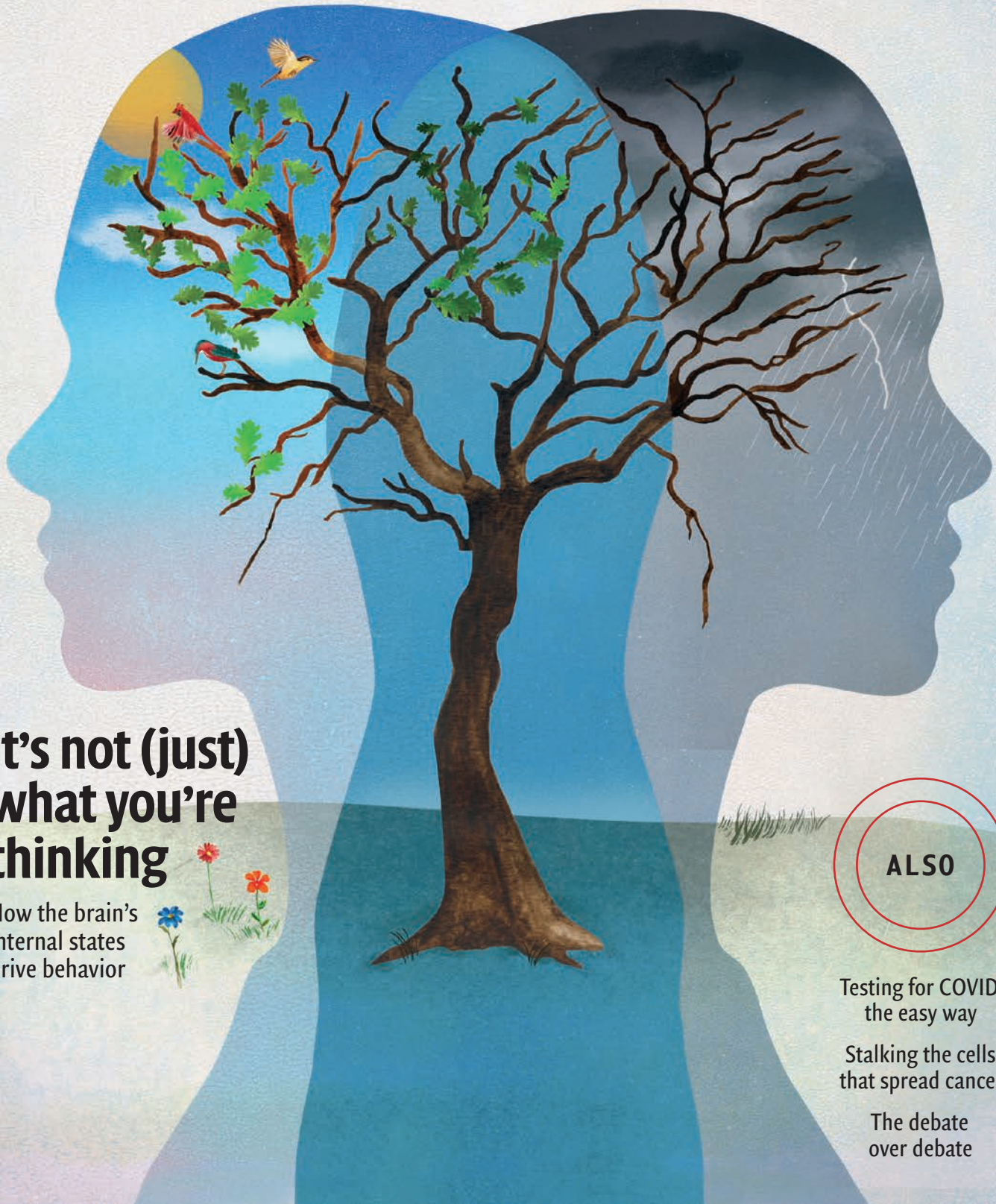
ISSUE
07
SPRING
2021

Seek

THE ROCKEFELLER UNIVERSITY



It's not (just) what you're thinking

How the brain's internal states drive behavior

ALSO

Testing for COVID, the easy way

Stalking the cells that spread cancer

The debate over debate

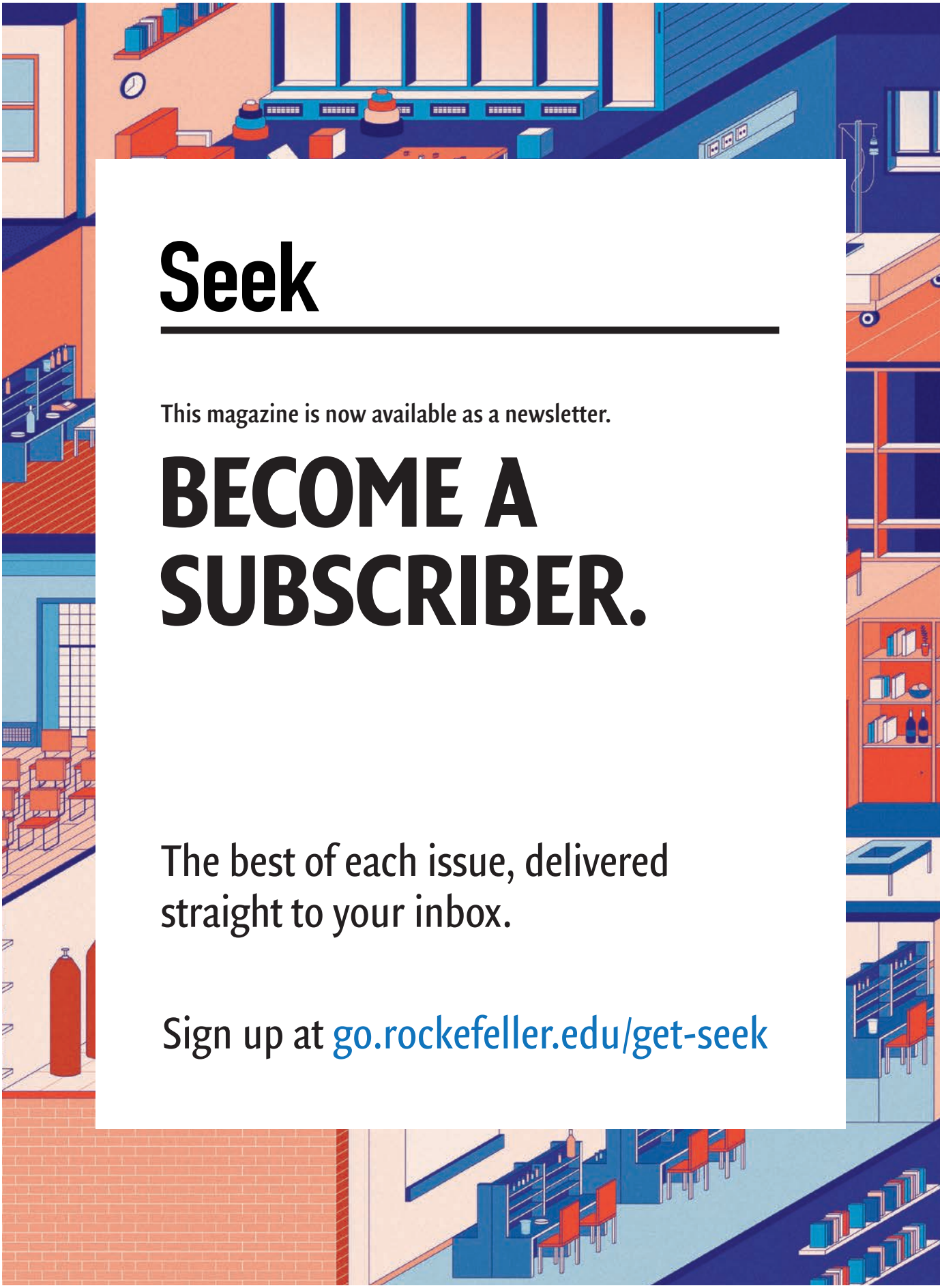
"We still don't know how the brain really works. How does information from thousands of firing neurons get organized, and how does this organization fluctuate over time?"

20

It's all about your frame of mind

We think of brains as computers—stimulus in, action out. But they're far more finicky than any iMac. Easily swayed by underlying internal states such as hunger, aggression, or arousal, our neurons are capable of incredible flexibility. For neuroscientists, it's yet another wrinkle in understanding our wrinkliest organ.

Illustration by Ellen Weinstein



Seek

This magazine is now available as a newsletter.

BECOME A SUBSCRIBER.

The best of each issue, delivered straight to your inbox.

Sign up at go.rockefeller.edu/get-seek

ALEKSANDAR SAVIĆ

CONTENTS

ISSUE 07 SPRING 2021

"Much of what we know comes from studies of HIV, but we now have the opportunity to go deeper, generating technologies to advance research on virtually any infectious disease."

Illustration by Daniel Lievano

FEATURES

34

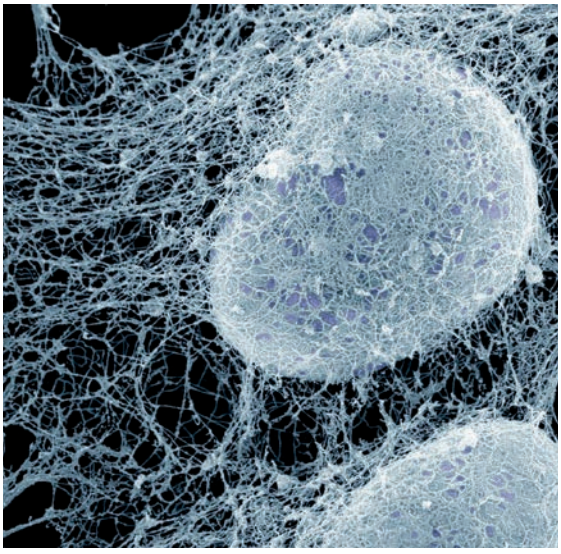
Testing, testing

Weekly COVID tests are critical to preventing community spread. But when state and city programs faltered, one Rockefeller group took matters into its own hands.

28

A mission to end metastasis

Not all cancer cells are killers. One lab is focusing its energy on only those that enable tumors to spread—and it may have found their kryptonite.



MATTHEW SEPTIMUS (2X); ERICK M. RAMOS; STEVE GSCHMEISSNER / SCIENCE PHOTO LIBRARY

FOREFRONT

"The scientific method is objective, but the enterprise of science is not necessarily so—it is done by humans, prone to their biases and errors."

PAGE 15

15

Scientists can seize their narrative

Erich D. Jarvis, scientist and advocate, wants his colleagues to know that it's ok to say what you think.

6

Scientists are learning more and more about this coronavirus. They're also waging war on sequencing errors, mapping short-term memories, and discovering why **some flies like only fresh fruit.**

ON THE BACK COVER

"The Rockefeller University" by Cornelia Li

Seek

Editor-in-Chief
Zachary Veilleux

Managing Editor
Eva Kiesler

Art Direction & Design
Point Five

Contributors
Lori Chertoff
Katherine Fenz
Bahar Gholipour
Joshua Krisch

Print Production
Kelly McLees

Digital Production
Mary Dang
Maureen Lynch

Seek is the research magazine of The Rockefeller University.

President
Richard P. Lifton

Executive Vice President
Timothy P. O'Connor

Associate Vice President for Communications and Public Affairs
Franklin Hoke

Seek is published twice a year by the
Office of Communications and Public Affairs.

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

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

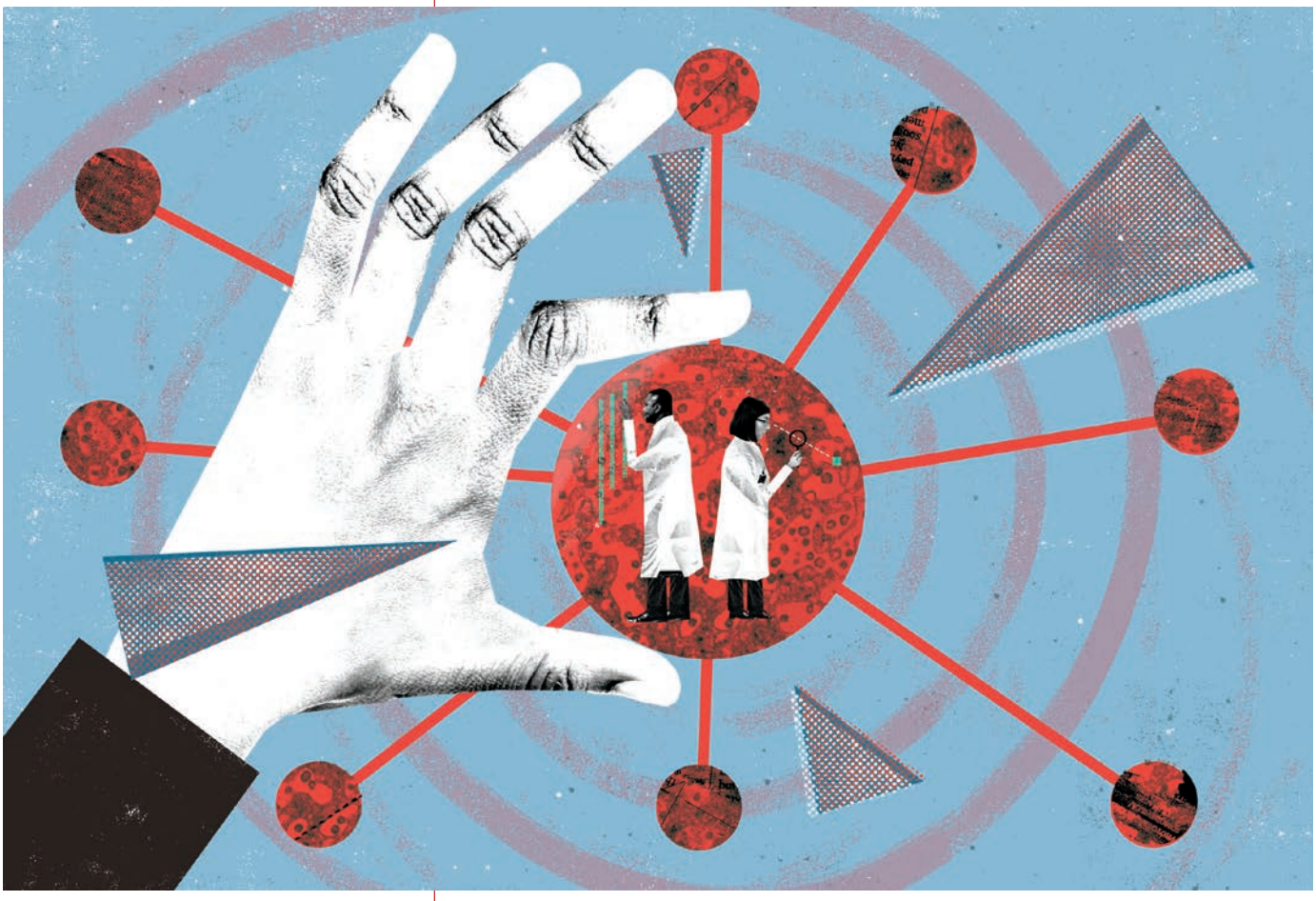
seek.rockefeller.edu

Wide-open spaces. At midday, the six-story atrium at the heart of the university's Collaborative Research Center, which houses about a third of Rockefeller's laboratories, is normally bustling with activity as scientists congregate to catch up, share data, and refine ideas. But places designed to spread knowledge are also good places to spread viruses. During the winter, when COVID policies required strict social distancing, in-person meetings were suspended and most of the furniture was removed. What remained was a calming quiet, and plenty of elbow room for those needing to catch up on reading or e-mail.

PHOTO BY MATTHEW SEPTIMUS

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

FOREFRONT



THE PANDEMIC

As society reopens, scientists aim to close in on COVID

FOR MANY, NEWS of the first "breakthrough" COVID cases was alarming. But for scientists, it was expected—and presented an opportunity.

"We always knew there would be a certain number of people who develop infections even after being fully vaccinated," says Robert B. Darnell. "What we didn't know was what those cases would look like." How severe would they be? Would some SARS-CoV-2 variants prove more adept at breaking through the vaccines' protection than others? How would these cases impact the course of the pandemic?

As the global health crisis enters its second summer, the nexus of COVID research is shifting. We've come to understand the basics of how the virus infects host cells and replicates, and we've learned enough about the body's immune response to create several good vaccines. But the world's long-term relationship with this coronavirus, and other viruses like it, is still an open question.

