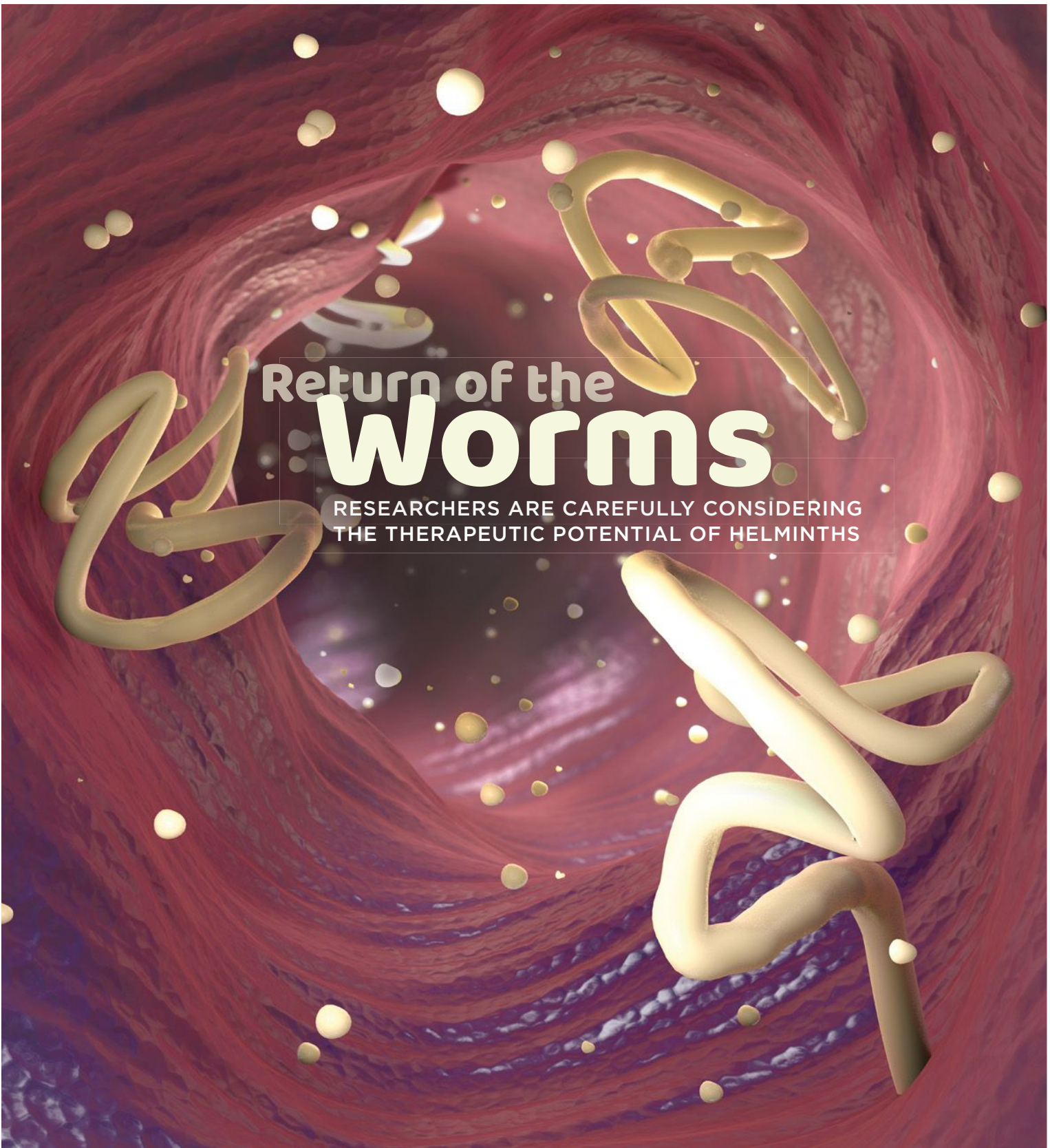
DECEMBER 2021 | VOL. 35, ISSUE 6 | WWW.THE-SCIENTIST.COM

