryos and deriving cell lines from them. They also developed a platform that made it possible to grow the embryos in petri dishes, even past day seven, when normal embryos need a uterus to survive. For the first time, they could watch the transformations taking place during the second week of human embryogenesis, up until around day 14. This is when a milestone called symmetry breaking occurs—characterized by the emergence of the body's three axes (head to foot, front to back, left to right).

To observe later developmental stages, Brivanlou's lab and Rockefeller physicist Eric Siggia created synthetic human embryos growing as self-organizing 3D cultures. These embryo models mimic the geometry of natural embryos and can be induced to undergo symmetry breaking, giving researchers a way to study the biological events occurring in the third week of development. They have also provided some of the most striking pictures of early human life ever seen.

50–70

Number of times a normal human cell may divide before it starts to deteriorate.

∞

Number of times a human stem cell may divide.

"Many recent breakthroughs are based on the knowledge that stem cells are not operating in a vacuum."

Synthetic embryos are not only answering questions about human development, however. Brivanlou's lab and others are also using them to study medical problems, including limitations in technologies to help couples conceive. In a study last year, for example, the team identified an avoidable bottleneck in in vitro fertilization procedures, a finding that "may change the way IVF has been done for the past two decades," Brivanlou says, "and make it succeed for many more people."

Currently, only a small fraction of embryos generated in fertility clinics are used for transfer to the uterus. Most are deemed deficient, flagged by a test detecting an abnormal number of chromosomes. But the team found that these supposedly nonviable embryos often self-correct as they grow, in a complex process that eliminates cells with irregular chromosome numbers from the fetus. These results are in line with earlier observations that when such abnormal embryos are implanted in a prospective parent, they survive just as often as embryos that test normal and produce healthy babies.

"These embryos are viable and should no longer be discarded," Brivanlou says.

Moreover, the synthetic embryos have created unprecedented opportunities for studying disease mechanisms and testing new drugs. One striking example is the lab's use of the technology to study Huntington's disease. This inherited neurodegenerative disorder was long thought to start in middle age, when symptoms typically emerge. But the researchers found that earlier signs of Huntington's arise before birth, in the first two weeks of embryonic development. "This suggests that what we've long thought of as an age-related condition may in fact be a developmental disease," Brivanlou says.

Another technology that emerged from stem cell research is the organoid. From their human embryonic stem cell lines, Brivanlou and his colleagues created brain organoids—tiny 3D cultures of embryonic neural tissue for studying Huntington's and screening for novel drugs. And when the pandemic hit, they created other organoids that model embryonic lung tissue, providing a system to investigate how SARS-CoV-2 damages airways and alveoli in people with severe COVID.

"This technology really opens a door to identifying the mechanisms by which organs like the brain or lungs normally develop, understanding how they go awry in disease, and testing drugs that set these mechanisms back on the right course," says Brivanlou.

50–150
Number of cells in an embryo five days after fertilization.

In the 1970s, a series of botched experiments led Howard Green, a researcher at the Massachusetts Institute of Technology, to a surprise discovery. While unsuccessfully trying to replicate a rare cancer in a petri dish, Green noticed that the tumor-derived cells were forming structures resembling the outer layer of the skin, or epidermis. This "test-tube skin," as he called it, was so similar to real skin that it could be used for skin grafting. In an oft-cited procedure in 1983, Green and others regenerated massive amounts of epidermis and saved the lives of two boys whose bodies were covered with third-degree burns. But Green didn't know why his cultured cells could magically generate new tissue. What was going on inside that dish?

Eventually, he figured it out: Some of the cells had the capacity to divide over and over without specializing.

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A century and a half of stem-cell science

1868
German biologist Ernst Haeckel coins the term *stamzelle* (stem cell in German) describing the hypothetical ancestral cell from which all multicellular organisms evolved.

1892
German scientists Theodor Boveri and Valentin Häcker pronounce the stem cell the mother cell of the germ line, based on the theory that there is a common cell capable of self-renewal and differentiation.

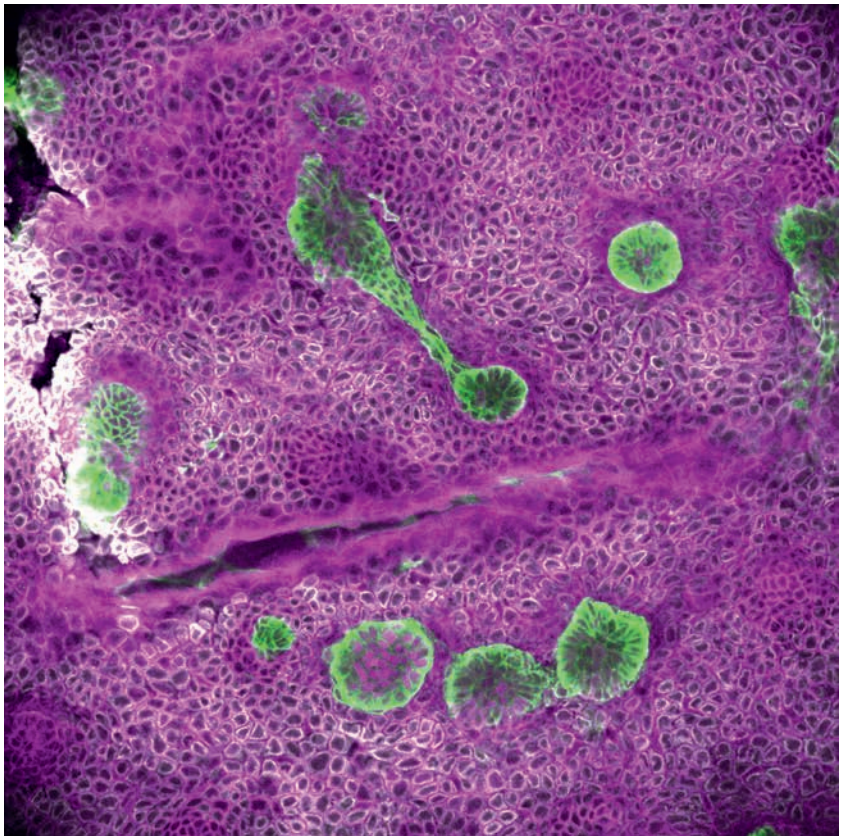
1958
The first allogeneic bone marrow transplant is performed in France, on survivors of a nuclear accident. Later, scientists will learn that stem cells are the key component of the lifesaving therapy.

1964
Canadian biologists Ernest McCulloch and James Till provide the first experimental evidence of the hematopoietic stem cell from which various types of blood cells originate.

1981
British scientists Martin Evans and Matthew Kaufman and American biologist Gail Martin isolate and culture the first embryonic stem cells from mouse embryos.

1983
Howard Green, an American physician-scientist, successfully treats burn victims with skin grafts derived from epidermal stem cells.

1988
The U.S. government places a moratorium on federally funded research on fetal tissue transplantation. Over the next 30 years, similar restrictions will be repeatedly repealed and reenacted.



Epidermal stem cells (green) form small buds that might later develop into tumors.

Without these cells, now known as adult stem cells, no epidermal structures would grow.

Adult stem cells can be found in virtually all the body's organs, where they live in localized homes called niches and self-generate to replenish the surrounding tissue. If it weren't for adult stem cells, these organs would wither and we wouldn't live long.

Elaine Fuchs was in graduate school when a lecture by Green upended her plans. Well on her way to becoming a microbiologist, she was so intrigued by the new discoveries that she switched gears and joined Green's lab as a postdoc. Today, four decades later, she continues to study epidermal stem cells, which have guided her to numerous discoveries in far-ranging fields, from inflammation to cancer.

1

Number of epidermal stem cells needed to generate enough skin to cover the adult body.

Fuchs realized early on that to understand the rejuvenating power of stem cells, she needed to study not only the cells themselves but also their niches, and the dynamic interactions taking place among stem cells, their neighbors, and their local environment. "A key discovery was that adult stem cells require other cells in their surroundings to be able to behave properly," she says. "Many recent breakthroughs in the field—like organoid cultures, for example—are based on the knowledge that stem cells are not operating in a vacuum."