One clue to how things will progress comes from surveillance within the Rockefeller community. Since January, mandatory weekly COVID testing of all on-site Rockefeller personnel has been conducted in-house by Darnell's lab, using a saliva-based PCR test

Illustration by Nate Kitch

he and his colleagues developed (for more on the test, see "Building a better COVID test," page 34). In addition to keeping the community safe, this program has produced a wealth of information, and it was among the first to document and explore what breakthrough cases look like at the clinical and genetic level.

The results suggest reasons for both confidence and caution. The vaccines are holding up well to known variants, such as those originating in Brazil and the United Kingdom, and they prevent severe disease. But even a highly successful vaccination program doesn't mean the end of COVID.

"Based on what we've seen, routine testing of any individual with flu-like symptoms, or those who have had contact with a positive case, will remain an important tool to prevent the spread of this disease for some time," says Darnell, who is *Robert and Harriet Heilbrunn Professor*.

Meanwhile, pursuing treatments for COVID remains as important as ever. Monoclonal antibodies have shown exceptional promise over the past year, and one version developed at Rockefeller—a combination of two antibodies originally isolated from COVID patients who successfully fought off the infection early in the pandemic—entered clinical trials this January.

Similar antibody-based drugs have been used experimentally in thousands of COVID patients, and these drugs help stop the infection in its early stages before it progresses to severe disease. The cocktail developed by Michel C. Nussenzweig, the *Zanvil A. Cohn and Ralph M. Steinman Professor*, and his collaborators including virologists Paul Bieniasz and Theodora Hatziioannou, recently licensed to Bristol Myers Squibb, is designed to help minimize the risk of the virus mutating and developing resistance to the therapy.

Scientists are also pursuing new antiviral drugs that, similarly to broad-spectrum antibiotics, might be effective against multiple pathogens. A group led by Nobel Prize–winning virologist Charles M. Rice, the *Maurice R. and Corinne P. Greenberg Professor in Virology*, mapped a network of more than a hundred human proteins that SARS-CoV-2 hijacks as it takes over a cell's replication machinery. One of them, a little-known protein called TMEM41B, stands out for its use by four different coronaviruses as well as by viruses that cause Zika, yellow fever, and other diseases. The team is investigating ways to disrupt TMEM41B's ability to support an infection.

Other researchers are studying how the virus impacts lung cells specifically. Because SARS-CoV-2 first enters the body via the lungs, its interaction with cells in the airways and alveoli is what allows it to establish a foothold in the body. A team led by Ali H. Brivanlou, *Robert and Harriet Heilbrunn Professor*, has used stem cell technology to produce tissue that mimics lung buds, the embryonic precursor to mature lungs (see "Synthetic micro lungs," page 18). Beyond providing a realistic model to investigate the mechanisms of viral infection, the method can quickly produce vast amounts of lung tissue for drug-screening purposes.

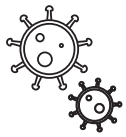
As the pandemic evolves, so do our questions. What does the immune response to SARS-CoV-2 look like months or a year after infection? How does vaccination impact people who have already been infected? How well do our antibodies adapt to deal with the emerging variants of the virus? Bieniasz and Hatziioannou are studying the shifting relationship between our antibodies and the virus. Working with Nussenzweig, their team has found that in those who recover from COVID, the immune system retains a memory of the coronavirus, building a long-lasting defense in which antibodies are continually refined and improved.

What's more, their work suggests that vaccination further boosts the neutralizing power of antibodies: Individuals who receive vaccines after having recovered from COVID should enjoy high levels of protection, even against emerging variants they haven't yet encountered. Vaccinated individuals who haven't been exposed to the virus, however, retain some vulnerability to the variants, their work shows.

"It's a complex situation," Bieniasz says. "And it suggests that vaccines may need occasional updates in the future to keep up with the mutating virus." ◎

Teresa Rozza and Salina Parveen prepare saliva samples for COVID-19 testing.

MATTHEW SEPTIMUS



DATA

There are many thousands of SARS-CoV-2 variants, and over 2,600 distinct lineages have been discovered so far. Four are considered "variants of concern" by the CDC.

Ancient and endangered, the kakapo is one of 25 species with a brand-new reference genome.

7,262

DATA

Average number of manual "edits" required to properly assemble a high-quality vertebrate genome sequence.

ANIMAL KINGDOM

Quality genomes for all

THERE WAS NO reliable genomic sequence for scientists to consult when studying the flightless kakapo of New Zealand. Nothing on the adorable vaquita porpoise or the blunt-snouted clingfish either. No error-free genetic database for bats or platypuses, Canada lynxes, or Goode's thornscrub tortoises.

When it comes to vertebrates—other than humans, of course, and popular lab animals such as mice and zebra fish—scientists are often stumbling in the dark. Reference genomes of tens of thousands of species either don't exist or are unusable, rife with errors and duplications.

"It is unconscionable to be working with some of these genomes," says Rockefeller's Erich D. Jarvis.

From the collective groan of frustrated scientists, the Vertebrate Genomes Project was born. Its goal is to build a library of more than 70,000 error-free reference genomes representing every vertebrate species alive today. Projected to take at least 12 years, the endeavor recently reached an early milestone with the release of its first 25 premium genomes. Reported in a series of papers in *Nature*, this work provides a proof of concept for a new method that merges several sequencing tools into one lean pipeline.

"We call it the kitchen sink approach, combining tools from several DNA sequencing companies to make one high-quality genome," says Jarvis, who chairs the project. Reference genomes that once took years to generate are now rolling out in weeks or months, and scientists at several institutions are already using the new approach in their research. "It often pays off to do some hard work on the front end so that we can get high-quality data on the back end," says Jarvis, whose Laboratory of Neurogenetics of Language studies vocal learning in songbirds, hummingbirds, and other species.

But plenty of work still lies ahead. "The next step is to sequence all 1,000 vertebrate genera, and then all 10,000 vertebrate families, and eventually every single vertebrate species."

Reference genomes of tens of thousands of species either don't exist or are unusable, rife with errors and duplications.

CHRIS BIRMINGHAM

CELEBRATIONS

A Nobel like no other

TO RECEIVE HIS 2020 Nobel Prize, Charles M. Rice had to travel no farther than midtown Manhattan. For the first time in decades, the event involved no trip to Stockholm, no lavish banquet, and certainly no handshake with the king of Sweden—only a quiet socially distanced ceremony at the Swedish Consulate.

Still, as is usually the case with Nobel laureates, Rice spent many long hours on camera; working double time as a spokesperson for basic science while conducting intensive investigations into COVID-19.

Rice, the *Maurice R. and Corinne P. Greenberg Professor in Virology*, shares his Nobel Prize in Physiology or Medicine with two other scientists for discoveries that led to the identification and characterization of the virus responsible for hepatitis C. After proving the pathogen's role in causing the disease, his continued research enabled the creation of new drugs, a combination of which was ultimately shown to cure it.

SANTA MARIA PECORARO DI VITTORIO

In lieu of holding a press conference, Rice recorded TV interviews.

CLINICAL CASES

The perils of long telomeres

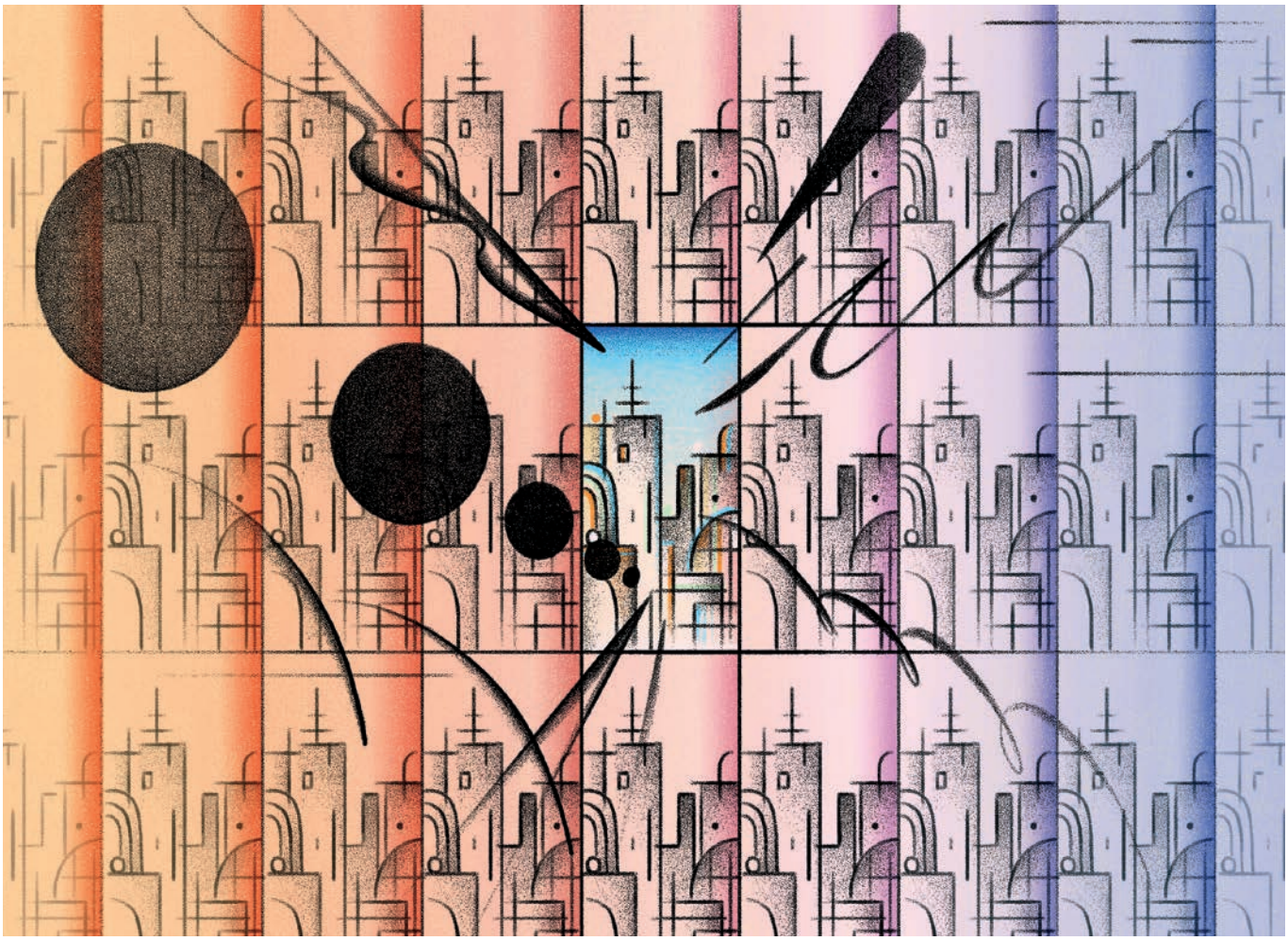
IT WAS ONCE thought that all cells were immortal, forever able to replicate and generate fresh copies of themselves. But a cell's days are, in fact, numbered, predestined by the length of its telomeres. Located at the tips of each chromosome, these structures shorten as they absorb the wear and tear of cell division. Eventually, a cell's telomeres wither away entirely, capping the number of times it can divide at about 50.

There may be good reasons why this threshold hovers consistently around 50 and not, say, 25 or 500. Scientists have for decades suspected that telomere shortening isn't just an unwanted side effect of cellular aging but a carefully calibrated process that proactively curtails cell division to prevent cancer. And the telomere reserve we are born with is key, with each telomere being long enough to allow normal development yet short enough to run out before rapidly proliferating cells start amassing into tumors.

Studying four Dutch families with striking cancer histories, scientists in the lab of Titia de Lange, the *Leon Hess Professor*, recently provided a real-world example of this theory.

The six individuals in the study had each developed one or several cancers of different types, including breast, colorectal, thyroid, and skin cancer. The researchers found that because these patients had mutations in TIN2, a protein that keeps telomere length in check, they had also been born with extremely long telomeres. The work was published last December in *eLife*.

All of which suggests you can thank your normal-sized telomeres for every single cell in your body that hasn't run amok. They may not seem to stand against the cruel passage of time, but they likely have prevented many cancers from occurring in your lifetime.



MODERN MATH

Predicting the unprecedented

STANDARD MATHEMATICS CAN predict how cancer cells will multiply, how crop yields will fluctuate, and how insects will swarm—in much the same way that statistics can determine the average human height. As long as measured quantities have finite averages and variances, figuring out the specifics is a simple matter of applying a formula known as Taylor's law, which relates a population's mean to its variance.

But what about extreme events with no finite limits—pandemics like COVID-19 or financial fluctuations like the GameStop short squeeze?

The data sets that describe extreme events are known as heavy-tailed distributions. While most aspects of our daily lives huddle around an average—a neat bell curve of mundane behavior, minor disease outbreaks, small blips in a stable market—extreme events are plotted at distant tails of the graph. When there's no finite limit to how extreme an event can be, then there's no limit to how far its tail can be flung or how "heavy" it can grow. One extreme event can stretch the entire graph into unpredictable territory. It follows that Taylor's law loses its footing in a heavy-tailed world.

Rockefeller's Joel E. Cohen disagrees. His recent work on heavy-tailed distributions, which he published with colleagues at Columbia University and Cornell University in *Proceedings of the Royal Society A*, describes how Taylor's law can predict even extreme outliers. The study proposes a novel way of looking at heavy-tailed variables that yields surprisingly orderly connections between the mean and the variance of a system.

Cohen's discovery does not mean that scientists can now simply plug their numbers into an equation and foresee the next market coup. But it does raise the prospect that mathematical modeling may one day help scientists anticipate and manage extreme occurrences, "from daily precipitation to microbial evolution, from cortical oscillations in the human brain to global pandemics," says Cohen, who is the *Abby Rockefeller Mauzé Professor*. "Advances like these are the mathematical analogue of bioimaging—they make it possible to see what was previously invisible." ◎

Illustration by Gizem Vural

ANTIBIOTICS

An old drug solves new problems

SORANGICIN A HAS never lived up to its potential. It's been three decades since the compound was discovered to have antibiotic properties, yet it languishes in obscurity—ignored by all but a few scientists.

Meanwhile, antibiotic resistance grows. Every year, about half a million people fall ill with tuberculosis that doesn't respond to conventional antibiotics such as rifampicin. Resistant TB strains, experts warn, are a ticking bomb.

It might be time to give sorangicin a second look. A recent study found that sorangicin, first discovered in the 1980s, can kill even drug-resistant TB. "Sorangicin inhibits regular strains in very much the same way as rifampicin, by targeting the molecular machinery that transcribes DNA to RNA," says Elizabeth Campbell, a research associate professor at Rockefeller. "But now we show that, through a different mechanism, it also traps those variants that escape rifampicin." The work was published in *Proceedings of the National Academy of Sciences*.

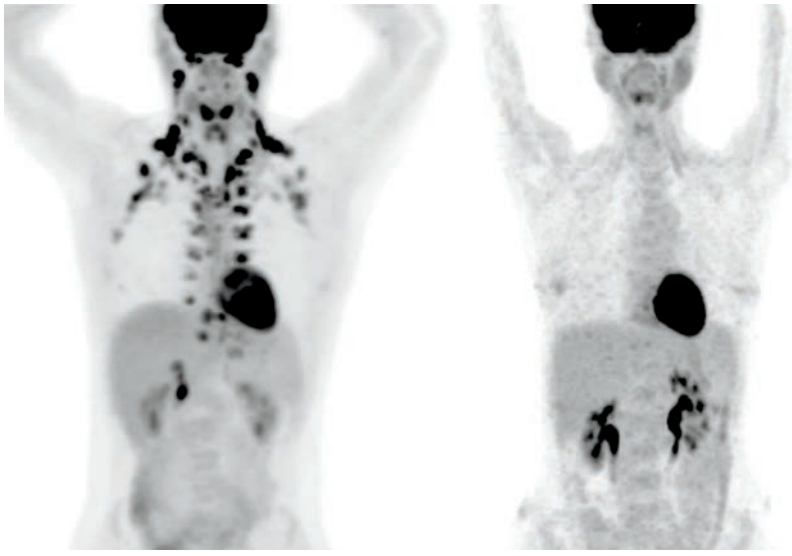
Campbell examines structural data.

Campbell and colleagues are particularly excited about sorangicin as a potential drug candidate because of its compatibility with other medications. Rifampicin, on the other hand, has been shown to reduce the efficacy of HIV medications by up to 90 percent.

"If sorangicin can be developed into a medication, it might be especially helpful for people with comorbidities," she says. ◎

METABOLICS

The weight you don't want to lose



Brown fat deposits are found between the shoulders and along the spine.