TheScientist



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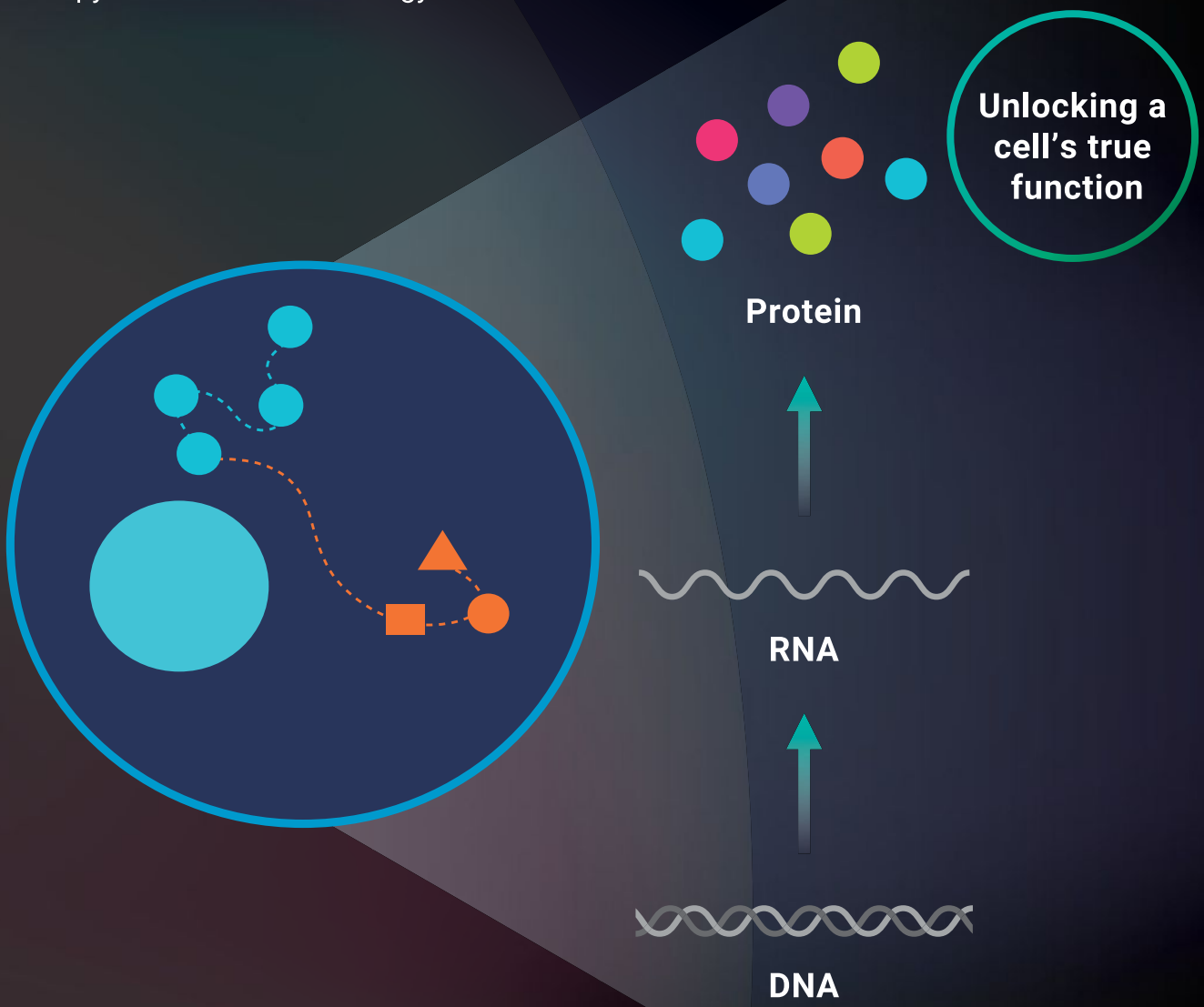

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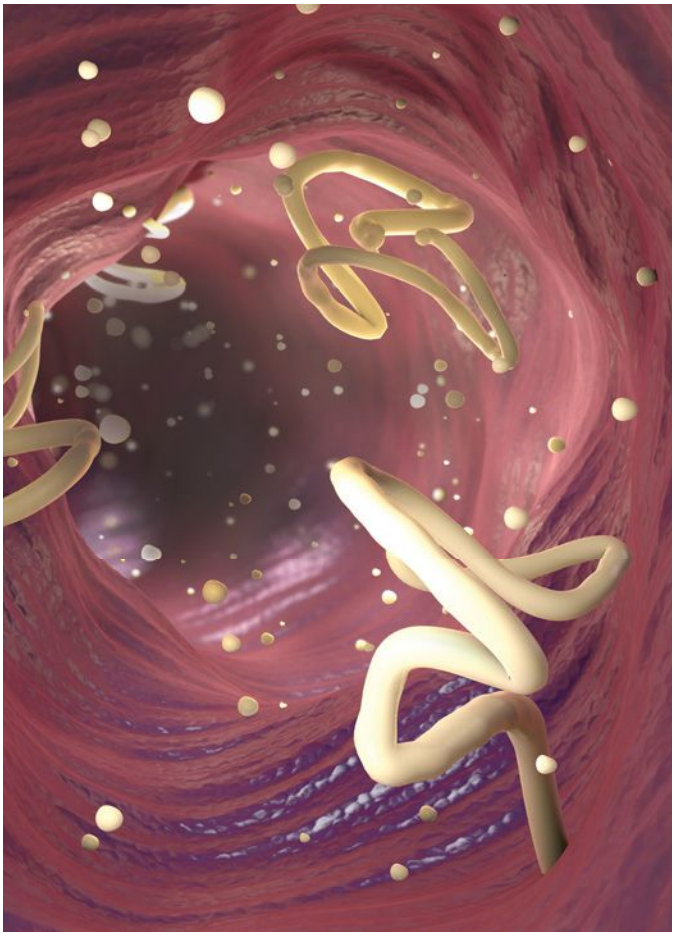

Features

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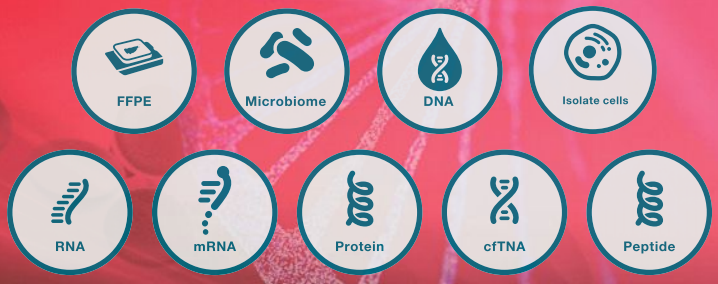