Because stem cells continually exchange signals with their surroundings, Fuchs adds, all efforts to grow tissue in a dish begin with decoding the signals those cells use to communicate.

In the skin, specialized stem cells make the epidermis, hair follicles, and sweat glands; and in other organs like the intestines and lungs, different epithelial stem cell types regenerate similar absorptive layers. Bit by bit, Fuchs's lab is piecing together what goes on in the niches of these different stem cells, pinpointing the specialized microenvironments that nourish and guide them, and how those environments change in injury, inflammation, and cancer.

"In the skin, there are 65 different cell types, and we believe a good number of them interact with stem cell niches in various ways and at different times," Fuchs says. Neurons, fibroblasts, adipocytes, muscle cells, and immune cells all communicate with skin stem cells to do the right thing at the right time. And recently, the lab discovered that the lymphatic

1998
A team led by American developmental biologist James Thomson isolates the first batch of human embryonic stem cells.

2006
A Japanese group led by stem-cell scientist Shinya Yamanaka creates induced pluripotent stem cells by reprogramming adult cells to an embryonic state.

2009
Stem cell lines derived at Rockefeller are among the first to be included in a National Institutes of Health repository.

2009
The first human trial of an embryonic stem cell-based therapy is cleared by the FDA to treat patients with severe spinal cord injuries.

2009
A team led by Dutch molecular geneticist Hans Clevers creates the first organoids from adult stem cells of the intestine.

2011
Elaine Fuchs identifies cancer stem cells of squamous cell carcinoma and shows that these cells make tumors more aggressive.

2014
Ali H. Brivanlou develops a microchip-based system to grow synthetic human embryos in culture.

Learn more about these milestones in the story.

Epithelial stem cells have led Fuchs to discoveries in far-ranging fields, from chronic inflammatory diseases to cancer.

vasculature wrapping around the niches synchronizes the activities of stem cells across a tissue. The dynamic interplay between stem cells and their niche is at the heart of the skin's routine maintenance and its ability to repair wounds, Fuchs says, and it also plays a role in many medical conditions—enough to keep her lab perpetually busy.

"How are stem cells suddenly called into action to regenerate tissue damaged by injury?" she says. "How do they defend against pathogens and cope with infections and inflammatory stress? And why do they sometimes malfunction?"

In eavesdropping on stem cells in their niche, the lab has made several surprising discoveries. Among them is the fact that many chronic conditions traditionally considered immune disorders—including psoriasis, atopic dermatitis, inflammatory bowel disease, and asthma—might be the result not of a malfunctioning immune system but rather what happens when epithelial stem cells lose the ability to mount a robust barrier against pathogens or when they miscommunicate with the immune system. Moreover, Fuchs and her coworkers suspect that chronic inflammatory diseases may occur because stem cells harbor memories of past inflammatory episodes, causing them to react too quickly to a new trigger.

It was Fuchs and her colleagues who discovered in 2017 that stem cells draw on their memories in responding to threats—a process similar to immune cells' ability to remember a pathogen and respond more quickly on subsequent encounters. It's generally a useful adaptive mechanism that strengthens the body's defenses throughout life. The problem, Fuchs explains, is that repeated assaults might cause the cells to overreact to irritants or pathogens, leading to runaway inflammation that may manifest as rashes, pain, and other symptoms typically seen in these disorders. And her research suggests that stem cell memories are long-lasting: In a lab mouse, they can persist for up to six months, the equivalent of about five human years.

Can these memories be erased? Fuchs thinks so, and her team is exploring this possibility. Ultimately, the researchers hope that their work will lead to the development of new treatments for chronic inflammatory conditions to replace current immunosuppressive drugs, which have undesirable side effects and are not always effective.

>200
Number of cell types in the human body.

65
Number of cell types in the skin.

There are other circumstances in which adult stem cells trigger disease instead of healing and protecting their native tissue. One is metastatic squamous cell carcinoma, a highly aggressive type of skin cancer that Fuchs and her coworkers have studied for many years.

Like most of the body's tissues, healthy skin epithelium contains many mutant cells, which is usually not a problem. When a mutant shows up, neighboring cells will keep it in check by curbing its proliferation, for example—and after a day or two, the cell will die. But if the mutant happens to be a stem cell, which is the case in squamous cell carcinoma, the scenario becomes far more dangerous. Because it can self-renew indefinitely, the stem cell will hold on to all the mutations it has gained in the past, building a vast repertoire of genetic errors and improving its chances of dodging the intrusive neighbors.

How does this cell dupe nearby cells, and how does it cut itself loose from its locale of origin to leak into the bloodstream and metastasize? "The epithelial stem cell has to manage to survive in order to give rise to cancer, and to do that it needs to change the way it communicates with other cells," Fuchs says.

Recently, her team found that squamous cell carcinoma cells exit their native nook by giving self-serving instructions to neighboring epithelial stem cells that maintain the surrounding epithelium. "Epithelial stem cells normally control the tissue architecture,"

JOHN ABBOTT

Soto-Feliciano wants to know how children's cancers differ from those affecting adults.

Fuchs explains. "But when a mutant cell induces them to change their gene-expression program, they begin to lose control of the mechanical properties needed to keep the tissue supple and healthy."

She and her coworkers found that the mutant cell can create an escape route by generating stiffer surrounding tissue. As a result, mechanical forces build up, allowing the mutant to break through and pierce into deeper layers of the skin, from where it can spread further throughout the body via capillaries. More benign skin cancers such as basal cell carcinomas, on the other hand, produce less rigid cell structures and are more likely to stay put.

The lab is now working to pinpoint which of the many mutations found in squamous cell carcinomas are responsible for hijacking the normal ability of epithelial stem cells to proliferate and heal wounds. "Figuring this out will get us much closer to understanding how the disease advances and develop effective interventions," says Fuchs, the *Rebecca C. Lancefield Professor*.

>1M

Number of stem-cell transplants performed to regenerate blood cells in patients.

LIKE FUCHS, C. David Allis followed the science where it led him—and became a cancer biologist along the way. A pioneer in the study of epigenetics, he began working in stem cells because they provide a convenient model, a cell-based system for studying an organism's entire genome in action. While specialized cells are more obscure—they've typically closed off all the genes they don't need—stem cells are a blank slate. Every part of their genome is flexible, allowing scientists to explore a vast universe of epigenetic programming taking place in development, dementia, depression, or your biological context of choice.

With his coworkers, Allis, who is Rockefeller's *Joy and Jack Fishman Professor*, has discovered several epigenetic mechanisms that normally keep stem cells in check but may be disrupted in cancer. Yadira Soto-Feliciano, a former postdoc in the lab who recently became an assistant professor at MIT, says this chain of events happens in pediatric cancers in particular.

Why pediatric cancers? And why are few adult cancers caused by these epigenetic mishaps? To answer these questions, Soto-Feliciano wants to know what else distinguishes a young person's cancer from that of an adult. Focusing on childhood blood cancers, she is pinpointing the mechanisms that mess up stem cells' epigenetic programming in the first place.

Recently, she and Allis found a clue. They discovered that in pediatric leukemia, the initial disease culprit is a molecular machine that normally prevents malignant transformation by quieting certain genes in stem cells of the blood. A DNA rearrangement creates a riotous version of the machine that switches the genes on rather than off. Cancer ensues.

"The cells begin to proliferate rapidly, but without actually reaching their final form," Soto-Feliciano says. "They become frozen in a stem cell-like state and can't mature into blood cells."

In her lab at MIT, she's looking for a way to free these trapped stem cells and help them grow out of their "stemness"; and she's exploring the possibility of developing new drugs based on that principle. The task may be accomplished by targeting the mutant machine with small molecules (several such molecules are currently in clinical trials for leukemia). But there is an additional layer of complexity: Soto-Feliciano has found that leukemia cells thrive not just by turning on growth-fueling genes, but also by shutting down other genes that normally suppress growth. She is now testing a combination therapy that, in addition to neutralizing the growth-fueling mutant, also switches suppressor genes back on, finishing off the cancerous cell once and for all.