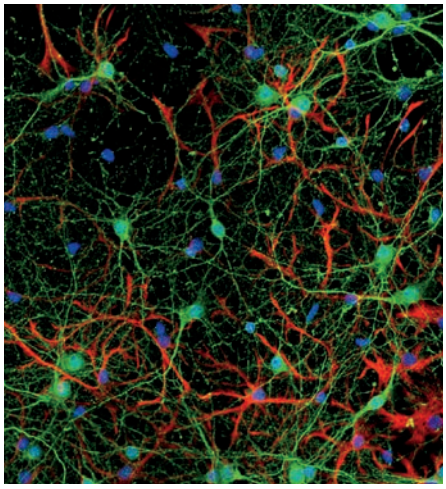
MARIO MORGADO; ANDREAS G. WIBMER AND HEIKO SCHÖDER, MEMORIAL SLOAN KETTERING

BROWN FAT CAN be just as hard to acquire as white fat is to lose. Newborns and animals have a surplus of the stuff, which burns rather than hoards calories. Adult humans, not so much.

"The natural question that everybody has is, 'What can I do to get more brown fat?'" says Paul Cohen, the *Albert Resnick, M.D. Associate Professor* and senior attending physician at The Rockefeller University Hospital. "We don't have a good answer to that yet."

Recently, however, Cohen's team discovered that brown fat has many benefits beyond waistline control. Published in *Nature Medicine*, their study of 52,000 people suggests that 10 percent of adults have detectable amounts of brown fat, and that these individuals are less likely to suffer from type 2 diabetes, heart disease, and hypertension. Brown fat also appeared to mitigate the negative health effects of white fat in those who were obese.

"We are looking into the possibility that brown fat tissue does more than consume glucose," Cohen says. ◎



TROUBLESHOOTING

The Prozac problem

PROZAC DOESN'T ALWAYS work—and when it does, it takes too long to kick in.

"The rate of suicides drops after nine days of treatment, and people start to feel better only after two to three weeks," says Revathy Chottekalapanda, a senior research associate in the laboratory of the late Paul Greengard.

Why selective serotonin reuptake inhibitors (SSRIs) like Prozac take so long to start working—and why they fail some people entirely—is a mystery that dates back over 40 years, to when the drugs were first introduced. Chottekalapanda and her colleagues have a new theory, centered on a single gene that, in mice, ramps up exactly on day nine of Prozac treatment. This molecular switch triggers a cascade of gene-expression changes that transform the animals' behavior, reducing symptoms of depression and anxiety.

"For the first time, we were able to put a number of molecular actors together at the crime scene in a time- and sequence-specific manner," Chottekalapanda says of the findings, which were published in *Molecular Psychiatry*.

DATA

122.4 MILLION

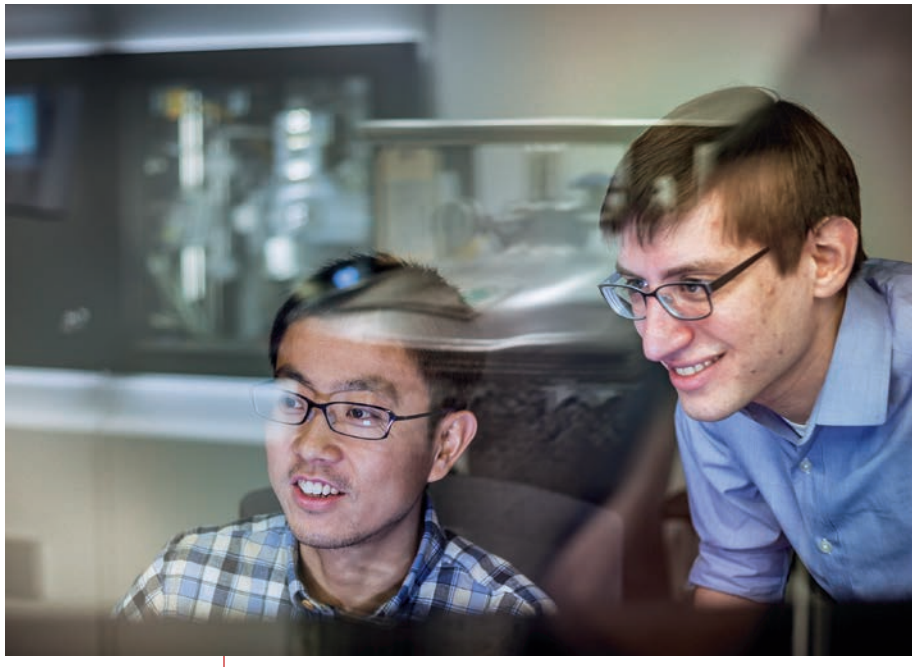
Number of SSRI prescriptions filled annually in the United States.

PHYSICAL FORCES

How cells feel their way

CELLS ARE TACTILE little things. Whether bumping up against their neighbors or clinging alone to the bottom of a scientist's Petri dish, they are able to sense their physical environment as well as we can feel the push of the ground beneath our feet. Scientists have long known that mechanical signals flow from the outside environment into the cell and inform its movements, but only recently have they acquired the technology to study this phenomenon, known as mechanosensation, in detail.

In Gregory M. Alushin's Laboratory of Structural Biophysics and Mechanobiology, scientists have taken a major step toward describing how mechanosensation plays out on a molecular level. It all comes down to actin, a protein involved in giving the cell its shape, and its biochemical ally α-catenin, a so-called adhesion protein found in the cell's outer rim. Using specially designed laser tweezers, the researchers were able to stretch out single actin filaments, which are about 15,000 times thinner than a human hair, to demonstrate that actin transmits a signal to α-catenin when stretched. The α-catenin protein heeds the call, responding to actin's transmission by either tightening or loosening its grip on the external environment.



Alushin (right) in the lab.

"The idea that actin filaments could potentially be tiny stretchy tension sensors in the cell has been banging around in the literature for a while, but we've proved it here," Alushin says.

Although α-catenin is known to be critical in brain development and is frequently mutated in cancer, scientists have had a hard time pinpointing its exact role. "We know that if you get rid of it, everything else in the cell breaks, but not much more," Alushin says. "But by defining the force-detector in α-catenin, we will enable researchers to manipulate the protein with better precision." The results were published in *eLife* in September.

LABORATORY OF MOLECULAR AND CELLULAR NEUROSCIENCE; FRANK VERONSKY

THE GENOME

Thriftiness is hardwired

RECYCLING IS GOOD. Microbes that eat plastic? That could be even better.

But when David Zeevi and Liat Shenhav set out in search of such organisms, they ended up making a surprising discovery about how cells conserve their own resources.

The plan was to scan microbes living in diverse areas of the Earth's oceans and identify which genes are essential to those that prosper in polluted areas rife with plastics. The telltale sign of such genes is high resistance to change: Being crucial to survival, the genes would not endure random mutations during evolution and would remain largely unchanged across a species.

But the analysis turned up an unexpectedly high number of genes that were stable in this way. Soon, the team had embarked on a new project. "The question became, Why are so many microbial genes intolerant of change?" says Zeevi.

As they sequenced more organisms, a pattern emerged: The most stable genes were often linked to the use of carbon or nitrogen, which microbes need to make proteins. In a sense, the bacteria were conserving scarce resources.

Zeevi and Shenhav, who are fellows in the Center for Studies in Physics and Biology, suggest there could be something in the structure of the genetic code itself that leads to this phenomenon. The genetic code, shared among all life forms, is composed of short segments of DNA called codons that specify the amino acids to be used in protein manufacturing—thereby affecting overall nutrient requirements.

Using computational modeling, the researchers simulated one million imaginary, randomized genetic codes and measured the overall nutrient cost of all possible mutations. It turns out that mutations to the randomized codes resulted in higher nutritional requirements than did mutations to the authentic one.

"The standard genetic code is set in a way that makes it less likely for mutations to cost the cell extra carbon and nitrogen," Zeevi says. "This is the case not only in microbes in the ocean but in all life." ◎

THE JACKSON LABORATORY

"It's rare to find a single gene with a strong influence on a complex cognitive function, but it happened in this case."

MIND MAPPING

How mice miss the exit

ANY MOUSE WORTH its whiskers can navigate a maze.

But mice are curious creatures, and they prefer to explore new arms of a maze rather than run the same route again and again. So they memorize the paths taken previously, and then on future maze runs, instead of turning down a well-trodden path, they make a point to seek out new adventures.

In the laboratory of Priya Rajasethupathy, however, a few forgetful mice often fail to find the road not taken. They hesitate at forks in the maze, wanting to turn down a new path but struggling to remember where they've previously scurried. They get it right half the time—which means they're guessing instead of relying on their short-term memory.

In recent work published in *Cell*, Rajasethupathy and colleagues discovered that variations in a single gene, which codes for a brain receptor called Gpr12, can explain to a great degree these differences in short-term memory among mice. They found that mice with excellent short-term memories have more than twice the Gpr12 receptors as forgetful mice and that, by boosting the expression of this one gene, scientists can help absentminded mice make the right turn 80 percent of the time.

"It's rare to find a single gene with a strong influence on a complex cognitive function like short-term memory," says Rajasethupathy, the *Jonathan M. Nelson Family Assistant Professor*. "But it happened in this case, and it led us to the unexpected mechanisms involved."

One such revelation came when the scientists began exploring the Gpr12 receptor, which they thought would be restricted to the prefrontal cortex, the brain region classically linked to short-term memory. Instead, the receptors primarily function in the thalamus and help establish synchronized brain activity during memory tasks. "These findings reveal a crucial dialogue between brain regions during short-term memory use," Rajasethupathy says. ◎

This fly likes its fruit fresh

COMPARED TO OTHER pests, the fruit fly is relatively docile. It is tiny, it is quiet, it doesn't bite—nor is it out to destroy anything of great value. In general, members of the *Drosophila* genus are attracted to rotting produce, food that nobody wants anyway.

The spotted wing drosophila, known to scientists as *Drosophila suzukii*, is an exception that has developed a taste for ripe summer fruits. It feasts in orchards and fields while fruit is still early in the ripening stage, damaging crops and leaving behind microscopic larvae. That box of fresh, plump cherries from the market? It looked good to *D. suzukii*, too.

"The preference for ripe fruit is a novel behavioral trait that is causing significant agricultural losses," says Li Zhao, assistant professor and head of the Laboratory of Evolutionary Genetics and Genomics. "Understanding how it emerged may lead to new ways of controlling the damage it causes."

D. suzukii arrived in North America about 12 years ago, seemingly out of nowhere. Long confined to East and Southeast Asia, it had voyaged all the way to California, where farmers were at first dumbfounded trying to figure out what was ruining their crops. It was around this time that Zhao, then a postdoc at the University of California, Davis, got involved in a USDA-funded project to put together an early draft of the fly's genome using next-generation sequencing technology.

Zhao's lab is devoted to the study of how novel genes develop. Now she is getting to the bottom of what exactly has caused *D. suzukii*'s preferences to change as it evolved. "*Suzukii*'s strange behavior is a perfect case study for us," Zhao says.

$308.9 MILLION

DATA

Cost of damage caused by *D. suzukii* to California strawberry crops in 2008, the first year the pest was observed in the state.

The obvious place to look is in genes that are important for sensory perception, such as those coding for smell receptors. Previously, scientists hypothesized that *D. suzukii*, too, parted ways with its evolutionary kin after a mutation. Perhaps its perception of a meal's sugar or alcohol content, which varies as fruits ripen and then rot, changed.

But when Zhao and her colleagues compared *D. suzukii* to its closest relatives, they found something quite different. It turns out that this fly picks fresh fruit over rotten not as a matter of taste or smell but based on the firmness of the fruit. When offered servings of a gelatinous milk shake containing varying amounts of alcohol, sugar, acetic acid, and agar, *D. suzukii* consistently chose the firmest, regardless of its chemistry. And a detailed genetic analysis of 200 individual flies revealed that some of the most rapidly evolving genes in *D. suzukii* are those coding for mechanosensory receptors.

"Our ability to control this invasive species could rely on a better understanding of mechanosensation—such as the processes by which flies are able to detect how much force is required to manipulate an object," says Zhao. "It's a new direction to explore."

Illustration by Erick M. Ramos

Q & A

Should scientists be more vocal?

With Erich D. Jarvis

MATTHEW SEPTIMUS

Jarvis's three-decade career in the neuroscience of language has provided many opportunities to speak out.

POLITICS HAS LONG been the third rail of science, charged and untouchable. No good can come from scientists espousing their views on world affairs, the thinking goes, or engaging in sociopolitical debate not directly related to their work.

But science underlies most anything worth debating—and who better to weigh in than scientists themselves? It's a fact made vividly clear by the onslaught of recent crises. From the pandemic to police brutality to climate change, our national arguments require informed views based on science and facts. Yet we live in an age where truth has become malleable.

It's time for scientists to step off the sidelines, Erich D. Jarvis says, especially when it comes to issues of social injustice.

A neuroscientist who studies vocal learning, Jarvis uses songbirds—which like humans and only a few other species, have developed the ability to mimic novel sounds—as model organisms to study the basic mechanisms involved in the development of speech. As head of the Laboratory of Neurogenetics of Language, his work has led to a broad understanding of how neural circuits for vocal learning evolved and became specialized for speech.

Jarvis has found his own political voice. Often the sole person of color in the room, Jarvis long ago learned to be an advocate—for Black people, for the disadvantaged, and for scientists as a group. We spoke with him about his experiences with activism in academia and how the next generation of scientists can build a more equitable community at home and a stronger relationship with society as a whole.

Is the stereotype true that scientists have traditionally been reluctant to engage in activism and politics?

I've certainly seen an entrenched culture in academia that feels science should stand on its own. That our role as scientists is to discover truths but it's up to other people to decide what to do with them. There's a fear that commenting on policy or engaging in controversial issues would be perceived as personal bias, raising doubts about our commitment to objective research. It isn't just at the individual level; historically, public engagement is not something that most academic institutions encouraged or even tolerated.

While we've gotten pretty good at certain types of advocacy—advocating for funding comes to mind, and that's a relatively recent phenomenon—many scientists also don't want to be seen by their peers as seeking attention. And to some extent we are uncomfortable in a messy world of viewpoints where some people's opinions don't make logical sense. It's easier to stay out of it and "let the work speak for itself." Say what you have to say in the paper.

Was that your experience when you began?

When I entered science, I saw my mentors and colleagues shy away from having public opinions, whether on politics or racism or anything else. This attitude was new to me. I had been a professional dancer, I was used to being in public, so it wasn't my inclination to keep my thoughts to myself. But I did adopt that stance. I was young and new to this community, and eager to fit in.

Soon, however, I started to see this as a mistake. I think the first time it hit me was when I was running the scholars program for the Society for Neuroscience. The program supports underrepresented minority students, and one year we had an application from a White woman whose disadvantage was that her family had cut her off when she was accepted to and decided to go to college, to work with "devil worshiping liberals who believe in evolution."

I thought, there's a miseducated public out there, and scientists are hiding behind our walls and talking to each other instead of trying to do something about it. I thought we need to reach out to this woman's family. Whether it's about race, discrimination, climate change denialism, or other matters, we need to be more proactive. There are plenty of voices, with all kinds of agendas, vying for the opinions of people like this woman's family. They need to hear from scientists, too.

What are some ways scientists can combat disinformation more effectively?

We all need to recognize that communication, that speaking on the state of evidence, offering expert interpretation, or endorsing the best available actions on, say, cutting carbon emissions, is not the same as advocating for a cause due to a personal bias. Rather, it's about sharing facts, information, and expertise, which one can argue is part of a scientist's job and responsibilities.

Jarvis speaks at the televised 2017 March for Science rally in Washington, D.C.: "You know something is really wrong when people must form a protest march for science, even in the rain."

EARTHDAY.ORG

That's why I think more scientists should strive to actively communicate the best scientific knowledge to policy makers and the public. The fear that this sort of activity jeopardizes one's credibility is likely overblown. There are studies and surveys showing that the majority of Americans do not view it as inappropriate for a scientist.

We can also look inward and tackle disinformation inside science itself: We can take a closer look into some of the historical scientific "facts" we take for granted, and ask where they come from. Does our understanding need an update? There are many examples of how racist ideology or implicit biases have influenced the biomedical and behavioral sciences. Sometimes such influences have led to wrong scientific claims.

Take the word *neocortex*, which refers to the outer layer of the brain and means "new cortex" in mammals. But it is actually not new in mammals—there are counterparts in other vertebrate lineages. The name comes from White European scientists who believed it was part of an evolutionary trajectory that would result in a superior White race, with the neocortex being biggest in European men and smallest in Africans. There are many examples of historical studies on racial or

gender differences that, despite suffering from biases and flawed methodology, are still routinely cited. The scientific method is objective, yes, but the enterprise of science is not necessarily so—it is done by humans, prone to their biases and errors.

How have things changed in the last few years?