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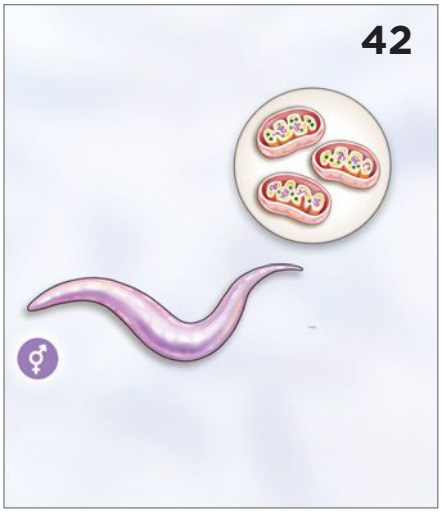

CORRECTIONS:
In the October 2021 issue, the article "Bianca Jones Marlin: Studying Genetic Memories" stated that Marlin had opened her lab at Columbia University in January 2020, instead of in January 2021. The article "Moments in Time" implied that time coding in mice was discovered in 2020, but scientists have been studying the phenomenon since at least 2007. The piece also suggested that the high degree of recall accuracy was caused by the time cells, but the study did not explicitly test such a link, although other studies have. *The Scientist* regrets these errors.

PUZZLE ON PAGE 12

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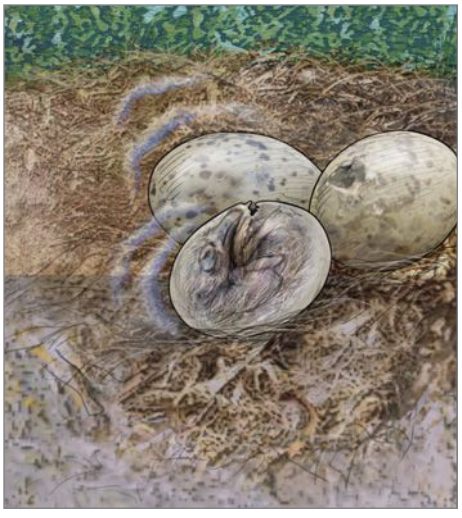

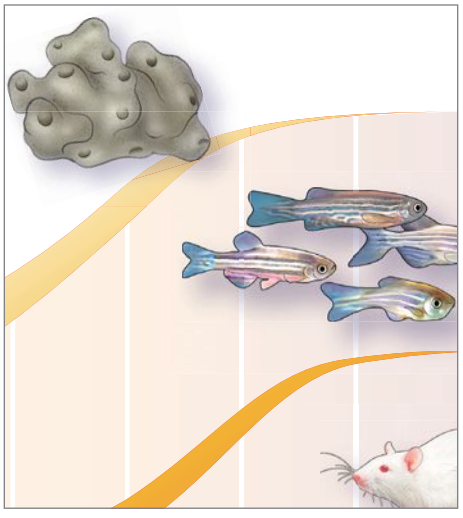

ONLINE AT THE-SCIENTIST.COM:

Embryonic Eavesdropping
Recent findings buck the traditional idea that embryos are passive agents and instead suggest that by tuning into vibrations, organisms can better prepare to enter the outside world.

Enhancers: Conserved in Activity, Not in Sequence
Certain stretches of DNA that regulate gene expression have evolved differently from protein-coding genes.

How Brexit Is Transforming the UK's STEM Community
Scientists face the ramifications of the country's departure from the European Union, from laboratory supply shortages to difficulties hiring international students and faculty.

Coming in 2022

Next year is bringing some exciting changes to *The Scientist*. We will continue producing refreshing life science news and opinions, but we'll be delivering these stories to you in an interactive format called the *TS Digest*. This digital publication will be published twice a month on the-scientist.com via a dynamic platform called FlipSnack. And every three months, starting at the end of March, *The Scientist*'s best content from the preceding three months of the *TS Digest* will be packaged into a beautiful magazine reminiscent of the periodical that print subscribers have been perusing for more than 35 years.

We here at *The Scientist* are looking very much forward to a new year and a new publication strategy, and we're excited for you to come along on the journey!

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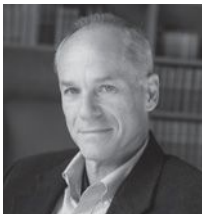
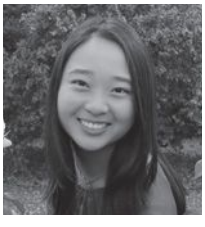


Marcelo Gleiser says he was always fascinated with the universe and its mysteries. He studied physics at the University of Rio de Janeiro for his undergrad and pursued a PhD in theoretical physics at King's College London. As a professor of physics and astronomy at Dartmouth College, Gleiser started a course he describes as "physics for poets," which emphasizes humanistic aspects of science for non-physics majors. A voracious reader of science books and articles, he wrote his first science book, *The Dancing Universe: From Creation Myths to the Big Bang*, in 1997 as a text for this course and has since authored several more award-winning nonfiction books. "I always felt the need to share the beautiful things I've learned in science with the general public because I place science on the same level as art," Gleiser says. "The same way that you want to share a poem, rock song, or a painting with people, you should be sharing the discoveries and inventions that we do in science." He created and currently directs the Institute for Cross-Disciplinary Engagement at Dartmouth College, where he continues to bridge scientific and humanistic perspectives in his work. On page TK, Gleiser, a 2019 Templeton Prize Laureate, penned an essay based on a new book he edited called *Great Minds Don't Think Alike*, a collection of conversations among scientists, philosophers, and others.