"Ultimately, the disease is a fight between two types of genes," she explains, "those that promote cancer by making stem cells unable to differentiate, and those that suppress tumor growth by making the stem cells proceed normally through development. An ideal cancer therapy will address both ends of that equation." ◎

LORI CHERTOFF

How did evolution come up with that most human of all human traits—our ability to imitate and produce speech? The answer has eluded scientists for centuries. But all along it may have lurked above their heads, in the treetops.

By Bahar Gholipour and Eva Kiesler

REPEAT AFTER ME

New insights challenge our very notion of what language is, and how it came to be.

HUMANS ARE EXTRAORDINARY. "Paragon of animals," as Hamlet put it, with "god-like intellect," Charles Darwin added. We will never run out of words to marvel at our own uniqueness. And we have modern medicine and atomic bombs to show for it.

But while testaments to our specialness abound, it's been harder to pinpoint what exactly makes us special. Is there something about the human brain that lifts us above our fellow vertebrates? So far, biologists have found no gene, no neural circuit, no anatomical structure that makes us meaningfully different from all other creatures. And one by one, new studies find that other animals possess traits once believed to be quintessentially human: tool use, culture, morality. Even our aptitude for spoken language has turned out to be not so unique.

"Humans are actually one of several animals capable of vocal learning," says neuroscientist Erich D. Jarvis, his words intermingling with the chirps and squeaks from a nearby cage.

As Jarvis continues to talk, he reaches inside the cage and gently retrieves the little noisemaker, a zebra finch small enough to nestle in the palm of his hand. Its cheeks are bright orange, a sign that it's a male. Jarvis gently deposits the bird into another cage, where two female finches huddle on a perch. The male flutters up to a nearby perch and resumes the concert, and Jarvis explains that the finch's song is a language comparable to human speech, passed on in its family for generations. Like other songbirds, the finch is capable of vocal learning, a component of language that allows newborn birds to learn to produce sounds with distinct meanings, the closest thing to words and sentences that we know of. It's a trait that songbirds share with humans and a few other animals—a seemingly arbitrary group that includes whales, bats, elephants, and parrots, among others.

Jarvis likes songbirds (who doesn't?), but he is not studying them for their own sake. With bird experiments and powerful genomics tools, he uses the finch as a window into the neural constituents of vocal learning, and as a lens through which to illuminate more than 60 million years of evolution. Bit by bit, his lab is reconstructing the trajectories in which birds, humans, and other vocal learners evolved their knack for vocal communication. Their work has already turned up surprising insights, some challenging our very notion of what language is and how it came to be. And ultimately, what the scientists are learning from birds might reshape our understanding of how the human brain produces not just speech, but any kind of behavior, emotion, or thought.

"The most complex brain function we know of is that which enables spoken language," Jarvis says. "If we can figure out how it works, maybe everything else will fall into place."

LANGUAGE IS THE bedrock of human culture. Without it, our ideas would mostly stay inside our heads, and all the knowledge that's been passed from one generation to another would be long lost. Whether we'd even be able to *think* without language is an open question, and there would certainly be no poetry, no stories, no politics, and no science.

But what's the essence of language, and where did it come from? These questions have been pondered by some of the world's most notable thinkers, from John Locke to Noam Chomsky, but no clear answers have materialized.

Here's another question, which Jarvis and his lab members like to chew on: Why us?

"Among the vocal learning species, humans appear to be the most advanced," he says. It's

PREVIOUS SPREAD: CARLOS A CARRENO / C3.PHOTOS / GETTY IMAGES

For Jarvis, the zebra finch is a lens illuminating 60 million years of evolution.

MATTHEW SEPTIMUS

tempting to conclude this is because we're smarter than other animals—until you consider who's in second place.

But first, let's consider that while vocal learning is rare, it is just one of several components of language—and some of these other components are widespread in the animal kingdom. "For example, many animals are capable of auditory learning, the trait that allows dogs to learn the meaning of sounds like 'sit,'" Jarvis says. "Dogs are also capable of vocal-usage learning, the ability to produce a specific sound in a given context, like whining to beg for food."

So, if humans are the gold medalists in vocal learning, who takes silver? Not the chimpanzee, nor the bonobo, nor the gorilla. Our closest relatives are poor speakers, even though they can be excellent auditory learners (case in point: Koko, a famous gorilla, learned thousands of words but used only sign language to communicate them). The fiercely intelligent dolphin? Closer. Dolphins are indeed vocal learners, but they're not in the top tier. Second best after humans is none other than the parrot, with the songbird coming in third.

Humans, parrots, songbirds: It's a strange club. Our ancestors parted ways with the ancestors of birds more than 300 million years ago. How can it be that, of all things, our strongest competitors in vocal learning—the trait that's supposed to be our edge as a species—are a bunch of feathery dinosaur descendants with brains no bigger than a grape?

"Evolution invented vocal learning several times independently, just like wings evolved separately in insects, bats, and birds."

For one thing, vocal learning has little to do with the size of an animal's brain. What matters is the brain's anatomy. Fernando Nottebohm, a Rockefeller professor emeritus and Jarvis's former Ph.D. adviser, discovered in the 1970s that vocal-learner birds have specialized brain regions he called song nuclei, whose sole function is to control the animals' ability to learn new sounds and produce them. These regions are missing in nonspeaking birds, like woodpeckers and chickens.

As a grad student, Jarvis discovered that when songbirds sing, the firing of neurons in their song nuclei causes certain genes to switch on—and for each burst of song, the expression of these genes increases. At the time, in the late 1990s, these findings challenged scientists' understanding of how learning and memory happens. "When the birds learn a song," Jarvis says, "this doesn't just involve changes in synapses, as once thought, but also changes in the neurons themselves."

Years later, as an associate professor at Duke University, Jarvis was able to expand on these findings and ask at what point a handful of birds evolved song nuclei in the first place. He co-led an international consortium of scientists that spent several years sequencing and analyzing the genomes of 48 contemporary bird species—finches and other vocal learners, and their nearest relatives that are not vocal learners. The result was a stack of 20 papers that, when published near-simultaneously in 2014, landed with a thud in the bioscience world. It gave researchers worldwide a wealth of new data to work on, including a new evolutionary family tree of bird species going back to the extinction of dinosaurs, when most modern birds arose. This exhaustive map upended much of what people thought they knew about birds (it turned out that grebes are more closely related to flamingos than to ducks, for example, and that swifts are closer to hummingbirds than to swallows), and for Jarvis, it confirmed what he had long suspected: that the three species of vocal-learner birds—songbirds, hummingbirds, and parrots—are evolutionarily distant. Each must have acquired song nuclei on its own.

"Over the past 66 million years, evolution invented vocal learning at least three times independently among birds," Jarvis says, "just like wings evolved separately in insects, bats, and birds."

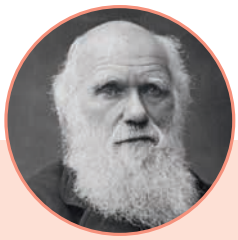
FROM LEFT: NATIONAL PORTRAIT GALLERY, LONDON; HERBERT ROSE BARRAUD / WIKIMEDIA COMMONS; ALAMY

Five ideas about language

The essence of human speech has been debated for centuries—and the mystery continues.

1690s
John Locke

God gave humans the capacity to articulate sounds. We're able to use these sounds "as marks for the ideas in [our] own mind."



1830s
Charles Darwin

Human language evolved from animal sounds, like birdsong. "Animals communicate to each other."

1860s
Max Müller

Darwin is wrong. Animals don't have language; in fact, language is "a barrier between the brute and man."

SKETCHED IN A diagram, song nuclei make the bird brain look like a flow chart with arrows crisscrossing among seven clouds, each representing a vocal brain region. The arrows trace two neural pathways, one for learning new sounds and another for producing them. When Jarvis showed that this complex neural architecture is practically a mirror image of the human brain's vocal pathways, some took his findings as evidence for the existence of God. How else, despite being separated by eons of evolution, could two species have been recently upgraded with new brain modules of the same design? Jarvis was perplexed, too, until he stumbled on a major clue in the process of studying birds hopping on an exercise wheel.