For as long as there has been politics there's been a tendency to manipulate facts to gain power and oppress populations, typically minority populations. But what we've seen recently, with one of our two major political parties embracing outright fabrications, in contradiction to overwhelming evidence, is new and alarming. That has undoubtedly led to a growing interest in the scientific community getting involved.

Another major shift is in our attention to issues of inequality. Race has always been used to establish a hierarchy in society and, by extension, in science. Many people are realizing that it is not right to ignore that any longer.

I've had a bit of an awakening in this last year with the Black Lives Matters movement, in that I've never seen people so angry. There's been a combination of factors coming together, starting with the uneven impact of COVID on communities of color, and culminating in the callous murder of George Floyd by a White police officer who, for a time, seemed likely to escape consequences. People age 30 and younger had never experienced this before, they had never seen the blatant racial biases laid out so clearly and originating from the very top of our country's power structure.

My parents, who grew up in the fifties, sixties, and seventies, they saw it. I was told these stories, I was told to watch myself in the street. Be careful when a police officer pulls you over, they warned, because you can get killed. This is the mindset I grew up in, but it felt like things were slowly changing for the better. Until Trump came to power in 2016, when it all got worse. The illusion was shattered.

How has your personal experience as a young Black scientist equipped you for this moment?

I grew up in the Bronx and went to undergrad at Hunter College, which is an ethnically diverse school that looks a lot like the rest of New York City. But when I started graduate school at Rockefeller in 1988, I got my first taste of imposter syndrome. I was accomplished, I had already published four or five papers as an undergraduate, but I didn't feel like I belonged. It was a shock.

As I came up the ranks, there started to be fewer and fewer people who looked like me. I became aware that some people believed I was there only because of some diversity quota. And you start to internalize these things. When your experiments don't work—which is most of the time in science—you blame yourself. I would go over my CV, go through the papers I had published and the grants I had received—that was tangible evidence that I was doing something right.

Today, about thirty years later, I see students and postdocs grappling with the same issues. There's still discrimination, but I no longer relate to it in the same way. I used to tell my students that the most important thing is to have resilience, to persevere in the face of daunting obstacles. Today, I no longer say that. The Black Lives Matter movement has made me think that the amount of resilience I had to have to survive should not be needed. We need to build a system where anyone with the right talent and determination can be a successful scientist.

What are the most important actions an institution can take to address inequity?

We need research to understand what the problem is, and we need training to present solutions to the community.

Following the killing of George Floyd, many professional scientific organizations published statements opposing it. That's great, but statements have little impact unless they are accompanied by effective actions. And to determine what these actions should be we need to understand the roots of institutional racism. We need more evidence-based research on the problem and how to address it. We need to have the findings and practices presented to us, especially to those in hiring and faculty search committees. We also need effective training for gender and racial biases, for all faculty, staff, and students.

We need the participation of everybody. It has to be part of institutional discussions, it has to be happening in labs, in academic departments, in staff meetings, in faculty meetings. There needs to be participation by as many people as possible, not only by women and people of color. It's not realistic or fair to automatically expect researchers who happen to be members of minority groups to take on the role of both scientist and de facto diversity officer. That is two jobs, and they won't be able to do either job well enough. ◎

10.6%

DATA

Average amount by which papers authored by ethnically diverse teams outperform other publications in impact.

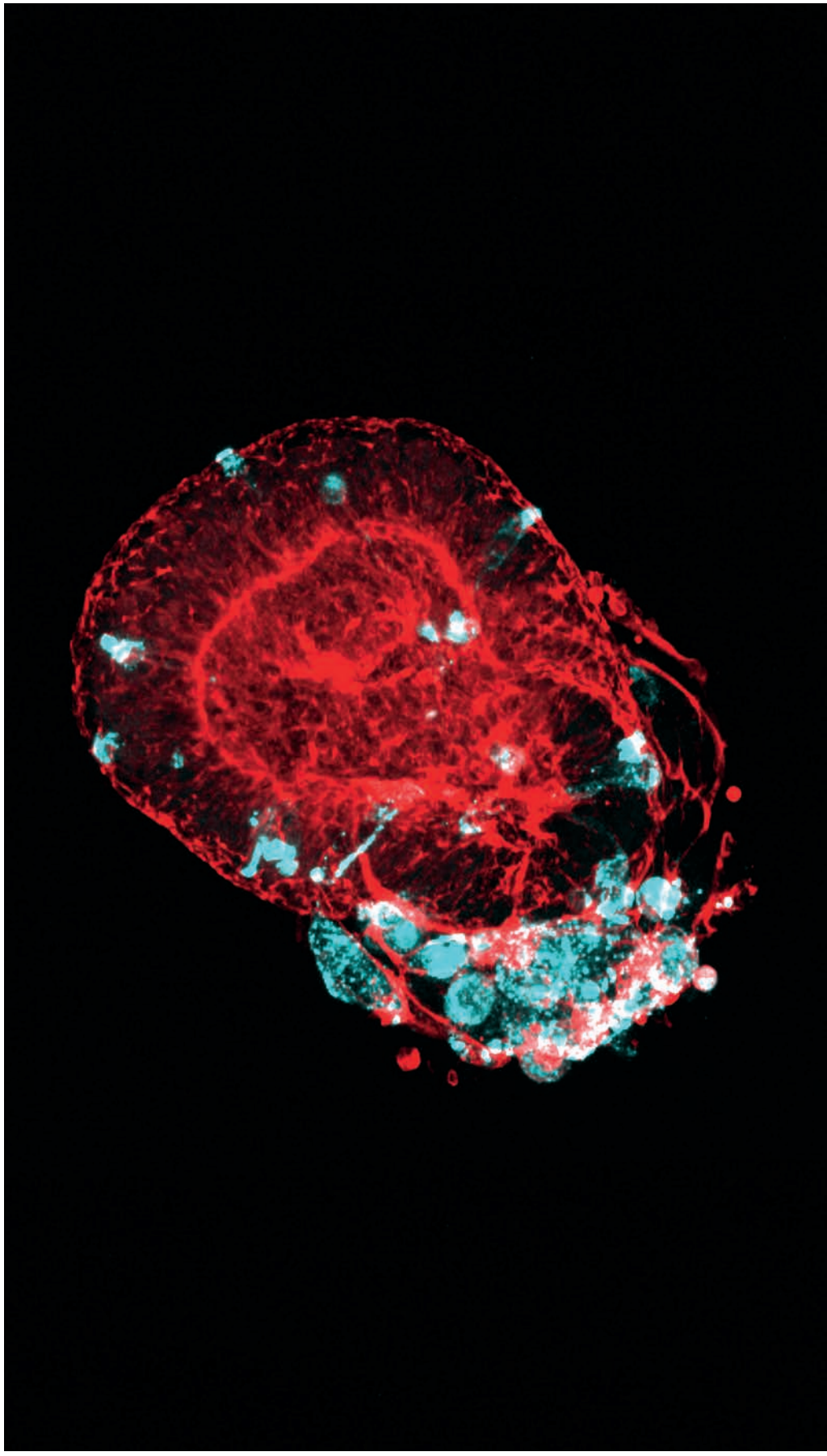
"I used to tell my students the most important thing is resilience, but I no longer say that. The amount of resilience I had to have to survive should not be needed."

Synthetic micro lungs

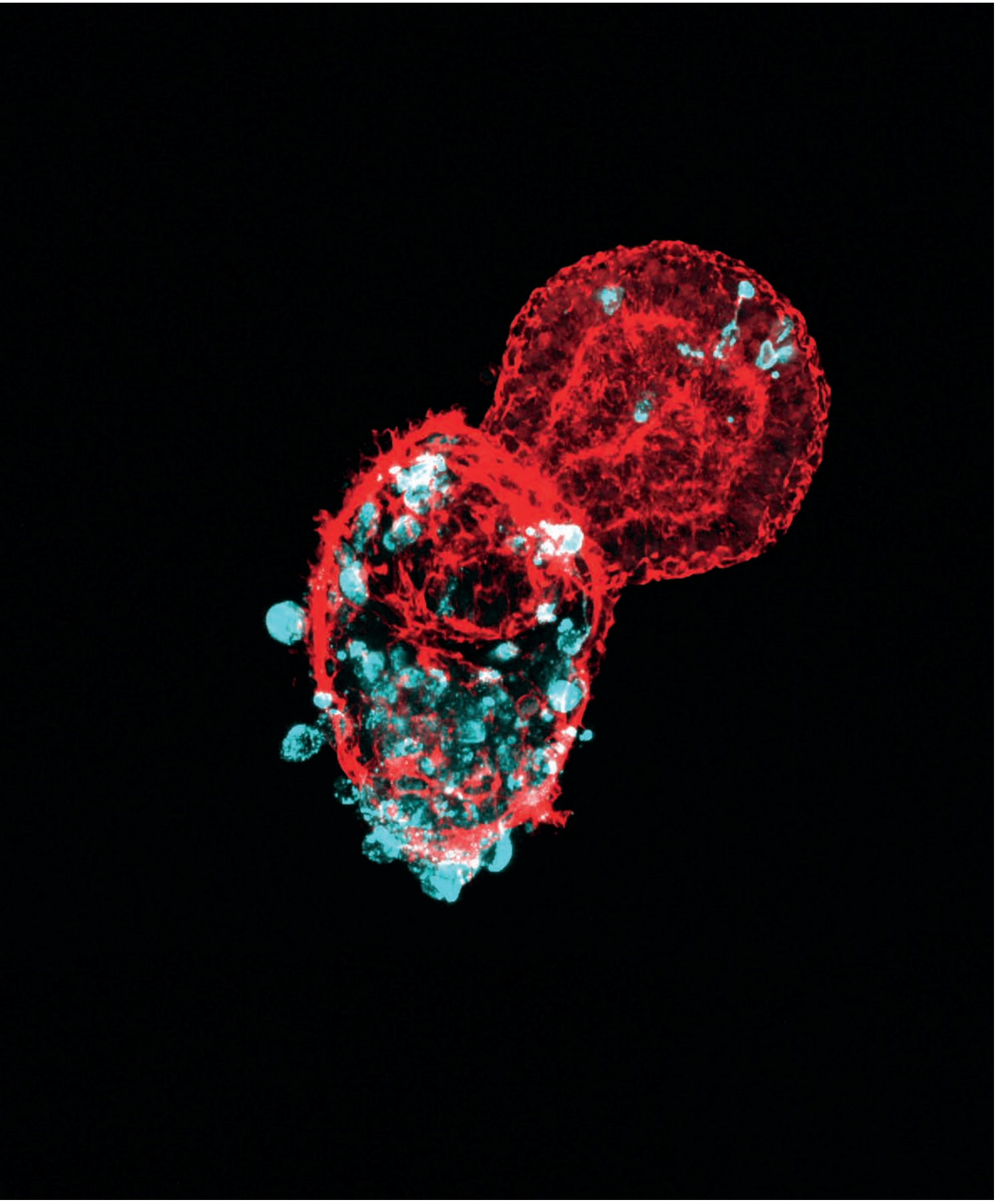
WHAT EXACTLY HAPPENS when a SARS-CoV-2 virus, catching a ride on a breath, flies through and lands on the cells of our lungs? To tease out the complex biological events playing out inside infected human cells scientists need models, the more realistic the better.

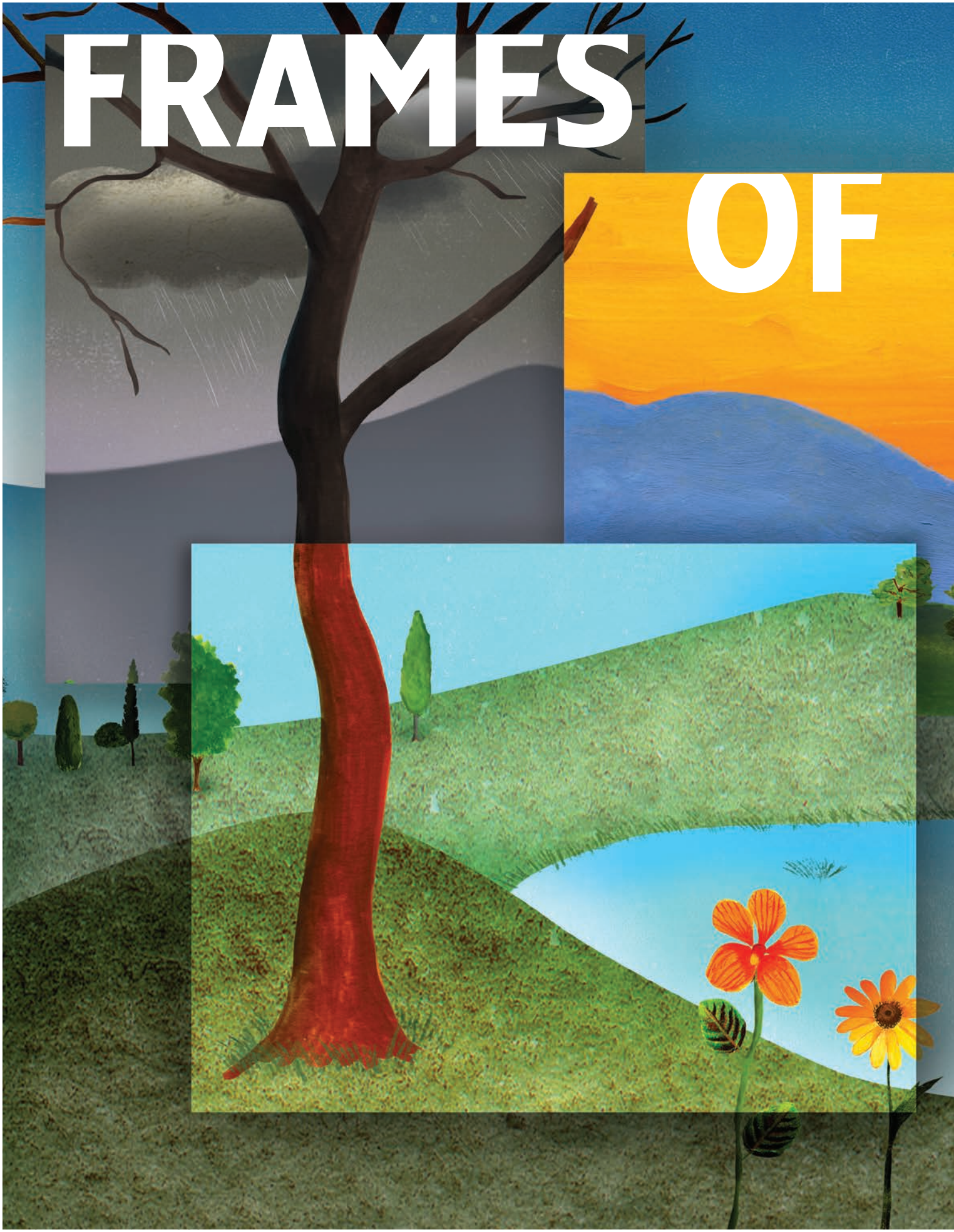
Researchers from Ali H. Brivanlou's Laboratory of Synthetic Embryology used stem cell technology to produce lung "buds" that self-organize into three-dimensional tissue akin to early lungs. The tiny synthetic lung (red) allows researchers to study how SARS-CoV-2 (blue) attacks the airways and alveoli, structures known to be damaged in COVID.

It's a realistic model for testing whether novel COVID-19 drugs curtail infection, says Brivanlou, who is *Robert and Harriet Heilbrunn Professor*.



LABORATORY OF SYNTHETIC EMBRYOLOGY




FRAMES
OF

MIND

If the key to a life well lived is good decisions, the key to good decisions is flexibility. Behind the brain's remarkable adaptability is its ability to reach vastly different conclusions based on the same information. Neuroscience, we are learning, is even more complex than we thought.

BY BAHAR GOULIPOUR AND ALEXANDER GELFAND

ILLUSTRATION BY ELLEN WEINSTEIN

Cori Bargmann

If there's one thing we know about insects, it's that they're drawn to light. It's why streetlights attract clouds of gnats, ceiling fixtures become insect graveyards, and bug zappers emit that purple glow. Take one dark summer night, add a little candlepower, and the nearest 200 insects will always appear.

Except, it turns out, they don't *always* appear. If you conduct a controlled experiment with a single fruit fly and a single light source, you'll discover that the fly sometimes flies toward the light and sometimes doesn't. It might instead wander aimlessly, find a dark space to rest, or not react at all. Although light attracts insects, it doesn't do so consistently.

This is a problem for neuroscientists.

The brain, we've been taught, is supposed to work like a web of circuits. A sight, sound, or smell goes in, connections are activated between neurons, and a behavioral response emerges. But why doesn't the same stimulus result in the same response every time? Even in the simplest organisms, neurologically speaking, those with only a few hundred neurons in their bodies, the exact same stimulus in the exact same situation can result in a range of behaviors.