Chloe Tenn, *The Scientist*'s current editorial intern, was "obsessed with dolphins" as a child in Miami, Florida, she says, and in high school she considered pursuing studies in marine biology or veterinary medicine. But a psychology course in her senior year hooked her on neurobiology, and she declared that as her major at North Carolina State University in 2017, picking up minors in English (she also loved writing) and forensic sciences (she envisioned a career in criminal psychology). After spending time studying histones in a lab, however, she realized that bench work was not for her, noting, "I did not like sitting there pipetting." That's when a former advisor suggested she look into science communication. In August 2019, Tenn started producing content for a lab at NC State, followed by a communications internship with North Carolina Sea Grant. In October 2020, she enrolled in a yearlong science communication master's program at the University of Manchester and began freelancing for the UK branch of the biopharma marketing company AZoNetwork. Tenn completed an internship at the Smithsonian Office of International Relations, writing about everything from space to environmental studies, before coming to *The Scientist* this fall. "It is so fast paced and so much fun all the time," says Tenn of her current role. "I just like talking to scientists about their research—that's my favorite part." On page 45, Tenn profiles University of California, Santa Barbara, cell biologist Brooke Gardner, writing about her research into organelles called peroxisomes.

Ashleigh Campsall says she has wanted to be a graphic designer since she was seven years old, when she'd play with design programs on the Apple computers at the graphic design company owned by her best friend's parents. She took cyber-art classes every year of high school, focusing her coursework on digital arts and design. She then pursued a yearlong Art and Design Fundamentals program at Georgian College in Barrie, Ontario, followed by a three-year graphic design program where she earned an advanced diploma. After working with dogs at a pet daycare—another passion of Campsall's—and with various media and advertising companies, she joined LabX Media Group as a graphic designer in 2019. She says her favorite part of this role is "having creative freedom and trust from the clients that we work with and . . . just getting to be creative and push the boundaries. I can go out of the box a bit more than I used to before." In this issue of *The Scientist*, she helped create infographics and custom designs for the magazine's editorial content. She also creates content for the creative services division and provides support for the other brands under the LabX Media Group umbrella. "I get to create really engaging things that help scientists make the world better," she says.

ELI BURAKIAN; MARTHENA PHAN; © VROOM PHOTOGRAPHY

New Year, New *TS*.

Announcing an exciting new offering from *The Scientist*, coming in January 2022:

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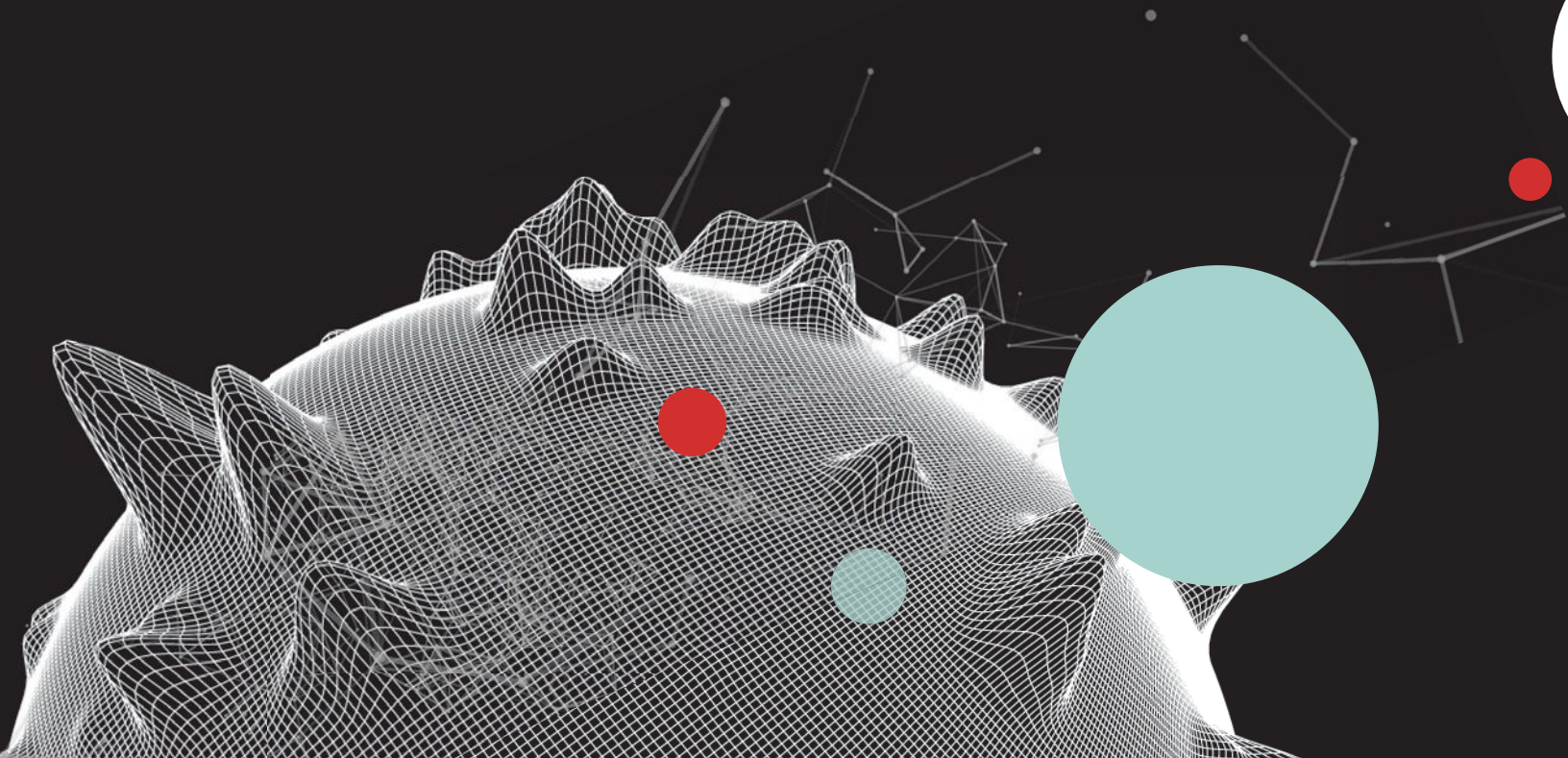


Innovations that Matter

Scientific advances almost always have the potential to benefit human lives. In times like these, they have the power to save them.