Birds are bundles of energy. During experiments, they will not only sing but also walk, sprint, and wing-whir. Jarvis and his colleagues monitor the birds' brains during these activities either by recording the firing of neurons using tiny electrodes, or by measuring the expression levels of genes known to be sensitive to increased neuronal activity.

This was how the team serendipitously discovered the bird brain's motor-control regions, which happened to sit right next to the song nuclei. Further experiments showed that in vocal learners, the song nuclei are deeply enmeshed with the motor regions, and they extend neural connections from the cortex all the way down to the neurons in the brain stem controlling the voice box. This could mean that the same pathways that enable chicks to learn to hop or fly also enable them to learn new sounds—a radical finding that other scientists later reported from studies of the human brain.

The upshot is a new way to think about speech: as a motor skill, not unlike riding a bike.

"We think the vocal pathway evolved out of the motor pathway," Jarvis says, adding that in embryos, motor pathways may duplicate multiple times to connect with various muscle groups. He believes that at one point in evolution, an extra duplication occurred in humans that yielded the specialized motor pathway that controls our voice box—and that such super-duplications also produced the vocal pathways of songbirds, parrots, and hummingbirds.

All of which begs the question: If evolution executed this process at least four times, might it also have made other animals like dolphins and bats capable of vocal learning?

Jarvis is not sure, but his team has found a way to peek inside the dolphin brain. When dead dolphins wash up on the shores of Jones Beach State Park on Long Island, graduate student Brigid Maloney hurries over to collect brain tissue. Her goal is to compare the anatomies of vocal pathways of different speaking animals.

"We know that songbirds and humans share a set of genes only expressed in vocal areas," Maloney says. "We're trying to see if the same genetic program exists in dolphins." She has developed a meticulous technique for preparing thin slices from the big dolphin brain, and jerry-rigged a microscope to examine these samples. Her study could illuminate the neurobiology of language in a vocal learner that's neither human nor bird. (Dolphins and other cetaceans such as orcas are rarely used in neuroscientific experiments, nor are whales or elephants. For starters, they don't fit in a lab.) And if the researchers' theory of the motor origin of language is correct, Maloney should trace the dolphin's vocal pathway to the brain's motor areas.

Maloney and Jarvis are hoping to find out soon. "Give us a year or two, and we might have the answer," Jarvis says.

IN THE MEANTIME, other members of the lab are making headway toward deciphering the genetic underpinnings of vocal learning. Former postdoc Lomax Boyd and graduate student César Vargas have been studying how neurons forming

1950s
Ludwig Wittgenstein

Language is a set of social games. The meaning of words and sentences depends on the rules of the game being played.

1960s
Noam Chomsky

The capacity to learn language is innate. Humans are born with "universal grammar," a basic understanding of how communication is structured.

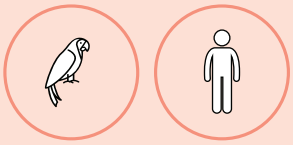
FROM LEFT: MORITZ NÄHR / WIKIMEDIA COMMONS; AP

The vocal learning spectrum

Animals have varying degrees of vocal learning. Many use innate vocalizations to communicate, but only a few are able to modify these sounds or learn new ones.

Vocal learning complexity

1 **High vocal learners**
Are able to learn new vocalizations throughout life.



2 **Complex vocal learners**
Can learn complex, syntactic-like vocalizations.

3 **Moderate vocal learners**
Are able to modify innate vocalizations or control them at will.

4 **Limited vocal learners**
Can make only subtle modifications to innate vocalizations.

5 **Vocal non-learners**
Can neither learn new vocalizations nor modify innate ones.

the forebrain-larynx connection regulate a group of genes that are similar across human and vocal-learning bird species, enabling us to learn new sounds from our elders.

Here's how they think it works: When a neuron expresses a gene named SLIT1, it will not connect with other neurons expressing a receptor called ROBO1 (this is a standard way for developing neurons to know which other cells to connect with and which cells to avoid). In animals that don't speak, an active SLIT1 gene prevents new pathways from forming between the cortical neurons and brain-stem vocal neurons, which control the larynx (or the bird version of the larynx, called the syrinx). However, Boyd, Vargas, and their colleagues propose that in the speaking species, SLIT1 is silent in the vocal areas. "When SLIT1 is off, the neurons can stay in touch," Vargas explains. "And that means the animal's brain can develop fine control over the laryngeal muscles."

A similar mechanism might exist in humans, although we have an additional layer of control over the formation of neural connections, controlled by a gene called SRGAP2. The result, Jarvis says, is that our brain networks don't become rigid and unable to continue learning once we grow up, as is the case with adult birds. The multiple layers of fine-tuning keep our brains in a plastic condition, while the brains of other animals become more "solidified," he says. "The system causes our neurons to stay in a more childlike state, allowing us to learn new vocalizations throughout our lives."

The genetic mechanisms that keep vocal neurons youthful might be a key additional ingredient of vocal learning, perhaps the very

Maloney is studying the biology of speech in dolphins.

Speaking mice may be up next.

MATTHEW SEPTIMUS (2)

thing that once allowed human ancestors to turn innate grunts into meaningful, complex expressions. Jarvis and his team are now working to test this theory. Would it be possible to reverse engineer the development of vocal pathways and bestow the gift of speech on a nonspeaking animal like, say, a mouse?

Until recently, scientists thought mice completely lacked vocal learning, but Jarvis's team has found otherwise. During courtship, male mice produce ultrasonic vocalizations that are not unlike birdsong, except for the fact that human ears can't hear them. The researchers discovered that the animals can sometimes make small modifications to these sounds. When housed in a socially competitive environment, for instance, different strains of mice with different songs will try to match each other's pitch.

Looking closely at these animals' brains, Jarvis and his coworkers also found a pale trace of the vocal anatomy of songbirds and humans. "Mice were thought not to have any neuroconnectivity from the cortex to the larynx motor neurons at all," he says. "But we found a rudimentary connection consisting of very sparse axons."

The researchers suspect these weak links could be enhanced by manipulating speech genes—for instance, by turning off SLIT1 in the vocal regions of the mouse brain, or by replacing the gene with the human version. If either approach works, the resulting mouse will presumably become more capable of speech-like communication.

A speaking mouse would be more than a scientific gimmick; it would provide an invaluable model for further studying speech as well as speech-related disorders such as stuttering. Because no such model currently exists, a speaking mouse could galvanize research in this area and make it possible to develop new drugs or other interventions.

"It would also give us the first model in which to study the neuroscience of vocal plasticity, or the advanced type of vocal learning that only humans and a few other mammals are capable of," Jarvis says. "This may well be the most exciting thing we've ever worked on." ◎

What do scientists do when their most pressing question cannot be answered with existing technology? Make their own from scratch, of course.

The tools that drive discovery

By Joshua A. Krisch

MATTHEW SEPTIMUS (2)

JEFFREY DEMAS ADJUSTED his protective eyewear and studied the bulky vertical band saw. He was about to shape a sheet of aluminum into a bracket—the potential solution to a problem he had been wrestling with for years.

A postdoc in the laboratory of Alipasha Vaziri, Demas was perfecting a new microscope that would make it possible to watch brain processes unfold with unprecedented speed and precision. The technique relied on a scanner that enabled researchers to capture crisp images at different depths in brain tissue and detect thousands of neurons firing at once. But as Demas had learned the hard way, the scanner had to be precisely aligned—so precisely, in fact, that even a few hundred microns off-kilter would turn precious data into meaningless noise. To keep the precarious scanner in place, he needed a bracket with the right dimensions. And it did not exist.