In other words, the brain has a talent that scientists have yet to wrap their heads around. It seems capable of toggling its own circuitry when the need arises. It makes sense, of course—the brain's ability to produce robust behaviors is critical for survival, but these behaviors also need to be flexible.

Otherwise we'd all be robots stuck in a loop.

FOR US HUMANS, THERE are words like "mood" and "feelings" to describe the brain's flexibility, depending on, for instance, how tired, hungry, lonely, stressed, or anxious we are. It's anyone's best guess whether flies *feel* changes in their internal weather the way we do, but all organisms have motivations. They may or may not suffer from hunger, for example, but they certainly have a stronger drive to find food when it's been a long time since their last meal.

Some scientists refer to these neurological milieus as "internal states," relatively long-lasting shifts in the brain's inner activity that temporarily change how one interacts with the world. The existence of internal states isn't news per se. A century ago, ethologists who famously described how certain behaviors are innate, hardwired into the nervous system prior to any learning, also noticed that these behaviors are too complex to be explained as chain reflexes. They observed in animals such as fish, birds, and bees innate patterns of behavior, such as foraging and fighting, that were triggered by specific sensory stimuli—the smell of a potential mate or the sight of a potential rival. But depending on the circumstances, the same sensory cue could sometimes elicit one type of behavior,

JOHN ABBOTT

sometimes another, sometimes none at all. These early pioneers could only hypothesize about what happens in the brain to create such versitality—and in the decades that followed, biologists had neither the basic understanding nor the tools to penetrate this question. Even with recent connectomics studies in which the wiring diagrams of some organisms' nervous systems were completely mapped out, scientists have been left with more data than answers.

Now, at long last, they may be ready to explore the brain in a new light.

Aided by new technology that makes it possible to peer deeper into the nervous system with more control and precision, a number of labs are venturing into the vast abyss separating our knowledge about the brain's hardware and an individual's fluid behavior. These researchers are now taking the first steps to outline the mechanisms by which internal states such as hunger and arousal influence the behavior of organisms as simple as worms and as complex as us, shaping the way we respond to our environments and to other creatures.

"We still don't know how the brain really works," says neuroscientist Cori Bargmann, who studies decision-making in the *Caenorhabditis elegans* nematode. "We are able to describe how information flows through an individual synapse, and we know that some brain regions are important for specific behavioral functions. But what happens in between? How does information from thousands of firing neurons get organized, and how does this organization fluctuate over time?"

Looking at individual neural circuits is useful, Bargmann says, but unless we consider the larger context in which those circuits are operating we will see only a tiny piece of the picture.

EVEN IN THE SIMPLEST ORGANISMS, THE SAME STIMULUS IN THE SAME SITUATION CAN RESULT IN A RANGE OF BEHAVIORS. THE BRAIN, IT SEEMS, IS CAPABLE OF TOGGLING ITS OWN CIRCUITRY WHEN THE NEED ARISES.

"Internal states are an incredibly important feature of the brain that has been understudied," she says. "And I believe it will have clinical relevance for mood disorders, drug addiction, and a host of other intractable brain diseases."

IN A SMALL, DARKENED ROOM deep inside Vanessa Ruta's lab, tens of thousands of flies are ready to surprise us. Bred in vials about the size of a glue stick, these *Drosophila melanogaster* flies—prime subjects of genetic studies for over a century—have all kinds of interesting traits not found in the wild. Some are missing genes tied to odorant receptors, some lack the neurons they need to process visual information, and so on.

By testing various flies in different behavioral scenarios, Ruta and her team can piece together how specific types of neurons work together to influence behavior. And by using flies whose neurons are modified to express a fluorescent protein when they fire, she can literally watch what happens in a male fly's brain when it encounters a female.

Flies are dogged suitors when they want to be: A male fruit fly will pursue a female for more than 26 yards—the equivalent, in human terms, of nine miles—if he's eager to mate. If he's not, he might not even notice her. Same individual, same conditions, same preprogrammed behavior—but a different response.

Ruta uses a fly-size virtual reality stage to watch this behavior unfold. Picture a male fruit fly tethered to a little foam ball covered in tiny black dots and floating atop a stream of air. A curved screen placed directly in front of the animal covers its entire field of view, and there is a camera behind the fly and a high-resolution microscope positioned directly above its head.

By projecting a dot onto the screen and mimicking the characteristic movements of a female fly, the researchers can trick a male into believing he has encountered a potential mate. An interested, aroused male will begin walking toward the fictive female, spinning the foam ball in the process. As a camera tracks its movement, the overhead microscope records the activity of his fluorescing neurons.

The neurons Ruta is interested in today, known as P1 neurons, are known to create the arousal that gets

the fly interested and moving. Turn them on and the fly pursues a simple moving dot in front of him. Shut them down and he won't pursue anything.

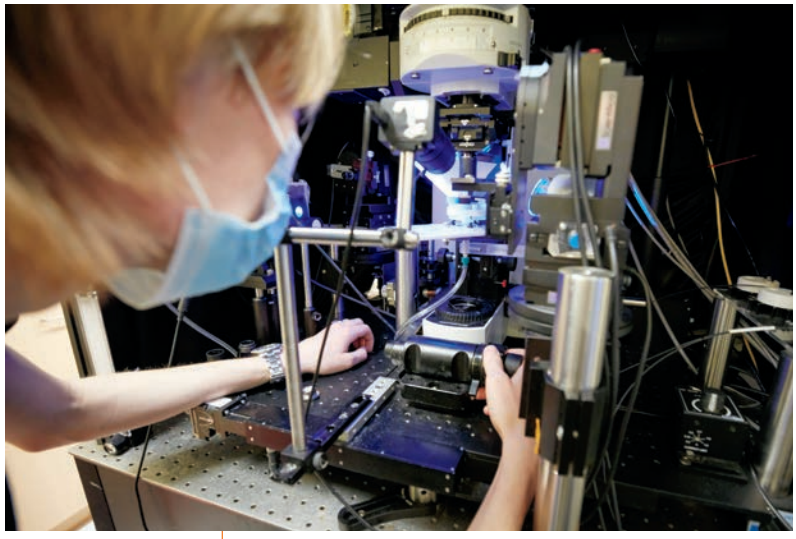
Ruta's graduate student, Tom Hindmarsh Sten, found that when P1 neurons are turned on, visual neurons sensitive to moving targets radically increase their firing and communicate with the fly's motor system, enabling him to follow them. The activity of P1 neurons and signaling of the visual neurons work hand in hand, the researchers found, fluctuating together up and down for many minutes. In a way, the P1 neurons function like a dimmable switch modulating the intensity of communication between the fly's vision and motor system.

"Even though these visual tracking neurons are detecting visual information all the time, we think they aren't able to transmit it to the motor system unless the males are in an appropriately aroused state, as decided by the P1 neurons," says Ruta, who is the *Gabrielle H. Reem and Herbert J. Kayden Associate Professor*.

How do the P1 neurons determine whether it's an appropriate time to be aroused? Ruta speculates that these neurons integrate signals from the flies' sensory organs—visual, auditory, odorant, etc.—suggesting the presence of a viable mate, plus, perhaps, the fly's own physiological state such as hunger or tiredness.

The design makes a lot of sense. This segregated circuit logic reconciles two competing needs of the fly: first, to enable the fly to reliably track and pursue a mate and, second, to remain sensitive to moment-to-moment feedback, allowing him to change his behavior if it's no longer appropriate. The internal state that we call arousal is nature's solution for producing behaviors that are at once robust and flexible.

Taken together, these findings present one of the clearest and most detailed pictures to date of how a motivational state is created, how it changes the way information is processed and routed in the brain, and how it ultimately influences behavior.



Hindmarsh Sten (top) makes adjustments to virtual reality equipment used in the Ruta lab. Cameras allow the researchers to monitor the fly's movements in response to stimuli.

MATTHEW SEPTIMUS

FOR SOME NEUROSCIENTISTS, flies are far too complicated, with their 100,000 neurons. A simpler model organism, they contend, is the best way to link individual neurons to individual behaviors. For these researchers, there is the 302-neuron *C. elegans* worm, in which every neuron has been mapped and every gene decoded.

With *C. elegans*, scientists such as Bargmann have the whole brain and the entire animal. They can see the whole system at once. And indeed, in many cases, the worm's response to a stimulus is quite simple.

For instance, take their response to diacetyl. Worms are easy to attract with this common chemical—the same compound that gives popcorn its buttery smell. Bargmann knows how to manipulate the receptor molecule for this odor in a neuron. Using genetic tools, she can make the molecule turn on in a different neuron; when that happens, the worms that detect diacetyl slither away from the source rather than toward it. This simple experiment—moving receptors around in neurons—shows how genes can be linked to specific neurons, and how neurons are prewired to behavior.

But not all the worm's responses are as uncomplicated. *C. elegans*, like many other organisms, uses pheromones to guide its social and sexual pursuits. But pheromones, mysteriously, sometimes attract companions into social groups and sometimes repel them.

Vanessa Ruta

It turns out that the sensory neuron that detects pheromones is the same, but three circuits are involved in the decision: Bargmann's lab found that one promotes attraction, another triggers repulsion, and the third decides which of the first two to listen to. By pitting the first two circuits against each other, boosting or suppressing their signals, this third input provides richer behavioral choices than those generated by the sensory neuron alone.

Pheromone responses are regulated not just by external sensory cues but by internal biochemistry—signaling molecules that "lobby" the neurons for one decision or another. The presence or absence of specific signaling molecules, which reflects the internal state of the worm's tiny nervous system, helps shape the worm's ultimate behavior.

"We wouldn't know how this circuit computes just by looking at its wiring diagram. Despite having the complete anatomical description and connectome of *C. elegans*, we can't see it as a set of absolute instructions," says Bargmann, who is the *Torsten N. Wiesel Professor*. "Instead, the anatomy represents a set of potential connections that are shaped by context to allow different paths of information flow."

It's not just a maze. It's a maze in which the paths between entrance and exit are reconfiguring themselves as you try to trace them.

APPETITE IS PERHAPS the classic example of an internal state. Feeling hungry? That smell drifting over from the neighbor's barbecue is generating saliva, causing rumbles, and perhaps fostering a sudden desire to make new friends. Feeling full? Get that cheesecake out of sight before I puke. Figuring out the internal state of humans is exponentially more difficult than that of worms and flies. One reason for this is the layered, hierarchical nature of internal states. Our internal states have their own internal states.

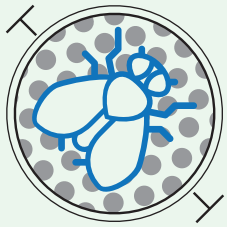
MARIO MORGADO

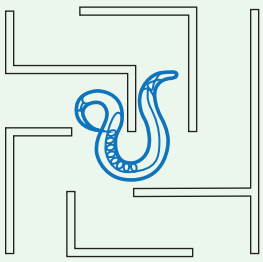
What are they thinking?

Context matters. To try to understand how, neuroscientists have developed several unique experimental protocols for use with the animals they study.

FLY ON A BALL

In the Ruta lab, scientists wanted to explore how fruit flies decide whether or not to engage in courtship. A male fly, tethered to a spherical treadmill, is shown a fictive female on a screen. He'll either walk toward her, wings fluttering in a courtship song, or he'll mind his own business. Meanwhile, the researchers record neural activity.



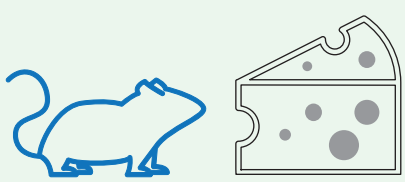


WORM IN A MAZE

Nematode worms are widely used in behavioral experiments, not only because of their simple nervous system of 302 neurons but because they move decisively toward or away from attractive and repulsive odors. Etch multiple pathways onto a plate and you can administer a multiple-choice quiz. Scientists have shown that *C. Elgans*' responses are generated by flexible circuits that integrate sensory signals with its internal state.

MOUSE AT A MEAL

Working with mammals is more complex. The neural networks that govern behaviors such as hunger and feeding can be widely distributed throughout the brain. To better understand feeding behavior, scientists trained their mice to associate hunger with a specific, unique place. Later, when the animals are returned to that space, they were observed eating even when they were fully sated.



MENTAL DISORDERS—ANXIETY AND POSTTRAUMATIC STRESS DISORDER, FOR INSTANCE—LOOK TO NEUROSCIENTISTS VERY MUCH LIKE INTERNAL STATES RUN AMOK.

Jeffrey M. Friedman

For example, hunger is controlled in the hypothalamus by, among other factors, the hormone leptin. But conscious decisions can override hunger. People may decide to eat less to lose weight, or to eat more to bulk up. Every organism keeps tabs on its nutritional state and calorie reserve, and although much of this regulation is driven by circuits in the brain stem and in particular the hypothalamus—parts of the brain that highly resemble one another in all vertebrates—mammals have other parts of the brain that are (we like to think) more evolved.

"What's different about mammals—in particular, humans—is we have a big cortex on top of these basic structures that regulates all the basic drives," says Jeffrey M. Friedman. "The cortex then makes all kinds of other judgments that might to some extent influence the function of that basic simpler circuitry."

Studying that simpler circuitry of hunger regulation, Friedman identified leptin 25 years ago. It was a breakthrough: a hormone that regulates food consumption and body weight. Manipulating leptin levels was the magic bullet that was going to allow us humans to adjust our appetites. But—and this is a recurring theme in neuroscience—it turned out not to be so simple.

Leptin is produced by fat cells and suppresses appetite. Some people who suffer from obesity are in fact leptin deficient; thanks to Friedman's discovery, leptin replacement therapy can help them lose weight. (Friedman derived the name leptin from the Greek word *leptos*, or "thin.") But leptin affects more than food intake. Animals that are deficient in leptin are also less aggressive, less active, and less likely to engage in sex. And leptin isn't the only player. Like arousal, hunger appears to be modulated by a complex web of interconnected biochemical and neural pathways.

"The hormone orchestrates a profound set of behavioral responses—and probably emotional responses—that then influence your response to all kinds of other stimuli," says Friedman, who is the *Marilyn M. Simpson Professor*.

Friedman and others have identified a population of neurons in the hypothalamus that inhibits food intake when suppressed by leptin. In Friedman's experiments in leptin-deficient mice, these neurons become hyperactive, ramping up food consumption. But in the cerebral cortex, which carries out higher brain functions, Friedman and his colleagues have recently discovered that a population of neurons can override the hunger circuitry of the hypothalamus, causing the animals to overeat even when they aren't hungry.

And the nucleus accumbens, a key reward center in the brain that is implicated in addiction disorders, likely also plays a role in feeding behaviors. Neurons in the nucleus accumbens are activated by natural rewards like food and water as well as by drugs of abuse such as cocaine and heroin. By imaging large numbers of neurons in the nuclei of mice that were in withdrawal from cocaine and morphine, Bowen Tan, a student in the Friedman laboratory, and Tobias Noebauer, a fellow in the lab of Alipasha Vaziri, observed clear differences in the animals' neural responses to food. The work explains how drug withdrawal, an internal state, influences the pleasure derived from eating.

Like Ruta's findings regarding arousal in fruit flies, Friedman's results provide an elegant illustration of how an internal state created by one neural circuit can be modified or inhibited by signals from another, adding flexibility to innate behaviors.

And because those behaviors are directly related to consumption, it's still quite possible that manipulating these signals could lead to treatments for eating disorders and related conditions such as obesity and type 2 diabetes.

AS FRIEDMAN'S WORK SUGGESTS, unpacking the myriad neural and biochemical pathways that create and regulate internal states could have major implications for human health.

Yet much work remains to be done.

Bargmann notes that, for the most part, scientists still do not know what initially triggers the internal states they have identified in various model animals.

ZACHARY VEILLEUX

Members of the Friedman lab—Jordan Shaked (top), Violet Ivan, and Han Tan—use mouse models to investigate the mechanisms behind feeding behaviors.

MATTHEW SEPTIMUS

Even more mysterious is how these states are maintained over time after the initial trigger has done its part. Neurons operate on millisecond timescales. Sometimes groups of neurons reinforce each other and stretch their activity to several seconds. But internal states persist on much longer timescales of minutes to hours.

"One of our biggest questions is how long-lasting internal states are generated and perpetuated," Ruta says. "How do you maintain an arousal state that lasts for tens of minutes?" The answer may lie in the ability to look at larger and larger populations of neurons, observing activity across regions to gain a fuller view of the gears that keep a state running.