BY BOB GRANT

We've come to the close of another year. Unfortunately, if not unpredictably, the COVID-19 pandemic eclipsed 2021 after severely disrupting most of 2020 for most of the world. But while we may be entering the third year of this new and shifting reality, at least we are now equipped with safe vaccines that are effective against the pandemic virus—a scientific feat that was achieved remarkably fast.

Even with the recent upticks in political divisiveness and misinformation spread that have attended this milestone in the course of a challenging pandemic, it's hard to overstate the triumph of creating a COVID-19 vaccine within a year of the pandemic's outbreak. For context, vaccines against polio—which first sparked an epidemic in the US in 1894, later paralyzing and killing millions of people in the first half of the 20th century—took two decades from the start of their development in the 1930s to the mid-1950s, when Jonas Salk's formulation was widely distributed throughout the US and led to a precipitous drop in the number of annual cases. To be fair, science has made great strides in its understanding of basic biology and medicine in the intervening seven decades. But still, the fact that researchers were able to go from detecting and isolating a novel pathogen to effective vaccines in about a year should be considered a marvel.

Almost equally impressive, though, is that with so much research effort and funding bent toward combating a shared foe in SARS-CoV-2, the global biomedical enterprise was still able to pursue lines of inquiry well underway before everything changed in the first part of 2020. Goals such as improving precision gene editing, enhancing the acuity with which single cells can be observed and biologically inventoried, and penetrating ever deeper into neurological structures to characterize their function were not abandoned because of the COVID-19 pandemic, and the results of our annual Top 10 Innovations competition celebrate the fruits of that tenacious labor.

Those well-rounded efforts include products that directly address COVID-19-related challenges, including a SARS-CoV-2 neutralization assay development kit to more accurately measure antibody binding, and a service to characterize single-cell gene expression that could help researchers interrogate exactly how the virus operates. Other spots go to a new platform for brain imaging in freely behaving animals and a couple of organ-on-a-chip systems that could facilitate in vitro insights that better recapitulate in vivo biology.

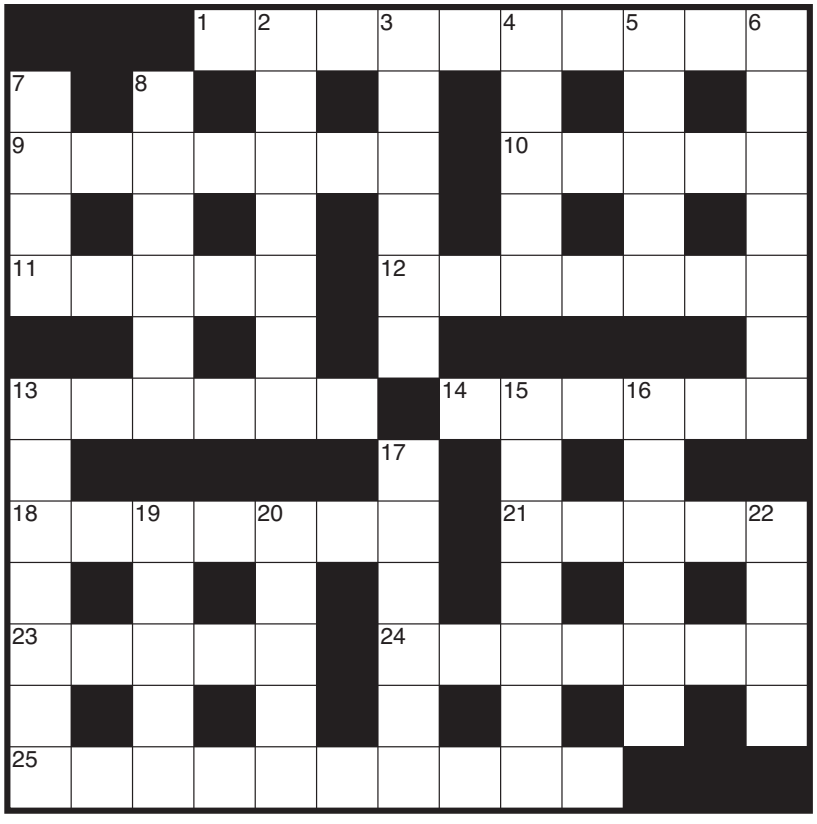
The scientific community's balance of inventions that could save human lives now and those that could improve health and save lives far into the future fills me with hope—not just that biomedicine will keep up the fight against this globe-plaguing virus, but that when the next pandemic comes along, biologists will be ready for it as well. And as life scientists continue to improve the tools and techniques for peering into the intricacies of living organisms, humanity will continue to expand its understanding of life, disease, medicine, and health.

This year has shown us that science has come a very long way in a relatively short time since the years when polio stalked children the world over. As we sit on the precipice of another pandemic year, I am confident that science and its practitioners will continue to rise to the challenge. And for our part, *The Scientist* will continue faithfully and honestly reporting developments as they occur. ■

Editor-in-Chief
eic@the-scientist.com

ANDRZEJ KRAUZE

Speaking of Science



Note: The answer grid will include every letter of the alphabet.