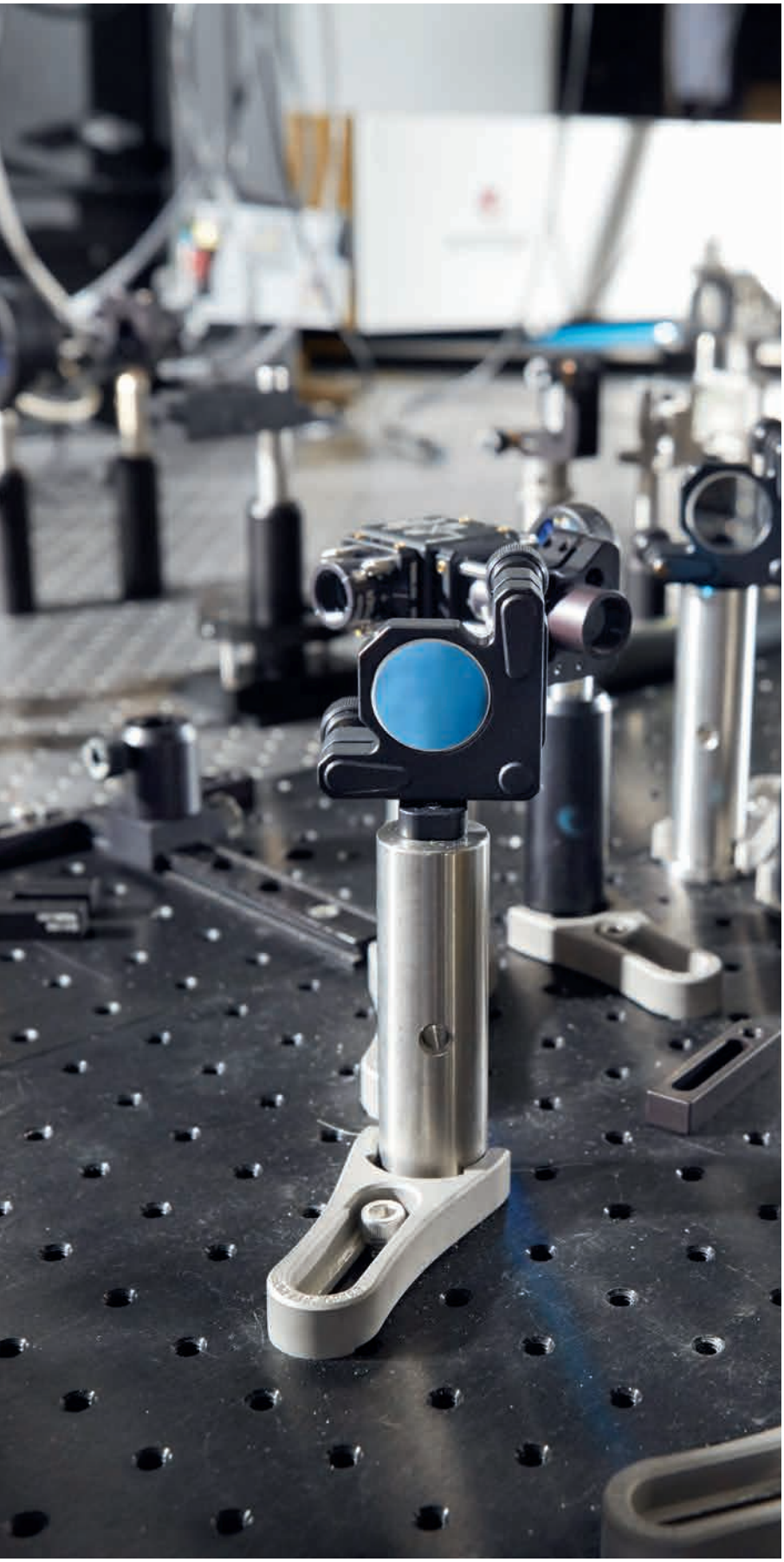
In the Precision Instrumentation Technologies (PIT) resource center at The Rockefeller University, under the watchful eye of the center's instrumentation engineer, James Petrillo, Demas made a few final adjustments to the band saw. "Let's give it a shot," said Petrillo, raising his voice over the thrum of the saw whirring to life. "Hopefully we don't–"

A buzz, a crash. Silence. "Not your fault," Petrillo laughed, raising his safety goggles to survey the damage. "It's a temperamental machine." With a shrug, Demas retired to a nearby desk, reconsidering with pen and paper how he might manufacture his elusive bracket.

Biologists need highly sophisticated tools to answer their most burning questions—and more often than not, those tools cannot be found on any vendor website. This means researchers must venture on elaborate detours, working with engineers—or moonlighting as engineers themselves—to create instruments that fit their needs. Sometimes a new device can be fashioned with a band saw; in other cases, solving problems at the cutting edge requires a less conventional tool—a novel biochemistry approach or advanced computer software, for example. And fashioning a new instrument or technique is almost never straightforward.

"Only a handful of scientists are committed enough to develop new tools when they hit a barrier in their research," says Nathaniel Heintz, the *James and Marilyn Simons Professor*, a neuroscientist and innovator. "But if they do it well, it pays off. For a period of time—before their technology is replaced with a better one—these people are allowed to see things that others have never been able to see before."

Here are four tools in the bioscience vanguard.



The no-compromise microscope

Alipasha Vaziri

THIS JUMBLE OF cables, lenses, and power supplies may not look like much. But the light-beads microscope is one of the most powerful imaging tools ever invented, capable of capturing activities of neurons within large swaths of the mammalian brain.

"Each neuron can have up to ten thousand connections to other neurons, so superior imaging is crucial if we wish to capture the brain's structure and function in action," Vaziri says.

The ideal microscope will be able to visualize neurons with crystal clear resolution as they actively call out from distant corners of the cortex, within a fraction of a second of one another. The fundamental difficulty comes down to resolution, scale, and speed—parameters that tend to be mutually exclusive. Traditional imaging tools often sacrifice scale for resolution, or vice versa. When scientists insist on having both, they typically circumvent the problem by taking snapshots of separate parts of the brain that are later stitched together, a workaround that sacrifices speed and makes it difficult to see action in remote nooks of the brain. And the deeper inside the brain they look, the more challenging the experiment becomes, because dense tissue has a nasty habit of scattering light.

The lab's latest feat of engineering, light-beads microscopy is so refined that it can record the activity of more than one million neurons across the mouse cortex. It involves breaking one strong photon pulse into 30 smaller subpulses that descend to various depths, each separated by a billionth of a second, making it possible to image very dense tissue. A cavity of mirrors ensures that each pulse reaches its target, causing nearby neurons to fluoresce no matter how deep inside the tissue they are embedded. The method also eliminates the dead time between sequential laser pulses, speeding up the entire process.

Like other imaging techniques developed in Vaziri's lab, light-beads microscopy is designed to retrieve as much data from the brain as possible. "That's the spirit of our approach," he says. "With the minimum number of photons, how can we extract the maximum amount of information?"

The optical portion of the light-beads microscope.

The mRNA trap

Nathaniel Heintz

WITHIN ORDINARY MICROTUBES, a remarkably potent tool is brewing. Dubbed "translating ribosomal affinity purification"—TRAP for short—the method was among the first to allow scientists to identify and sort the hundreds of cell types that make up the human body.

Before TRAP, researchers often studied diseases by isolating a patch of problem tissue, mashing it up, and analyzing all the genes expressed in the hodgepodge of cells. Although this method could identify faulty genes, there was no way to pinpoint the individual cells that produced those genes, so the actual disease culprits remained unknown.

"When you have a collection of 10,000 genes expressed in a tissue sample, figuring out which of those thousands are driving pathology can be very difficult," Heintz says. "We wanted to know everything happening to the individual cell types in the tissue."

Along with the late Paul Greengard and then postdoc Myriam Heiman, Heintz realized that they could distinguish one cell type from its neighbor by tracking the repertoire of proteins that each cell type produces—its so-called translational profile. Their TRAP tool literally traps a cell's mRNAs molecules—the actionable readouts from its genes—as they reach ribosomes, the cellular machinery responsible for turning the RNA sequence into protein. A fluorescent tag makes it possible to extract ribosomes from tissue samples and analyze their associated mRNAs. The result is a unique molecular signature that distinguishes otherwise similar cells from one another.

Individual cell types can be identified based on the proteins they make.

"TRAP told us everything," Heintz says.