Getting at the underlying mechanisms that regulate the internal states could be tricky, says Michael W. Young, the *Richard and Jeanne Fisher Professor*. In 2017, Young shared the Nobel Prize in Physiology or Medicine for his work on biological clocks. Back in the 1980s, when he first began looking for genes that control *Drosophila*'s sleep-wake cycles, he could not have known that he would eventually discover a whole host of proteins that form a self-regulating clockwork in the fly's cells. The discovery revealed biological principles that were later shown to apply to other animals, as well as plants, and control metabolism, development, and response to disease.

Those mechanisms establish what amounts to internal states of their own, driving behaviors that vary throughout a 24-hour cycle. And it turns out that the mechanisms of timekeeping are present in cells throughout the body, not just in the brain, suggesting a further level of complexity.

As our knowledge of the brain's internal state mechanisms expands, it could have similarly sweeping implications, advancing our understanding of phenomena far beyond ravenous mice and lustful flies.

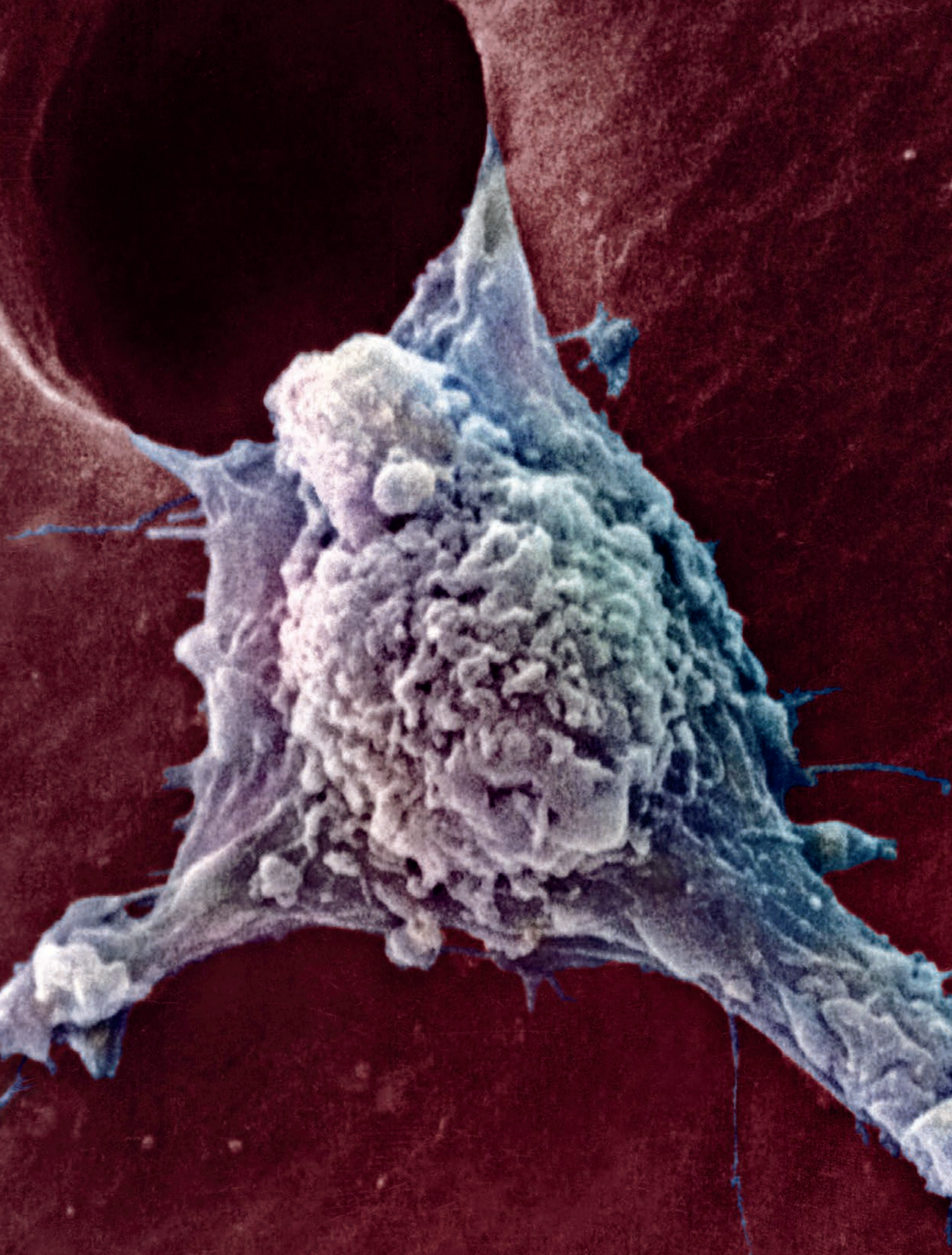
Bargmann, who in addition to her work at Rockefeller serves as head of science at the Chan Zuckerberg Initiative, points out that while mental health disorders such as anxiety and posttraumatic stress disorder impose a tremendous global health burden, neuroscientists are not nearly as good at understanding or remedying them as they could be.

Yet such disorders look very much like internal states run amok: a brain programmed to maintain a heightened state of alertness even when one is no longer necessary.

Similarly, Ruta suspects that decoding the neural basis for the regulation—and dysregulation—of internal states could have implications for attention deficit disorders. One of the principal functions of internal states, of course, is to focus our attention on and prioritize particular sensory inputs while filtering out others. And the same is true for autism spectrum disorder, which involves difficulty perceiving social cues, depression, anorexia, obsessive-compulsive disorder, and more.

Unraveling the factors that ordinarily initiate and terminate internal states could in time shed light on what causes them to be triggered too easily or to last longer than they should.

"We always have needs," Ruta says. "Presumably, the brain is just continuously fluctuating from one state to the next." ◎



AMI IMAGES / SCIENCE PHOTO LIBRARY

ONE CELL IN 10,000

Most cancer cells can't spread. Find those that can, and the disease could be a lot less deadly.

By Joshua Krisch

It's not a single alteration that makes a cell capable of the varied tasks required to metastasize. It's more like a perfect storm of them that accumulate in exactly the right way.

CANCER CELLS AREN'T good citizens—they're selfish and lazy, loitering around in clusters, stealing resources from neighboring cells, and refusing to contribute to the good of the community. But the vast majority aren't murderers. Out of every 10,000 cells that make up a tumor, 9,999 are basically innocent. Deadbeats, sure, but not criminals.

That one, though? It's capable of real mayhem.

It can evade the surgeon's scalpel. Irradiation can't touch it, and traditional chemotherapies have no effect on it. It's the cell that breaks away from the initial tumor, setting off on a dogged mission to establish new colonies in critical locations, eventually spreading far and wide. Even when cancer goes into remission, it lives on as a seedling, sprouting months or years later—bringing the disease back with a vengeance. It's the one cell that's capable of metastasis.

Metastasis is the reason cancer is so dangerous. But if you can stop that single cell, you can basically beat cancer, reasons Sohail Tavazoie. Trained as an oncologist and now running a busy lab devoted to the study of cancer biology, Tavazoie's premise is simple: If we can figure out why a tiny minority of cancer cells have the ability to trigger metastasis, and devise a way to stop them, cancer becomes a lot less scary.

IT WAS THE mid-2000s when Tavazoie began his oncology fellowship at Memorial Sloan Kettering Cancer Center. The big new thing at that time was precision oncology—the idea that, by rendering a tumor's genetic fingerprint, it might be possible to tailor drugs and other treatments for individual patients. Yet Tavazoie could never shake the feeling that focusing on the differences, instead of the similarities, among cancers might get in the way of finding broadly acting cures.

His patients' cancers had sophisticated names, classified into subtypes with technical nomenclature reflecting each cancer's anatomical birthplace and genetic features. And sure enough, researchers were coming up with increasingly sophisticated ways to attack tumors. Modern oncology had built a standard of care around the singular goal of shrinking the primary tumor. The approach was not wrong per se. It often worked. In some cases, it had resulted in new treatments with spectacular outcomes. The smaller the tumor, the less opportunity for metastasis.

But this school of thought skirted the central problem of cancer medicine rather than addressing it head-on. It is metastasis, not the primary tumor, that kills patients. So why was the focus always on the primary tumor? What we needed, Tavazoie felt, was a better understanding of metastasis.

Now, more than a decade after Tavazoie opened his laboratory, this novel approach is beginning to bear fruit in the form of two promising drug candidates specifically for the treatment and prevention of metastasis. Both drugs are based on an early discovery made by Tavazoie that upended the study of metastasis—that, regardless of what

The Tavazoie lab.

MATTHEW SEPTIMUS

cancer we're talking about, single genes could strongly drive or suppress metastasis.

The discovery emerged from a series of molecular experiments to separate cells that were capable of metastasis from those that were not. To do this, Tavazoie injected cancer cells into mice and then removed the tumors that formed outside of the initial injection site. After dicing, spinning, and mixing the metastatic tumors, he used traditional techniques to isolate single cells for analysis.

Tavazoie, who is the *Leon Hess Professor*, knew that there had to be some molecular anomaly that made these metastatic cells unique. But cancer cells are full of genetic anomalies—that's what makes them cancer cells. Figuring out which specific aberration was allowing these cells to metastasize meant digging through a litany of alterations; some genes were abnormally active, others abnormally inactive. There were lots of clues to why this was the case, but no clear culprit, and certainly no useful information for cancer patients.

"At this point, I wasn't even thinking of therapies. I just wanted to find genes linked to metastasis," Tavazoie recalls. "We hoped that what we found might help others, in the future, identify drugs that could target those key genes."

Eventually, by painstakingly winnowing a long list of candidates, they landed on the specific gene that would end up defining much of the lab's work and would form the basis of their drug candidates: *ApoE*.

Tavazoie's lab, the Elizabeth and Vincent Meyer Laboratory of Systems Cancer Biology, was not the first to look for answers in metastatic cells. As early as 1889, English physician Stephen Paget proposed his "seed in soil" hypothesis based on autopsies of hundreds of women who had died of breast cancer. Paget suggested that metastatic "seeds" spread out from tumors but only form colonies in areas with opportune "soil" for further growth.

This basic model stood the test of time, and nearly a century later it led to pioneering work by cancer scientist Isaiah Joshua Fidler in the 1970s. Like Tavazoie, Fidler injected cancer cells into mice, monitored for metastasis, and isolated those that managed to spread. Despite lacking the kinds of genetic tools available to Tavazoie, Fidler nevertheless arrived at a high-level picture of metastasis that has endured.

A lot has to happen for a cancer cell to break bad. Most cells in a tumor are perfectly content to remain in place, and, of those cells that do escape, precious few are able to survive. Metastatic cells must change to penetrate healthy tissue or otherwise sneak their way into the circulatory or lymph system. They must develop the ability to fend off immune cells, and, upon arrival in an end organ, they must figure out how to grow their own blood vessels for nourishment.

And it's not a single alteration that makes a cell capable of the varied tasks required to metastasize. It's more like a perfect storm of them that accumulate in exactly the right way. Given this reality, it's remarkable that it happens as often as it does.

Until you consider the possibility that metastasis, like many complex life processes, isn't actually random.

"We're now attacking metastasis from intersecting angles. We have experts in RNA, immunology, metabolism, mitochondrial biology, all focused on the same problems."

APOE WAS THE linchpin," Tavazoie says.

A well-studied gene, *ApoE* has been linked to multiple diseases, including Alzheimer's. But in melanoma cells, Tavazoie found that *ApoE* was at the center of a suppressive pathway that prevents a cascade of metastatic events from occurring.

The winding road that led Tavazoie and his team to *ApoE* began with experiments involving microRNA, small segments of RNA that turn specific genes on or off. Since the early 2000s, researchers had known that one of the main features of cancer cells is the total collapse of the microRNA system. Predicting that wayward microRNA might be linked to metastasis, Tavazoie isolated metastatic melanoma cells from mice and scoured the samples for microRNA anomalies, eventually finding a cluster that was entirely out of control.

In metastatic cells, these hyperactive microRNA were shutting down the *ApoE* gene.

Bit by bit, the mystery of metastasis began to unravel. The Tavazoie lab continued to pursue *ApoE*, demonstrating that three of the necessities for metastasis—forming new blood vessels, fending off immune cells, and penetrating healthy tissue—were regulated by that one gene. They found that melanoma cells with active *ApoE* never metastasized and that cells that did spread from the primary tumor invariably had inactive *ApoE*.

Eventually they came full circle, demonstrating that microRNAs function as a switch to turn *ApoE*, and metastasis, on and off. Disabling these microRNAs with a cocktail of nucleic acids revived *ApoE* and prevented metastasis in mice.

"These were very exciting years for us," Tavazoie says. "We had pinpointed a critical gene that strongly regulates metastasis and could manipulate it to prevent melanoma cancer cells from spreading."

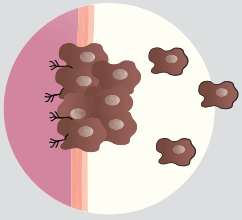
THE DISCOVERY OF *ApoE* at the heart of metastasis led to a flurry of activity from the Tavazoie lab, adding more detail to the emerging picture.

One recent Tavazoie lab investigation, spearheaded by Benjamin Ostendorf, a physician-scientist; Jana Bilanovic, a graduate student; and Nneoma Adaku, an M.D.-Ph.D. student, revealed how the three versions of *ApoE* that exist in humans impact cancer progression differently. They found that *ApoE4*—a variant that, paradoxically, increases the risk of Alzheimer's disease—appears to be the most effective subtype when it comes to preventing melanoma metastasis in mice.

A retrospective human study suggested humans benefit from *ApoE4* as well. According to medical records of 300 patients with melanoma, those lucky enough to have had the *ApoE4* variant were those that survived the longest. This was the first discovery that human hereditary genetics regulate metastasis, and it solved a long-standing

How to metastasize in four somewhat tricky steps

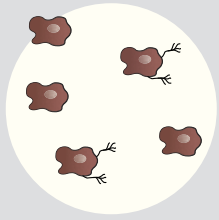
The deadliest cancer cells are those that successfully break away from the primary tumor, survive the wilds of the circulatory system, and put down roots in a suitable end organ. The process goes like this:



1

Escape

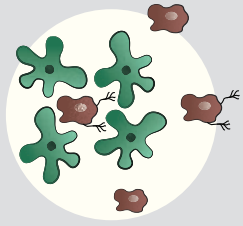
Through a series of mutations, metastatic cells become able to navigate a labyrinth of connective tissue surrounding their original tumor. A small population of newly motile cancer cells are well suited to this endeavor.



2

Build a network

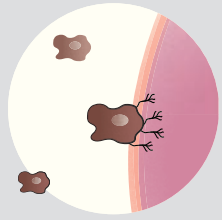
Traveling, transitioning cells need nutritional support. Metastasizing cells must form their own vasculature to deliver nutrients and remove waste. As cancer cells tunnel through the matrix of macromolecules within the extracellular environment, they must harness growth factors to form blood and lymph vessels.



3

Adopt a disguise

The majority of metastasizing cells will die en route, assaulted by the immune system. If they are to survive, they must rally fibroblasts, platelets, and other immune factors to their cause, armoring themselves with protective buffers made from the body's own cells and using them to evade attack.



4

Pick a new home

Metastatic cells are like seeds in soil—they cannot proliferate unless they happen to land in an ideal microenvironment containing the right ingredients for growth.

conundrum in the field regarding the genetic basis for metastasis.

"We're now attacking metastasis from intersecting angles," says Adaku. "We have experts in RNA, immunology, metabolism, mitochondrial biology, all focused on the same problems."

And the work is now beginning to coalesce around the development of new drugs capable of reviving *ApoE*. If they are shown to be effective in preventing metastasis in the future, it would be highly impactful for cancer patients. "If we had a therapy that could ensure that the cancer would not spread, a surgeon could take out the primary tumor—and you're done," Tavazoie says. "There would be no further treatment, and the cancer would not recur."

Ostendorf (right) and his colleagues explore how *ApoE* variants impact metastasis of melanoma.

MATTHEW SEPTIMUS

A FIRST ATTEMPT AT developing such a drug was based on the liver X receptor, which limits the expression of *ApoE*. Tavazoie and his colleagues showed in 2014 that an oral medication that acts on this receptor prevented metastasis of melanoma in mice. The mechanism was simple and matched years of observations: with the liver X receptor off-line, *ApoE* was free to produce proteins that block metastasis in healthy cells. One year later, similar techniques led to the identification of another critical metastasis gene that regulates creatine metabolism, and the lab has since discovered a compound that inhibits this gene and prevents colorectal cancer metastasis in mice.

A drug development company co-founded by Tavazoie has begun work on a clinical-grade version of these compounds to test in humans. Phase I trials of the compound, designed to arrest melanoma metastasis, confirm that it activates *ApoE* in humans just as it had in mice, and clinicians anecdotally report that the compound appears to stabilize the progression of metastasis in many patients with various cancer types. They have also observed examples of metastasis shrinkage responses in multiple patients. A separate drug designed to arrest metastasis in colorectal cancer patients recently began Phase I trials as well.