BY EMILY COX AND HENRY RATHVON

ACROSS

1. Country home to Miombo woodlands
9. Item on a chemist's table
10. Family relative of lilac and jasmine
11. Vessel connecting ventricle and abdomen
12. Upper fixed bone of the jaw
13. Apple or potato variety
14. Max of constant fame
18. Nicotiana member
21. Olfactory channels
23. Strongest bone in the human body
24. Most abundant monosaccharide
25. Secretion that nourishes queen bees (2 wds.)

DOWN

2. Perform in a surgical theater
3. Time for a foliage tour
4. Zoological park site since 1899
5. Hollow shaft of a feather
6. Where corneas may be found (2 wds.)
7. Some intravenous injections
8. Features of wheat or barbets
13. "Wheel animalcule"
15. Pertaining to the 17-Down
16. Descriptor of the small intestine
17. Site of gustatory cells
19. Like skin with keratosis pilaris
20. Calcareous deposit
22. Undergo molting

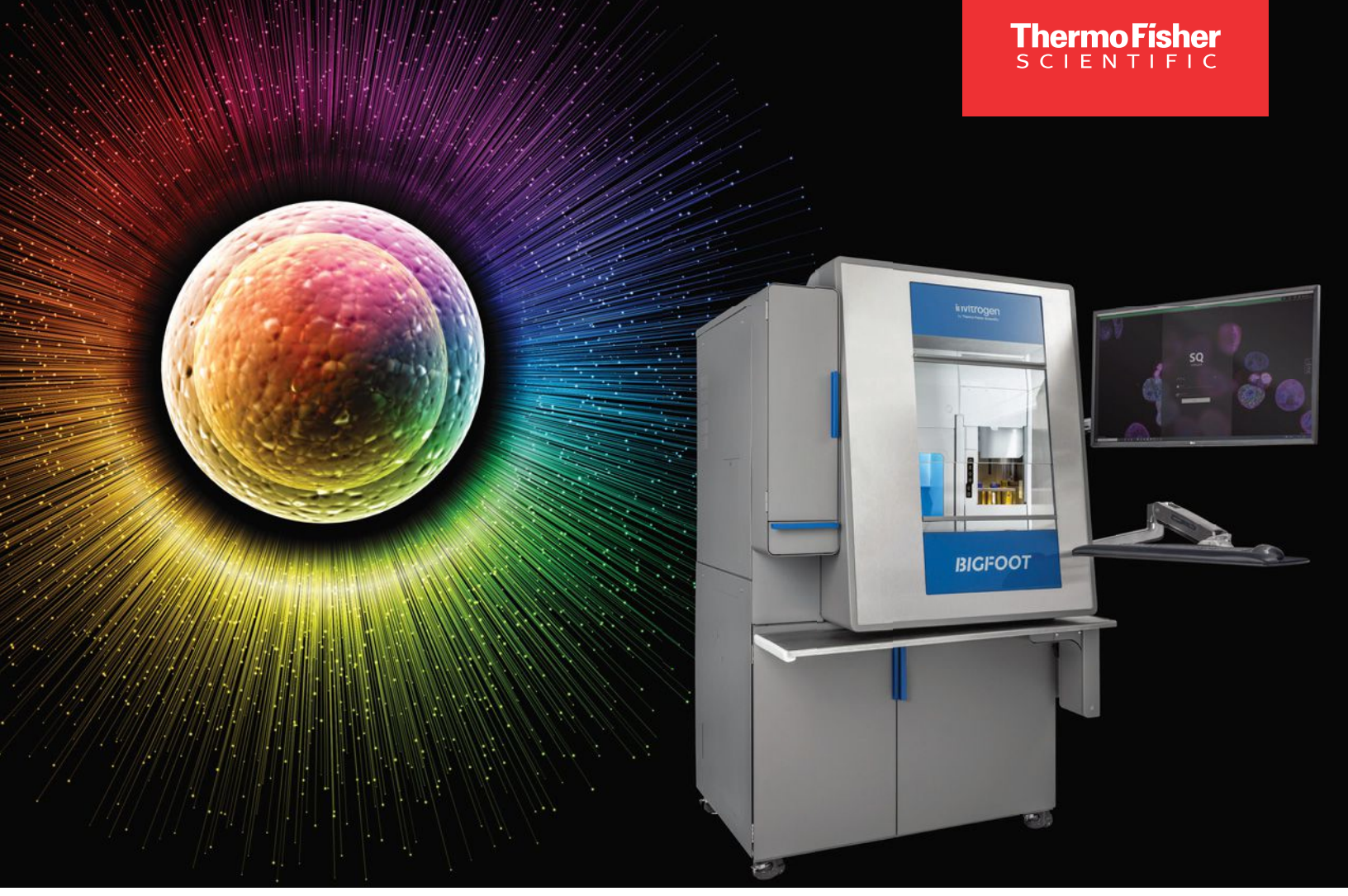
Answer key on page 5

I think there needs to be a balance between fundamental curiosity-driven science and applied science, because we need each other. The fundamental science often goes in unexpected directions and leads to advances that wouldn't have been made otherwise, and CRISPR is certainly in that category.

—University of California, Berkeley, researcher **Jennifer Doudna**, Nobel Prize–winning codeveloper of CRISPR gene editing, speaking with the Association of American Medical Colleges about the most pressing issues facing the academic medical community (November 8)

We found that when researchers report that males and females respond differently to a manipulation such as a drug treatment, 70 percent of the time the researchers have not actually compared those responses statistically at all. In other words, an alarming percentage of claims of sex differences are not backed by sufficient evidence.