But optimizing the technique turned out to be a marathon of trial and error. "Decent ideas almost always work in cell culture," Heintz says. "But making the same idea work in a living organism, or even postmortem brain tissue, can take years." The team tested different bits of the ribosome to figure out

Microcutters, mills, and screwdrivers

In a basement machine shop, a ten-ton milling machine the size of a studio apartment whirs, coolant splashing a gleaming suite of blades and drill bits that can cut grooves in metal down to the micron. Nearby, a laser cutter hums, and a 3D printer swipes to a preprogrammed rhythm. Rockefeller scientists pop in and out, their prototypes changing hands, suffering yet another round of revisions or, on occasion, working just right.

Surrounding the PIT's state-of-the-art instrumentation, walls and shelves are cluttered with low-tech tools—adhesives and abrasives, sanding stones, glass cutters, drill bits. And screwdrivers. So many screwdrivers.

"The design space that screws occupy is incredible," Petrillo says. "No matter how technologically advanced a project is, it will almost always require a simple screwdriver."

When researchers approach Petrillo with an idea for a new scientific instrument, he considers the entire array of machines and tools at his disposal. "Almost all of our projects go through an extended

PIT manager Peer Strogies at the shop's standard milling machine.

JOHN ABBOTT; ALEXANDRA ROWLEY; ZACHARY VEILLEUX

which would be the easiest to tag. And they tested various tags looking for one with strong fluorescence to produce a signal sharp enough to record and profile the cells.

Heintz's lab now uses TRAP to identify the cells and molecular events driving neurological conditions such as autism spectrum disorders, Parkinson's disease, addiction, anxiety, and depression. In each case, the team's approach is to ask how cells differ from one another and what happens to them in disease. "I don't have any particular favorites," says Heintz. "I just want to look at as many cell types as possible."

A harness, built on the fly

Gaby Maimon

TO THE UNINITIATED, it's a shallow upside-down cup marred by a tiny hole in its base. To behavioral neurophysiologists, it is an invention that transformed the field—a harness that allowed scientists to, for the first time, reliably observe activity in the brains of living, behaving fruit flies.

It was once impossible to study the neurophysiology of fruit flies without immobilizing them. In 2007, when Gaby Maimon was a postdoc at the California Institute of Technology, the method of choice involved cutting a tiny fly-shaped hole in aluminum foil, stuffing the fly into it, and adhering the insect in place with wax. Scientists would then remove a piece of cuticle over the fly's head and, while bathing the brain in saline, take measurements from neurons as they presented odors to the dry antennae ensconced safely below the saltwater.

But Maimon wanted to study the brains of naturally behaving fruit flies. "The hope was that we could one day make similar recordings, but in a fly that could also flap its wings in tethered flight or perform walking navigation on a tiny, fly-sized treadmill," he says.

In the laboratory of Michael Dickinson, Maimon began a long process of trial and error to improve on the crude foil harness. If it fit just right, Maimon imagined that scientists would still be able to keep the brain in a saline bath while the animal went (or flew) about its business.

"We contacted several microfabrication companies to make this harness for us, and they asked us to commit to making ten or twenty thousand," Maimon says.

MATTHEW SEPTIMUS (2)

Petrillo working on the eight-ton Hermle C22 CNC mill.

troubleshooting phase," he says. "We make something, the scientists try it, but it doesn't work. So the scientists come back, and we adjust it until it does."

For example, neuroscientist Vanessa Ruta once brought Petrillo an idea for a setup to study how fruit flies respond to olfactory inputs—a tiny arena on which the animals would be fed various odors from different directions. Petrillo's initial offering was a spartan dish made from white plastic; and with every round of feedback, it became more sophisticated. The final product incorporates a camera tracking a fly navigating a virtual odor environment, all under the lens of a two-photon microscope.

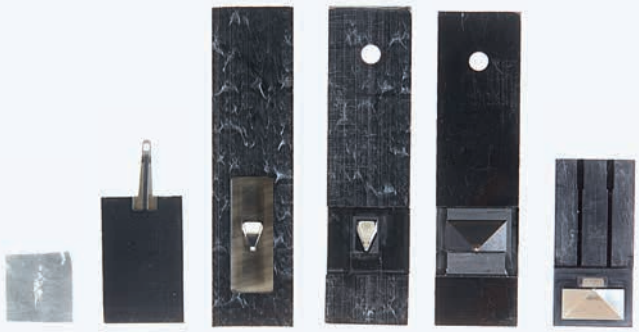
When working with less forgiving material than plastic, there's not as much creative latitude—which is why Vaziri and his coworkers typically bring Petrillo precise parameters for their microscopes. "We start by creating a 3D model of the original microscope to make sure all of the new parts we make will interface with it properly," Petrillo says. "Only after receiving feedback from the lab, and going through several iterations and adjustments, are we ready to machine a component. Because once we sculpt that piece from a giant block of aluminum, there is no room for error."

Evolution of the fly harness

The original fly harness was simple: aluminum foil with a hole that fit the fly's head (1). But the fly wasn't able to move, so Gaby Maimon, who wanted to keep the fly's head still without significantly impeding its natural movements, began to innovate.

Placing the hole at the end of a metal plank (2) gave the animal more visibility to look around but still restricted movement, a problem solved by putting the hole on the apex of a pyramid-shaped cup (3 and 4). Now only the crown of the fly's head was tethered to the harness, leaving the rest of the body free. Still, experimenters found it difficult to fit forceps into such a narrow design, so subsequent prototypes featured broader pyramids (5 and 6).

The current version includes tiny magnets that attach the harness to a microscope as well as two small light sources that illuminate the fly during experiments (6).



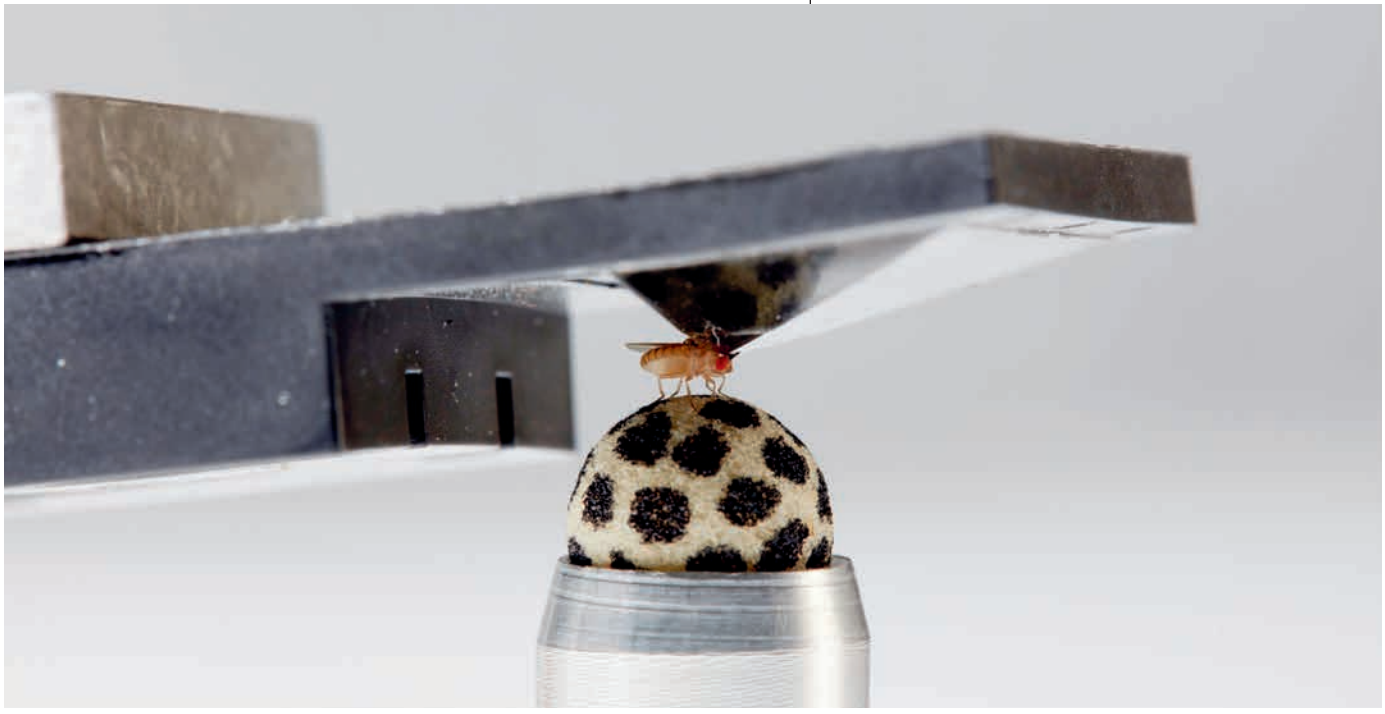
1 2 3 4 5 6

"But mass fabrication made little sense, because we didn't know if our designs would even work." So, with help from postdoctoral colleague Wyatt Korff, Maimon set out to make the harness himself on a milling machine.