"Both drugs appear to be safe and well-tolerated, and they are providing proofs of concept of what we observed in mice. We're also excited to hear that clinicians are observing examples of anti-metastatic activity in patients with advanced disease," Tavazoie says, while cautioning that preliminary reports from early trials designed only to evaluate safety are not scientific claims of efficacy.

Although further trials are in the works, there's a thorny problem when it comes to launching clinical trials on metastasis drugs. New cancer therapies are typically tested first in patients who are already very sick, for whom more established treatments have failed—it wouldn't be ethical to deny traditional treatments to people who need them in the hopes that a new drug would work better. But if you wait until those treatments have failed, it's difficult to evaluate a drug designed to prevent metastasis—such patients already have metastatic cancer. The upshot is that two compounds designed to prevent metastasis from ever happening are being tested in patients who already have advanced metastatic disease.

Nevertheless, Tavazoie is optimistic.

"Metastasis is a real bottleneck event," he says. "There are millions of cells within a tumor, and only one in 10,000 has what it takes to spread to another organ and form a colony. That we're up against such an extreme, rare event gives me hope that we will one day learn to prevent it."

But he does not expect any single drug to spell the imminent end of metastasis. Tavazoie envisions a more prudent future, in which a new generation of anti-metastatic drugs that activate *ApoE* work alongside existing treatments.

"We don't intend to replace targeted cancer therapies," Tavazoie says. "Our work exists parallel to other effective therapies such as hormonal therapies for breast and prostate cancer and immunotherapy for melanoma and lung cancer. Our long-term goal is to find a key pathway that could reduce the likelihood of metastasis by, say, 80 percent. Targeted therapies would extend the patient's life and remove the cancer, and our therapy would keep it from coming back." ◎

How a small study of arthritis patients gave birth to the pandemic's most innovative virus test

BUILDING A BETTER COVID TEST

By Joshua Krisch

Photographs by Matthew Septimus

1 Test kits are assembled in a conference room near the Darnell lab, each containing a vial of buffer, a biohazard bag, two paper cups for spitting, a bulb syringe, and illustrated instructions. Over 1,500 kits are distributed on campus each week.

LOOKING BACK, VACCINE coursing through our veins, it's easy to forget just how chaotic those early days of the pandemic were. But there was a time when it was hard to even find out if you were sick.

For months, COVID testing was a mess. There were dozens of testing protocols, many of them hastily released and unregulated. Tests themselves were scarce, slow, overpriced, and frequently unreliable. The testing process was risky for those collecting and processing samples. And the disease was spreading at busy testing sites. Testing failures weren't the only reason the SARS-CoV-2 virus was surging out of control. But they certainly helped.

Robert B. Darnell looked on in horror. "Every aspect of COVID-19 testing fell apart," he says.

Darnell isn't a virologist. He's not an epidemiologist or an expert in infectious disease. Before COVID-19 upended his research, Darnell's lab was primarily focused on RNA biology and autoimmune inflammatory diseases, in the lab and in the clinic. But thanks to a genetic testing procedure he developed as part of that research, Darnell found himself in the perfect position to take on the COVID clinical testing problem.

IN THE SUMMER of 2020, the consequences of our failed national testing apparatus were piling up. Doctors were guessing at diagnoses. Hospitals were struggling to isolate infectious patients. Public health officials were unable to track trends and identify hot spots, let alone trace contacts and establish effective quarantine programs. Reopening schools and workplaces was unthinkable.

2

KELLY MCLEES

Long before the virus struck, Darnell was a proponent of self-tests—the kind you can do at home with a kit and send in the mail. Many patients with autoimmune disorders such as rheumatoid arthritis, which Darnell studies along with his collaborator Dana Orange, an assistant professor of clinical investigation, suffer unpredictable spikes in severity. The researchers had a long-term project to collect patients' blood samples over time, tracking how levels of certain RNA might change in the days and weeks leading to a flare-up.

Over five years, Darnell's lab perfected a system by which arthritis patients could prick their fingers at home on a regular basis, collect the blood in a tube containing a solution that stabilizes RNA, and ship it to the lab for analysis. It was an easy, cost-effective way of acquiring samples directly from patients. And, although he didn't know it at the time, it was a pretty good proof of concept for a community testing scheme.

"We had RNA and molecular biology expertise, a distribution system, and an idea for collecting samples from individuals over time," Darnell says. "Then the novel coronavirus—an RNA virus—blew up." Suddenly, one of the most pressing needs of biomedicine was for a safe and inexpensive way to collect samples from patients remotely and screen them for viral RNA.

Darnell, who is *Robert and Harriet Heilbrunn Professor,* hatched a plan to develop a simple COVID test that could be administered easily at home, processed with readily available supplies, and deployed rapidly. The best part: no deep nasal swabs. All you have to do is spit in a cup.

By mid-fall, Darnell's operation, run from his own Rockefeller lab, had become a well-oiled machine, both technologically advanced and charmingly scrappy. His staff worked alongside newly hired laboratory consultants, graduate students, postdocs, and faculty and administrative staff reassigned from other departments. The sophisticated trappings of a clinical laboratory juxtaposed with a revolving door of Darnell's colleagues asking questions or lending a hand. Alongside a robotic arm that uncapped vials and mechanically mixed buffers, volunteers stuffed homemade testing kits into plastic baggies.

2 Adults, kids, and even toddlers and infants take the test at home or in their offices or classrooms. Saliva is transferred from the cup to the vial using an eyedropper.

3 Samples that are ready for processing, sealed in biohazard bags, are picked up daily by the staff of the glasswashing facility from secure drop-off sites in building lobbies.

4 At the height of the pandemic, up to 400 completed test kits were arriving in the Darnell lab every weekday. They are unloaded and sterilized in an oven before being handled further.

"It takes a village," Darnell says. "But we ended up with an extremely sensitive, safe, and inexpensive test that, logistically, could be done at home or in the office."

IT WAS CLEAR early on that a saliva-based test was the way to go. Studies from the 2003 SARS pandemic had demonstrated that coronaviruses infect salivary glands quite efficiently, and that whatever traces of virus could be collected by an uncomfortable and occasionally painful half-inch nasal swab could be obtained just as easily by having people spit into a cup.

Indeed, the Hong Kong government adopted a saliva test early on that showed promising results. "Together, these observations piqued our interest in trying to put together a saliva test that could be performed at home, placed in a buffer solution, and easily brought to the lab for analysis," Darnell says.

Although several groups jumped on the saliva bandwagon, Darnell's prior work with RNA gave the Rockefeller team an edge. And while many others focused on developing buffer solutions that would culture the virus and keep it alive long enough for testing, Darnell understood that spitting into a solution designed to grow more virus, or even just sending possibly contaminated "raw saliva," put everyone along the testing pipeline in danger. It was an unnecessary risk—you don't need live virus to detect the coronavirus's unique RNA signature.

"We developed a simple buffer, which uses inexpensive off-the-shelf reagents and, instead of culturing the virus, kills it on contact," Darnell says. "At the same time the solution stabilizes RNA, which is what we need to detect the virus."

Critically, the use of saliva meant the test could be self-administered. No nurses, no techs, no need for disposable PPE.

"At the time, there were shortages of swabs and gloves and masks and reagents," says Mayu Frank, clinical research coordinator in Rockefeller's Clinical Genomics Lab. "Our goal was to get around those obstacles, too, by designing a home collection kit."

By the time Darnell's saliva-based home-collection protocol rolled out in April, New York State had


3

4

5 Each vial is labeled with a unique barcode, ensuring that Darnell's team can track the samples accurately as they make their way through the testing process.

6 A robotic arm uncaps, mixes, and prepares 96 vials at a time for extraction and PCR analysis.

7 The vials are now ready for PCR sequencing, which will screen each sample for the presence of viral RNA. The DRUL assay can detect even one viral copy in a microliter of fluid.

clinically certified the lab and green-lighted the test, and preliminary results suggested his assay was at least ten times more sensitive than comparable tests. Practically, this meant that the Darnell test could likely detect much less virus, screening even asymptomatic patients and catching infections earlier on.

Darnell's test, cleverly dubbed the DRUL (Darnell Rockefeller University Laboratory) saliva assay, soon became the centerpiece of the university's reopening plan. It circumvented many of the shortages, dangers, and impracticalities of screening for the virus, and by the beginning of 2021, the Darnell lab was processing over 1,500 clinically certified saliva tests each week, ensuring that everyone reporting to work at Rockefeller's campus could be screened every seven days.

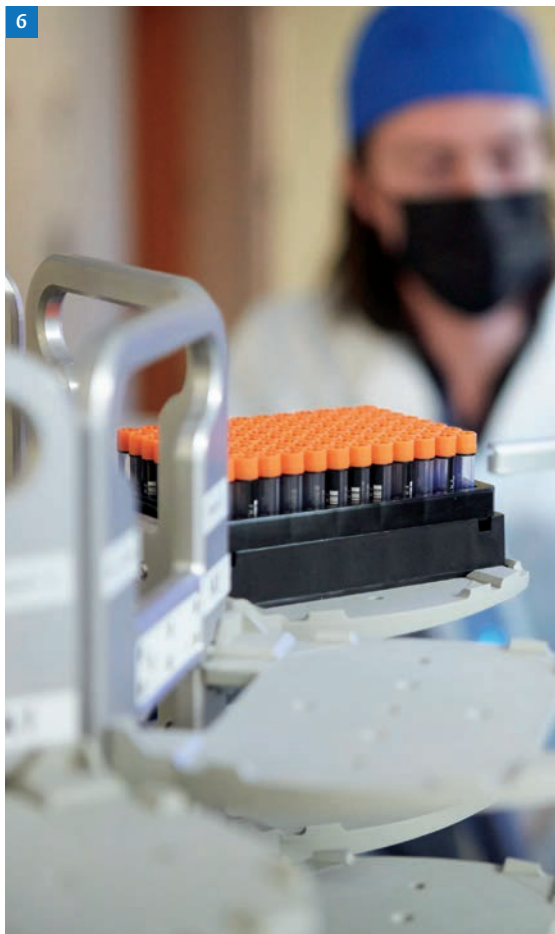
A SALIVA TEST at Rockefeller begins its life in the Darnell lab, where members of the team take turns assembling 1,500 homemade kits each week. Every kit contains a barcoded vial of virus-killing, RNA-stabilizing buffer loaded by a robot, together with a biohazard bag, two paper cups, and a small plastic bulb, along with illustrated instructions resembling an airplane emergency evacuation guide.

The instructions are simple: Spit barely one-fiftieth of a teaspoon into the cup, use the bulb to transfer it to the vial, and seal the contents in the biohazard bag. It is also relatively stress-free. Comparable saliva tests, Darnell says, demand as much as an entire teaspoon of saliva—nearly ten minute's worth. (For more on producing spit, see "The lemon aid," page 48.)

From Darnell's lab, the kits can be sent anywhere. But most are disseminated around Rockefeller's campus by staff from the university's centralized glasswashing facility, which already makes daily rounds to pick up and deliver glassware. Alice Dyer, who runs the service, and her colleagues generally pick up a week's worth of test kits from the Darnell lab on Mondays and divide the bounty among six red bins placed at strategic locations around campus. Individuals can take a kit from a red bin, self-administer the test, and drop their samples in a corresponding green bin.

"It is very important that the samples are picked up


5


6

In a head-to-head comparison with two commerical laboratories, the DRUL assay detected four positive cases that the nasal swab had missed entirely.

in a timely manner, so the test can be completed as soon as possible," says Dyer. "If we did not have this system, we could have a COVID-positive person walking around campus unwittingly infecting other people for days while they wait for their results."

During the peak of the testing program, between 300 and 400 completed test kits were arriving in the Darnell lab each weekday, courtesy of Dyer's team. Once received, the entire collection is placed in an oven for ten minutes to sterilize the outside of the kits, thin the saliva for testing, and kill any residual virus. Lab members then load a robotic arm with 96 vials at a time, where the samples are automatically uncapped and mixed with more solutions that prepare the RNA for extraction and PCR analysis.

Samples are finally loaded into a PCR machine, which amplifies the genetic material in the sample, allowing for easy detection of known coronavirus signatures. The results are usually negative, showing only natural saliva RNA. But there are occasional positives. "When it's positive for viral RNA, it is very clearly positive," Darnell says. After testing, the samples are destroyed and the results are reported back to the patients as well as to state public health authorities. If a test comes back positive, the university's nursing staff are notified so that contact tracing can begin.

In order for the results to be clinically useful, the Darnell testing lab went through the onerous process of becoming clinically certified, signifying a level of rigor well above that required for research. Work conducted in clinically certified labs requires all members directly involved in the research to undergo training with each assay. Every test must be certified by New York state, and even the most minor upgrades in method or material require fresh approval. A state-licensed clinical director checks in regularly, and the members of the lab are held accountable for their work.

These extra steps mean that the tens of thousands of results processed through the Darnell lab are reported daily to the state and can be studied by health officials and used to track the progress of the pandemic. (Nonclinical research results, by contrast, are considered preliminary and cannot contribute to the development of public health guidelines.)

7

8 Data from the PCR test is analyzed and positive cases are flagged.

9 The results are sent directly to employees by email and any positive cases are flagged for Rockefeller's Occupational Health Services office, where nursing staff provide counseling.

10 The OHS team, led by COVID-19 program director Ann Campbell, carefully traces on-campus contacts that infected patients have had in the previous hours. They also follow up with those who become sick to provide guidance and support, and stay in touch by text message.

As part of the test's validation process, Darnell put it head-to-head against tests processed by two commercial laboratories using protocols operating under the FDA's emergency use authorization program. Some 162 individuals at two sites received nasal swabs and provided saliva at the same time. In four cases, the DRUL assay detected positive cases that the nasal swab had missed. Overall, the study concluded that Darnell's test detected the virus as well as—or better—than competing tests.

ONE OF THE first positive results on campus was a member of the Child and Family Center, which provides childcare and education to families affiliated with Rockefeller. The test had been taken Friday morning, and the results were in that evening.

"We knew immediately," says Pamela Stark, director of the CFC. With help from OHS and the CFC nurse, the individual's known contacts were traced and quarantined. By Monday, they were confident the outbreak was contained and no one else was infected.

The program was also critical for the safe continuation of essential operations, such as animal care. Before vaccinations began, the 100 members of Ravi Tolwani's team at the Comparative Bioscience Center submitted samples for screening each week. When a few positive results turned up in his group, Tolwani collaborated with Darnell to rapidly screen anyone who may have been exposed, and the contagion was contained. "Many of our staff use public transportation, and even though we follow staggered schedules and distancing guidelines, we want CBC members to know that they aren't spreading the disease to their family members when they go home," Tolwani says.

Beyond the laboratories, the saliva test has allowed Rockefeller families to return to some degree of normalcy. Thanks to weekly testing of children, teachers, and parents, the CFC reopened in July 2020, months before other schools and childcare programs were able to do the same. Later, testing was expanded to include a K–8 "learning pod" program that provided oversight for small groups of older children attending school remotely. This meant that parents could

8


9

return to work and that science—including COVID research—could continue. "We have parents who are scientists doing groundbreaking work with COVID-19, but they needed reliable childcare in order to do that work," Stark says.

BETWEEN MARCH 2020 and May 2021, 150 members of the Rockefeller community tested positive for COVID. There were no deaths and only one hospitalization. Remarkably, no on-campus transmission was detected. Early implementation of Darnell's testing program, along with rapid quarantining of individuals who tested positive and strict adherence to social distancing, mask wearing, and other infection-control policies, allowed cases to be quickly identified and isolated. The university's 16-acre campus may well have been the largest chunk of land in the city from which the virus found itself excluded.

Moreover, Rockefeller provided the testing ground for the feasibility of the project and the efficacy of the assay. Darnell's saliva test has since been distributed far beyond campus.

The technology has been shared with groups at the Howard Hughes Medical Institute research campus, scientists at Stop COVID-19, staff working at New York City's Department of Health and Economic Development Corporation, and in the city's integrated health care system. Darnell has also shared his testing protocol with colleagues working in laboratories at the University of Washington, the University of California, Berkeley, Columbia University, Michigan State University, MIT, and the Max Planck Institute.

The team's daily efforts are still paying off as the pandemic recedes, protecting the Rockefeller community and others. Although high vaccination rates on campus made it possible to relax social distancing policies in the late spring, weekly testing continues, in order to catch both new cases among those who haven't been fully vaccinated and breakthrough cases in those who have been.