—Emory University neuroscientist **Donna Maney**, in a press release reporting the publication of a recent *eLife* paper she coauthored analyzing data from dozens of 2019 studies (November 9)



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IsoPlexis' single-cell proteomics helps characterize T cell properties for tumor-infiltrating lymphocyte (TIL) therapy against non-small cell lung cancer

IsoPlexis technology gives scientists the ability to examine and characterize immune cell populations. In particular, IsoPlexis' single-cell functional proteomics allows researchers to perform secretory and intracellular proteomic screening of heterogeneous cell populations at single-cell resolution. Recently, IsoPlexis' platform was instrumental in helping researchers identify T cell polyfunctionality as a key factor in positive responses to checkpoint inhibitor-adoptive cell transfer (ACT) combination therapy against metastatic non-small cell lung cancer (NSCLC). Their findings, published in *Nature Medicine*,[1] indicated manageable toxicity with tumor regressions—including complete responses—in several patients.

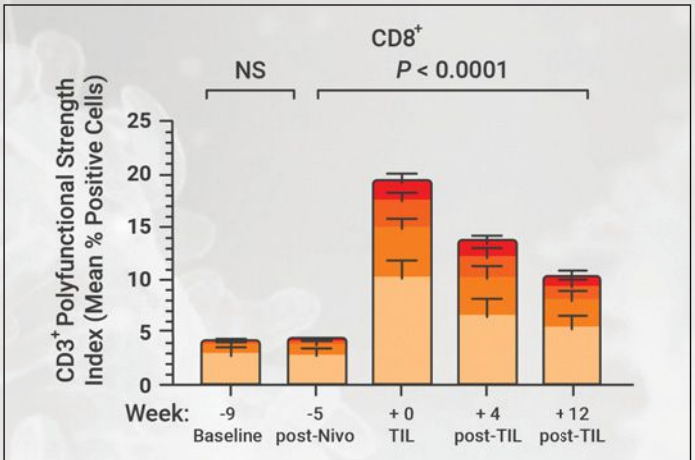
Augmenting checkpoint inhibition with adoptive cell therapy

The discovery of immune-checkpoint inhibitors provided an important breakthrough in cancer therapeutics. These drugs prevent tumor cells from escaping T cell-mediated cytotoxicity; however, NSCLC tumors are often immunologically "cold," meaning that they contain very few activated, tumor-specific T cells. To overcome this challenge, the team administered combination immunotherapy consisting of the checkpoint inhibitor nivolumab, followed by tumor-infiltrating lymphocyte (TIL) infusion, in 16 metastatic NSCLC patients.

Eleven of these 16 patients showed tumor regression at the first CT scan performed one month following TIL administration while two patients showed complete responses that remained ongoing 1.5 years later.

Characterizing T cell functional behavior with single-cell functional proteomics

The team employed IsoPlexis' platform to examine T cell polyfunctionality. Polyfunctionality is defined as the ability of an individual T cell to secrete multiple different cytokines after stimulation. Measured using a metric called the polyfunctional strength index (PSI), T cell polyfunctionality has been positively linked with cell therapy potency and vaccine efficacy. In this study, the researchers used IsoPlexis' technology to run a single-cell proteome panel measuring 12 human cytokines at once. The IsoPlexis platform enabled the team to test up to 1,000 individual cells across 12 patients. They found $CD4^+$ and $CD8^+$ T cell PSI to be dramatically elevated immediately after TIL administration, with a significant proportion of T cells capable of secreting three or more cytokines. Moreover, while $CD4^+$ T cell PSI largely returned to baseline levels by the fourth day post-TIL infusion, $CD8^+$ T cell PSI continued to be elevated beyond day 12 after TIL treatment.