He churned out one prototype after another. A pyramid-shaped cup too narrow to allow the experimenter to dissect the cuticle. Another pyramid too shallow to allow the fly to flap its wings without hitting plastic. And then, at long last, an efficient harness that allowed the insects to perform tethered walking or flight, as researchers measured signals in the brain.

In this new design, the fly's head and the front tip of its body were the only parts glued to the "harness" (a hole at the base of the pyramid). The rest of the body remained free, allowing the animal to flap its wings or walk. With this model, Maimon and colleagues have made great strides in their behavioral neurophysiology investigations, including recent work describing how parts of the fly's brain allow it to calculate its direction of travel or guide it toward a distant goal.

"Even halfway through developing this approach, it wasn't clear whether it would work, or work reliably, which made life a bit stressful as a postdoc," Maimon says. But when it finally came together, the novel harness launched a new way to study brains and behavior.



The fly can walk or flap its wings while its neurons are being recorded.

MATTHEW SEPTIMUS

A cell atlas based on data from more than four million fetal cells.

MONTY RAKUSEN/GETTY IMAGES; MATTHEW SEPTIMUS

Software for supersized data

YOU ARE LOOKING at one of the most powerful artificial intelligence programs in the world, software tweaked by Junyue Cao into a scientific tool that analyzes enormous biological data sets. It's innovation by adaptation—a repurposed tool, drawn into the lab from nonacademic sources.

Cao began working with artificial intelligence when the sheer size of his data sets became unmanageable. An expert on single-cell genomics, he and his coworkers are sequencing the genomes of millions of individual cells and analyzing subtle links between them, seeking to piece together a biological puzzle that may provide insights into many human diseases.

"Single-cell genomics faces two key challenges," Cao says. "One is to visualize huge numbers of diverse cells in low-dimensional space; the other is to identify the gene regulatory networks that operate within multiple layers of cells."

Cao recently published gene-expression information for four million individual cells in more than 100 types of fetal tissue—the largest single-cell genomic data set to date, which the lab is now scouring to identify cellular interactions that may herald disease. Cao stores the data and runs computations using the high-performance computing systems on campus. "If we used your laptop, it would take weeks to process this data," he says.

Junyue Cao

Cao and his colleagues tested and compared many machine-learning algorithms to visualize and classify the main cell types represented in large-scale data sets comprising thousands of genes and millions of cells. Many algorithms worked for normal data sets with hundreds or thousands of cells, but whenever the experiment was scaled up to the millions, the software came crashing down. And even when the researchers arrived at a clustering technique that worked, the program kept making mistakes, detecting links between cells that turned out to have low correlation.

"We again tried many approaches, until we landed on a machine-learning approach that let us manually remove weak links," Cao says. Apparently, there are some biological questions that AI isn't ready to answer without at least a little human supervision.

Cao is not a software engineer, and he does not develop the machine learning algorithms from scratch. But he's learned enough to adapt existing tools to his own needs. The machine-learning approach that his lab finally settled on was based on a technique that social media companies use to distill and track communities among users.

"It turns out, tracking millions of cell interactions is not entirely different from tracking millions of 'likes' on Facebook," Cao says.

INTERVIEW

Bioscience needs all the help it can get, says Cori Bargmann. And private foundations are in a unique position to open new doors to discovery, create stronger scientific communities, and make medical innovations accessible to people everywhere.

Philanthropy 2.0

By Eva Kiesler

John D. Rockefeller wasn't born rich. At age 16, working long hours as an assistant bookkeeper, he earned just $16 a month, the equivalent of $500 today. A conscientious young man, he nevertheless donated six percent of his earnings to the Northern Baptist church he attended each Sunday—and as his income grew, so did his charity.

By the turn of the 20th century, Rockefeller had become the wealthiest man in modern history and a keen patron of medicine, research, and the arts. In creating The Rockefeller Institute, the country's first biomedical research institute now known as The Rockefeller University, and supporting other scientific enterprises, he was among the first private citizens to position the nation for leadership in basic science.

Illustration by Matt Chinworth

A diverse ecosystem of funders makes us less likely to get stuck, and allows us to try new things.

Today, a new generation of donors is rewriting the playbook for biomedical research. For example, several foundations spun off from the tech boom, including the Bill and Melinda Gates Foundation and the Chan Zuckerberg Initiative (CZI), are pumping billions into bioscience labs to support high-risk, high-reward projects, many of which wouldn't happen without their sponsorship. "We are seeing a new flowering of philanthropy that will allow us to do brand new things in science," says Rockefeller's Cori Bargmann, the *Torsten N. Wiesel Professor*, who for the past five years has split her time between her own neuroscience lab in New York City and CZI in California.

As Head of Science at CZI, she oversees a grant program that thus far has awarded close to $1 billion with the goal to support research to cure, prevent, or manage all human diseases by the end of the century. We spoke with Bargmann about the growing impact of philanthropies and their opportunity to make science more efficient, useful, and just.

Here in the United States, the federal government provides ample support for basic research. Why do we need contributions from private donors, as well?
I think it's inherently a good thing that labs get support from several types of sources. Different funding organizations operate under different strategies, and philanthropists often bring expertise from other areas like the tech or business sectors, introducing new ways to drive science forward and maximize its impact. The result is a more diverse ecosystem of funders that will ultimately allow us to get more done. It makes us less likely to get stuck and gives us the freedom to try new things.

It's wonderful that we have the National Institutes of Health and the National Science Foundation, which fund a vast spectrum of biomedical science. Yet there are limitations to what areas these agencies will support and the time frame of their commitments, and philanthropies will often seek to complement government priorities. At CZI, we invest in key areas that will not get adequately funded by federal agencies but are nevertheless poised to yield transformative discoveries, the kind of projects that might unlock progress in many fields at once. And we take a longer view, supporting research whose impact may take 20 or more years to show up.

What are some areas where philanthropic support can have a big impact?
The NIH organizes its support under disease categories, and diseases affecting many Americans, like cancer or cardiovascular disease, tend to have the biggest budgets. This means that rare conditions—or conditions that are rare in the United States but more common in other parts of the world—may get sidelined unless private donors or nonprofits step in. Also, a system focused on diseases may fail to support other important things, like the development of new technologies.

Take microscopy, for example. It has enabled progress in research on virtually every disease, yet no major government program is explicitly dedicated to it. Who, then, will make critical investments in the development of new techniques? It's one of the ways philanthropies like CZI can make vital contributions.

Another is to invest in young scientists and make it easier for them to switch fields. Traditional funding systems tend to reward established researchers for their past successes, encouraging them to keep doing what they're doing, while being less supportive of new talent and new endeavors.

What are the typical roadblocks to innovation?
It depends on the project, and the only way to find out is to speak with the scientists involved. Sometimes their answer will surprise you—it's not always about money.

A project may stall because of intellectual hurdles, for example, in which case the scientists may need help finding collaborators with the right expertise. So, to maximize their impact, funding organizations must do more than write checks. Community building is key, as has been beautifully demonstrated by the Simons Foundation, among others. At a time when autism research was mainly pursued by childhood education experts, the foundation transformed it into a burgeoning bioscience field by bringing together expert clinicians, patients, and scientists studying everything from developmental biology to electrophysiology. You almost can't help learning from each other in such an environment.

How might funding organizations do a better job of supporting the most promising scientists?
A commitment to equity is a good starting point. There will always be more great scientists than there are resources to support them, and you need to do a great deal of work before you can make informed decisions. You need to look at everything each applicant has done in order to create a level playing field, a system in which everyone

Cori Bargmann in her home in Manhattan's Morningside Heights.