Meanwhile, work to refine the protocol, improve the testing process, and make it more widely accessible continues. When the next virus strikes, the testers will be ready. ◎

INTERVIEW

When the virus struck, researchers responded with unrivaled focus, creativity, and cooperation. Will science maintain its newfound momentum even as this pandemic recedes?

America is getting vaccinated. Now what?

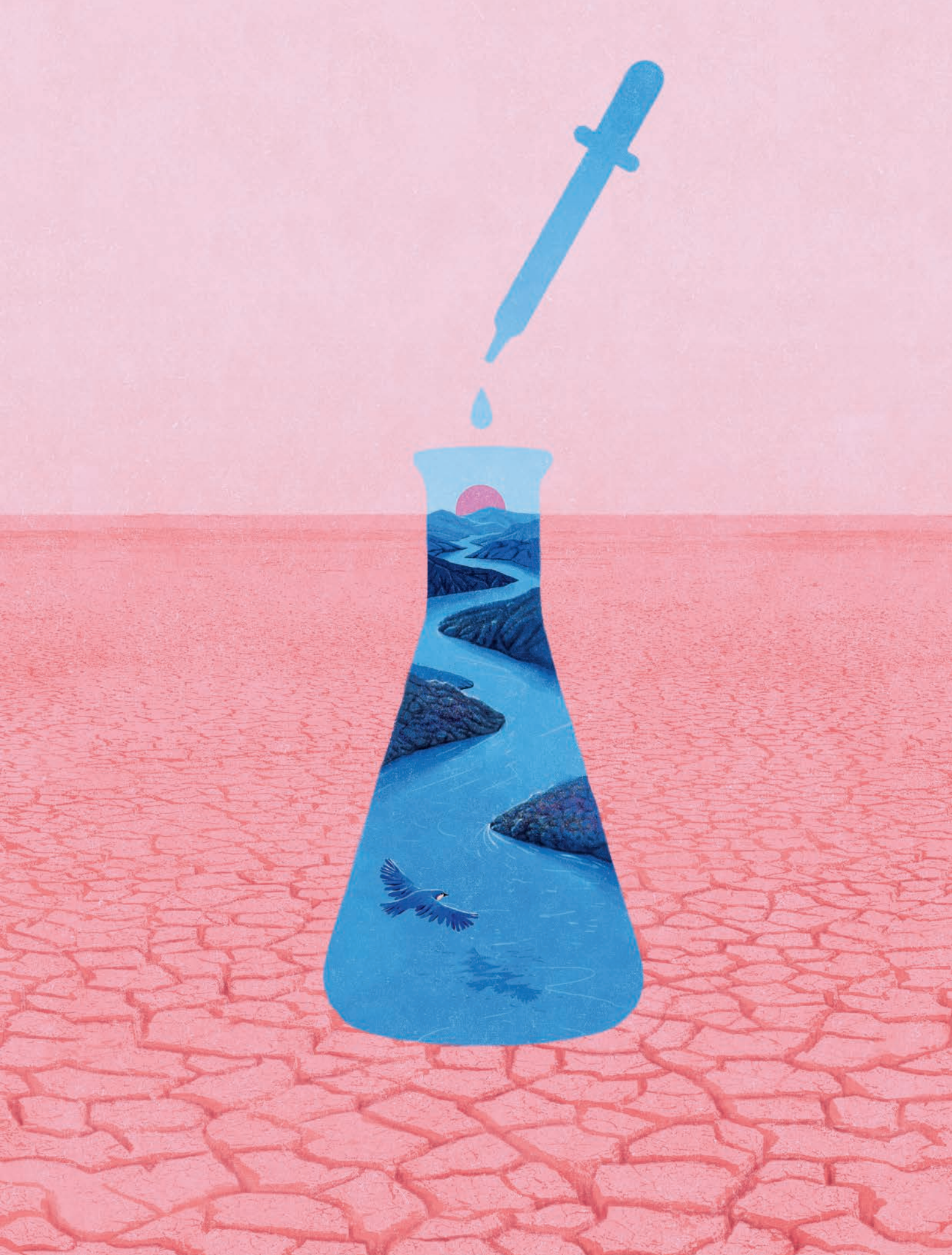
By Eva Kiesler

For the past 18 months, the pandemic has wrecked lives, crushed businesses, and tanked national economies. Meanwhile, science has flourished.

In particular, SARS-CoV-2 has become among the best studied pathogens in the history of medicine. And the urgency of the crisis has created focused attention on biomedical research and its importance to our lives, leading to new modes of discovery and launching unprecedented collaborations. The results—vaccines with unmatched efficacy, innovative monoclonal antibody drugs, novel research tools, and more—suggest the possibility of a new normal for science.

How did we get here so quickly, and what challenges lie ahead? Is biomedical research on the cusp of a continuing renaissance, or will the passing of the crisis bring a return of old habits? How will we prepare for the next virus?

Illustration by Daniel Liévano



We discussed these and other questions with three experts participating in efforts to mitigate COVID.

THEODORA HATZIIOANNOU is a research associate professor in the Laboratory of Retrovirology and coauthor of *Principles of Virology: Fifth Edition*, a leading virology textbook. An expert on HIV, Hatziioannou is now studying how SARS-CoV-2 variants interact with antibodies. She has also played a key role in developing a monoclonal antibody drug against COVID now in clinical trials.

RICHARD P. LIFTON, the *Carson Family Professor*, is president of The Rockefeller University and head of the Laboratory of Human Genetics and Genomics. As president, Lifton put in place rules and practices that allowed COVID research, and science as a whole, to move forward safely and swiftly at Rockefeller.

CHARLES M. RICE is the *Maurice R. and Corinne P. Greenberg Professor in Virology* and a winner of the 2020 Nobel Prize in Physiology or Medicine (see "A Nobel like no other," page 9). Best known for his work on hepatitis C, which led to a cure, Rice has worked on numerous viral diseases including Zika, dengue, yellow fever, and hepatitis B. Recently his lab identified human proteins that SARS-CoV-2 needs to survive inside host cells, a discovery they hope will translate into new broad-spectrum antiviral drugs.

What has most impressed you about scientists' response to the pandemic?

TH: The response has been amazing. Here at our institution and across the world, labs began forming tight collaborations, working almost as one, with each person contributing the types of experiments they were good at. The results are remarkable—an outpouring of publications has already impacted how we manage COVID at different levels, from slowing transmission to developing vaccines to treating infected patients. And on a personal level, being part of this movement has been an unforgettable experience.

CMR: I was struck by the sheer number of researchers who stepped up to help mitigate the crisis, many of whom had never before worked in infectious disease. By asking themselves how their expertise could be of help, they were able to come up with creative ideas and approaches. I suspect many of these "converts" will continue to work on coronaviruses or other aspects of COVID-19 after the pandemic is behind us.

Yet it's important to remember that the many scientific achievements we've witnessed over the past year didn't come out of nowhere. They are the direct result of decades of research aimed at understanding fundamental life processes.

Can you give an example?

RPL: One spectacular example is the development of highly effective mRNA vaccines like Pfizer's and Moderna's, which resulted from decades of molecular biology research. Only in recent years have scientists come up with clever ways to use RNA molecules for the development of vaccines and other kinds of therapeutics. They've had to overcome significant hurdles, including the fact that the immune system is built to recognize and destroy foreign RNAs when they get into cells.

When the pandemic hit, they had learned how to get around this problem by biochemically modifying RNA molecules. Just in time, the technology was ripe for the opportunity to develop lifesaving COVID vaccines.

Theodora Hatziioannou

Richard P. Lifton

Charles M. Rice

FRANK VERONSKY; FRANK VERONSKY; JEFF HENNEFELD

CMR: Another thing that turned out to be critical was past research on the SARS-1 and MERS viruses, relatives of the novel coronavirus that also jumped from animals to humans. Historically, very few labs focused on coronaviruses, studying mainly mouse hepatitis coronavirus, but the field was invigorated during the 2003 SARS outbreak and then again with the 2012 MERS outbreak, when there was fear that these highly pathogenic viruses would spread across the globe. These viruses use the spike protein on their surface to gain entry into host cells—which, as we now know, is also the case for SARS-CoV-2.

When the present pandemic hit, scientists didn't have to begin studying a new pathogen from scratch but could immediately focus on the spike protein, which indeed has turned out to be an excellent target for vaccines as well as for treatments such as monoclonal antibodies. Imagine how much time might have been lost if it weren't for the early work on mouse hepatitis coronavirus, SARS, and MERS.

With that lesson in mind, do you think the pandemic will cause a lasting shift in scientific priorities? Will more resources be allocated toward virology and infectious disease?

TH: I really hope so. Before COVID, virology was not very well funded. Unless you worked on a major disease-causing virus like HIV, it was very hard to get grants. Now, of course, money is streaming in to support research on SARS-CoV-2.

CMR: Coronavirologists have become the superstars of the day! Whether or not the COVID-19 pandemic experience will lead to sustained funding and changes in public health preparedness remains to be seen.

TH: But it's hard to tell what will happen once the pandemic is under control. As we saw with SARS and MERS, science policy tends to be rather shortsighted. A new virus emerges, and everyone panics—but once the threat has passed, things have a tendency to go back to how they were.

Maybe this time will be different. There are more viruses on Earth than stars in the universe, so we can't possibly study them all. But if governments and institutions were to allocate more resources to this field, we'd be able to cast a wider net. This would put us in a better position to respond the next time a coronavirus jumps from bats to humans or some other type of virus shows up in the human population. Because it will happen again, no doubt.

How might we get better at tackling coronaviruses we haven't yet encountered?

CMR: Among other things, more research into this viral family might allow us to develop an antiviral akin to a broad-spectrum antibiotic—a drug people could safely take if there are outbreaks with viruses similar to SARS-CoV-2. Ideally, one could use such a drug to treat people in the earliest stages of infection to prevent disease and curb further transmission among those who've been exposed to the pathogen.

TH: As with interventions against other pathogens, multiple drugs will need to be developed since viruses can acquire

resistance to individual compounds. Having multiple drugs also increases the potential for tackling future emergent coronaviruses.

So far the vaccines developed for COVID have been extremely effective. Will they be enough to get us permanently past this pandemic?

CMR: I think vaccines will make a big difference, but we cannot stop there. For one thing, we need to develop treatments and means of prevention for people who can't get the vaccine because they are immunocompromised, or who choose not to get vaccinated. To that end, there is much promise in modalities such as monoclonal antibodies and small-molecule drugs now being pursued at Rockefeller (read more about this work in "Inside the response" in the Fall 2020 issue of Seek).

TH: Also, the vaccines being used today will have to be tweaked and further studied going forward. We only began vaccinating people en masse in December, so it's way too early to know how immunity will evolve after vaccination. For how long do the vaccines work, and how effective will they be against new variants? And how will the virus evolve?

RPL: Beyond answering these scientific questions, there are urgent public health issues we need to address at the societal level. At the beginning of the pandemic, we were totally unprepared to diagnose the disease. We consequently failed to prevent people from getting infected and did not curb the spread of the virus.

Moreover, the pandemic has revealed deep health disparities based on income, race, and ethnicity, underscoring just how broken our public-health system is. This inequity is unacceptable, and we have a long way to go to make sure the vaccines become equally accessible to all citizens. We can do the greatest science in the world, develop the best diagnostic and therapeutic capabilities—but those agents will never have the impact they should if we can't deliver them to the most vulnerable members of our society.

If national and local governments faltered, the university responded proactively when the pandemic struck. How did this response unfold?

RPL: Our first goal was to keep all members of our community safe while also making sure buildings and facilities remained operational during statewide lockdown. In March of last year, we closed the university while retaining a skeleton staff of about 180 people whose presence on campus was indispensable. From day one, we implemented robust protocols for mask wearing, social distancing, and cleaning, as well as systems for testing and tracing. We had a committee of faculty experts monitoring developments to recommend changes to our practices as we learned more about how the virus works and how it spreads.

Our scientists had no intention of remaining idle. Nearly a third of our labs immediately pivoted to take on projects that would make meaningful scientific contributions to help combat the pandemic. Across campus, an inspiring effort took place to make this possible—reducing the density of scientists in the labs, improving air circulation, and establishing a testing and tracing program to prevent the spread of COVID on campus. We're fortunate to have a very dedicated workforce that helped keep our facilities secure, facilitated our essential operations, and ensured adequate supplies of PPE and other laboratory supplies we needed to do our work.

Last summer, we began reopening all labs with safety procedures in place, and as this scaled up, we instituted mandatory weekly COVID testing for everyone coming to campus using a robust saliva test developed on campus. With these precautions, we have had no cases of viral transmission

The pandemic demanded immediate sharing of data and showed that we can move much faster, and conduct investigations at a bigger scale, by working collectively.

DANIEL LIÉVANO

on campus throughout the pandemic, testimony to the impact each of us can have by working together to protect one another (for more about saliva testing, see "Building a better COVID test," page 34).

What implications might ongoing COVID research have for other diseases?

TH: The speed and detail at which we are learning how the immune system responds to virus infection and vaccination are just remarkable, and this knowledge is giving us a brand-new tool kit to study immune mechanisms in general. So far, much of what we know about antibody and T-cell responses to pathogens comes from decades of studies mainly of HIV-1, but now we have the opportunity to delve deeper, potentially generating knowledge and technologies that will advance research on virtually any infectious disease. The same is true for vaccines—novel platforms such as mRNA technology will find uses across many diseases in the future.

Also, there is so much we don't know about how viruses affect the body more broadly. For example, it will be very interesting to figure out the underlying causes of mysterious conditions such as long COVID, or PASC, in which people infected with SARS-CoV-2 may have symptoms for a year or more, and MIS-C, a rare and potentially dangerous side effect of COVID affecting young children. Research in these fields will likely yield knowledge applicable to a host of other post-viral diseases.

During the pandemic, scientists moved quickly to share their results, publishing a deluge of data on preprint servers ahead of peer review. Are cultural changes like this likely to stick?

RPL: The urgency of the pandemic demanded immediate sharing of data and showed that we can move much faster, and conduct investigations at a bigger scale, by working collectively—which we've long known but haven't always been able to operationalize. I'm sure that many collaborations that began in the pandemic will continue and that aspects of rapid dissemination of data via preprint servers will spread through much of the biomedical community. Nonetheless, not all science is advanced by large collaborations, which can get bogged down in bureaucracy and can stifle creativity. We need to be wise in fostering support for the most transformational ideas while selectively applying the advantages of scale when needed.

CMR: Preprint servers have really shown their value during the pandemic. Many researchers are now discussing what role they should play in the post-pandemic era versus how much we should continue to rely on for-profit publishers—especially on boutique journals where the peer review process can take months or years, and the cost of publishing a paper is more than many labs can afford.

I think most scientists would agree that peer review still has a role to play in the academic process, but we need to modernize the systems under which new research and young researchers are being appraised.

What worries you most about the future of the pandemic?

TH: I'm not worried about the science. We've seen incredible breakthroughs in a relatively short time, and I think researchers have done everything we could to stay on top of the virus. What worries me more is societal behavior and public health messaging. Simple things that would have helped mitigate the scale of this crisis didn't happen; measures to contain the spread were relaxed too early.

Vaccination is critical, and it's still under way. Variants of concern are circulating in the population, and we must keep the numbers down to limit the chance that these variants acquire further mutations that could reverse our progress. There is a way out of the pandemic, but we are not there yet—and many countries in the world aren't even close. So we need to convince policy makers and the public that, unfortunately, some effort is still required from each and every one of us. ◎

The lemon aid

THE SALIVA COVID test developed at Rockefeller is both highly accurate and very convenient—except when your mouth is dry. In that case, you need a lemon.

Studies have shown that sour foods increase saliva production more than sweet foods do, and that lemon in particular gets our juices flowing. In one experiment, undiluted lemon juice was shown to increase saliva production from 0.61 to 1.44 milliliters per minute. (The Rockefeller test requires just a tenth of a milliliter.)

Among the first members of the Rockefeller community to receive weekly COVID testing were the children who attend the university's faculty and staff childcare facility. While newborns typically don't have trouble generating drool, toddlers and preschoolers can find it difficult to spit.

"We started recommending lemon last summer when the testing program began and some parents were struggling to get their kids' saliva," says Samara Wright, the childcare center's nurse. "It definitely helps create more drool and speeds up the collection process."

With adults, just the thought of a lemon was often enough. "We found that imagining a lemon has nearly the same effect," says Myles Marshall, a technician in the Darnell lab who worked on the testing protocols.

For more on COVID testing with saliva, see "Building a better COVID test," page 34.

PICHEST

PRO · BONO · HUMANI · GENERIS · SCIENTIA
The Rockefeller University
1901

SCIENCE FOR THE BENEFIT OF HUMANITY

1230 York Avenue
New York, NY 10065
www.rockefeller.edu