MATTHEW SEPTIMUS

has the same chance to get funded—regardless of where in the world they come from, whether they've trained in a famous lab, or whether their papers have been published in prestigious journals.

One thing I'm particularly proud of is that CZI, rather than taking a U.S.-centric perspective, now funds scientists in 61 countries and counting. For example, we just launched a grant program across Africa, Latin America, and former Soviet countries—places where there are many terrific scientists but a dearth of science funding. We're also supporting Open Science, a movement to make scientific data freely available to access and free to publish, to benefit scientists in under-resourced countries.

It is also important to recognize that there are different ways to be a scientist. Science needs support for systematic work like the Human Cell Atlas, a massive ongoing project to map all human cells and their molecular features; and it needs eclectic, curiosity-driven investigations that may generate chance discoveries. For example, green fluorescent protein, a molecular marker used to study all kinds of biological processes, was discovered when a group of marine biologists wanted to know why a certain type of jellyfish glows in the dark.

Can you share more of your thinking around equity in science?

There is so much work to be done on this front. Equity must be achieved on many levels—not just in terms of who gets to do the science but also what kind of science gets done and how it gets done, who gets to be at the table making the decisions, and who ultimately gets access to new medical treatments and other scientific innovations.

We have a lot to learn from past inequities. For example, today women are at significantly higher risk for dying when they have heart failure, largely because of huge gaps in our understanding of how the condition develops and manifests in the female body. Researchers spent decades studying cardiovascular diseases, but all clinical trials were done in men.

Similarly, people of African descent are more likely than those of European descent to die from kidney diseases, raising the question of whether our current investment in this field is adequate. And when new treatments are developed, will they be affordable enough for the populations that most need them?

You mentioned that we need to reevaluate who gets to guide decisions about biomedical research. What role do you see for patients?

I think patients have tremendous power to build support for research on their own diseases. CZI and a number of other foundations are now supporting patient groups, and there is real magic in that partnership. This has been especially evident for rare diseases, where research has been kick-started as patients began to organize on internet platforms. Suddenly, hundreds of patients with the same poorly understood disease would come together and compare notes, making scientific observations possible.

What will be the next big thing in bioscience?

An interesting change is under way as technological achievements that would have been impossible five years ago—in single-cell biology, microscopy, and artificial intelligence, for example—are becoming routine. Increasingly, these techniques are making it possible to create a dynamic and unified understanding of human biology.

Until now, we've mainly been looking at snapshots of biological processes, observing them one moment at a time and one system at a time. But we know that the body's systems work together—our immune system communicates with our nervous system, for example, and we see that COVID affects anything from blood clotting to your sense of smell. Scientists will soon be able to take their research to the next level by studying the body in action, watching molecular events play out in real time and across spatial scales. Human biology is incredibly vast, and the next 10 years might utterly transform our understanding of health and disease—perhaps leading to medical advances we cannot even imagine yet. ◎

N95 recycler

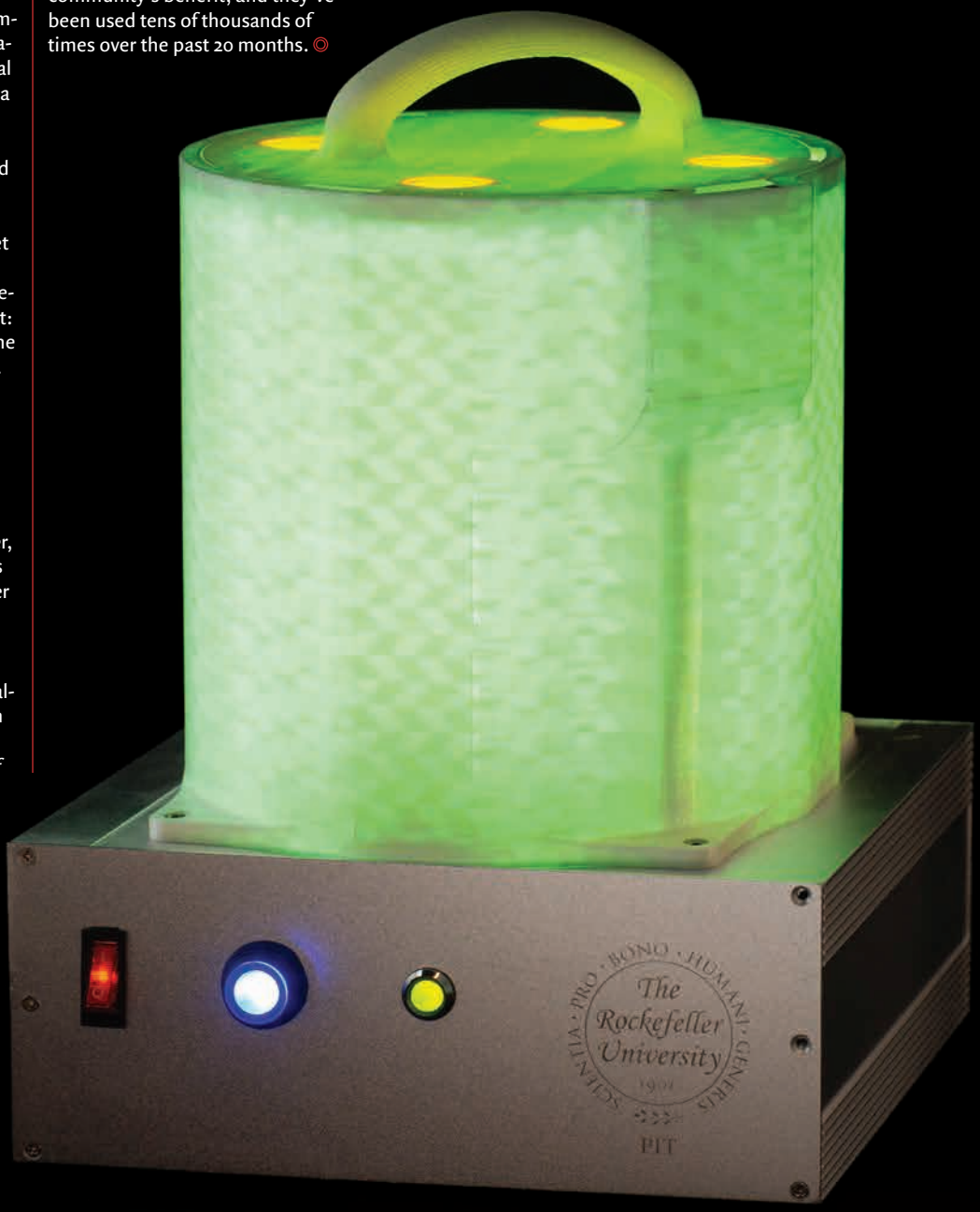
THERE WAS A time when face masks were considered disposable. Scientists, who needed them while working with infectious agents, would routinely toss them after each use.

This changed in 2020. As it became clear that disposable face masks would need to be reused, Thomas Huber and Alexander Epstein, members of Thomas P. Sakmar's Laboratory of Chemical Biology and Signal Transduction, set about designing a device to decontaminate masks.

Chemical disinfectants were ruled out, as was heat, which could damage the fragile fibers that trap virus particles. But ultraviolet light—specifically UVC radiation in the range of 200 to 280 nanometer wavelengths—seemed perfect: It kills pathogens by destroying the proteins that hold them together.

UV light is a brilliant disinfectant, long used in air ducts and water filters. For Huber and Epstein, the trick was to deliver UVC deep into the layers of fiber that make up N95 masks. With 36 watts of power, and translucent reflective surfaces covering 360 degrees, the chamber that the scientists built does the job, as validated with hundreds of UV-sensitive stickers and many rounds of fit testing. The most challenging part of the design, Epstein says, was the hook from which the mask hangs. "We tried a lot of designs but they all cast shadows," he says. "In the end we found that a bent paper clip works best."

Several chambers, the parts for which were 3D-printed in Rockefeller's Precision Instrumentation Technologies resource center, are stationed around campus for the community's benefit, and they've been used tens of thousands of times over the past 20 months.



Photograph by John Abbott

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