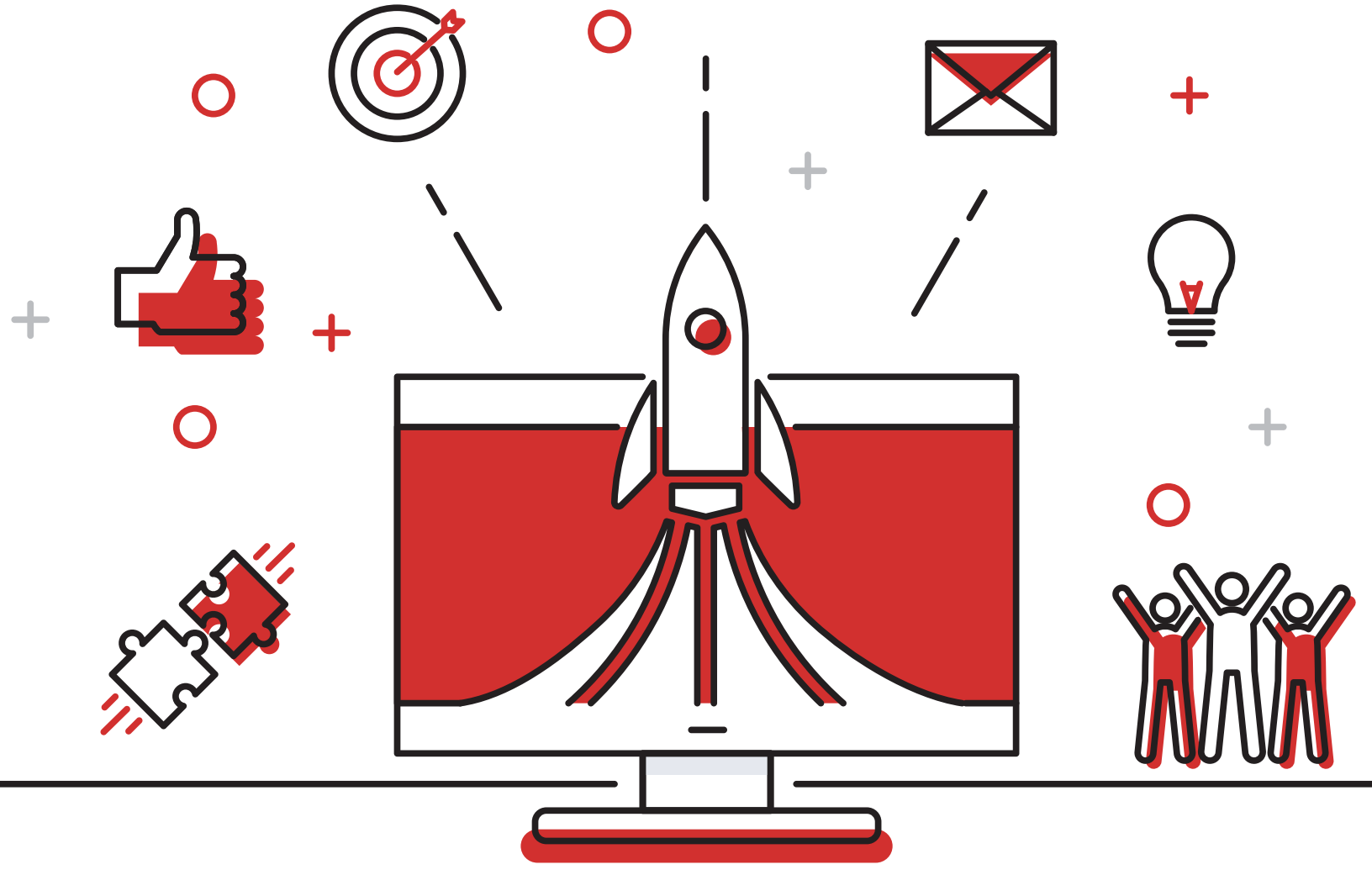
In a NSCLC checkpoint inhibitor/TIL combination therapy study, the polyfunctionality of CD8+ T cells shows the impact of TIL infusion on patient immune response.

Improving therapeutics through improving understanding

IsoPlexis' platform empowers researchers to discover the unique multi-functional cells via highly multiplexed single-cell functional proteomics which correlate to potency, persistence, and patient outcome in various studies. Here, it helped a research team better define how TIL-based ACT complemented or augmented nivolumab checkpoint inhibitor therapy against NSCLC. Following ACT, characterizing T cell activity and behavior that results in a positive outcome will aid scientists in identifying key driver phenotypes that boost cell therapy efficacy.

References

1. B.C. Creelan et al., "Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial," *Nat Med*, 27:1410-18, 2021.



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Data-Driven Hiring

Going beyond personal impressions is essential to hiring researchers who engage in meaningful and innovative work.

BY GEORGES BELFORT

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During these days of a hopefully declining pandemic, hiring new faculty has recently begun in earnest for many research universities. Hence, considering the most effective criteria for selecting new faculty is important, with long-term implications. So, what are the best criteria?

In his 2011 book, *Thinking, Fast and Slow*, Nobel Prize–winning economist and psychologist Daniel Kahneman contends that statistical analysis of data is an equal or even better measure of quality than intuitive judgments based on off-the-cuff interviews. These data can come from applicants' CVs—for example, college ranking, number of peer-reviewed publications, impact factor of journals, and the h-index of both the candidates and their mentors. They can also come from the job interview, according to Kahneman, who suggests that hiring committees ask candidates a few questions about each of six independent traits deemed to be prerequisites for success, then rank the answers on a scale from, say, 1 (poor) to 5 (excellent). To avoid systematic bias being carried over from one set of answers to another (called the halo effect), recruiters should collect the scores for each trait before moving on to the next trait, Kahneman suggests.

There are also qualitative considerations: the quality of the interview seminar and future research plans, interest in the faculty and their research, technical expertise to fit a presumed need, an engaging personality, and letters of recommendation from well-regarded references. Kahneman acknowledges these, and suggests that employers add up all the scores based on the interview and combine these with collegial discussion and intuition (called "delayed holistic judgment" by Kahneman and his coauthors in the 2021 book *Noise*). The candidate with both the highest final score and agreed intuition is the one for the job.

Every seven years, I have spent sabbatical leave at top-ranked US universities: Yale, Caltech, the University of California, Berkeley, and MIT. I have often wondered: What distinguishes faculty at such august institutions from the rest? Do the faculty at top universities have special insight in hiring new faculty that others do not? Or is it that they have the funds to recruit or to raid other universities and companies for proven superstars? The latter clearly helps, but there must be more to the story.

Highly ranked universities offer superior infrastructure, including major computers, large and expensive analytical equipment, and specialized core facilities. They also provide an exciting and

Considering the most effective criteria for selecting new faculty is important, with long-term implications.

supportive environment with low teaching loads, quality faculty and students, and frequent visits from renowned scientists. These features attract more-exceptional academics, who garner more funding, supporting the institution's growth and reputation. When a researcher is hired by a highly ranked department, there is a clear expectation that the new hire will perform well or will not get tenure. Having colleagues who are standouts in their respective fields can be intimidating, but they can provide critical advice, encouragement, and support whereby a rising tide lifts all boats.

A critical and sometimes overlooked issue for prospective faculty is their choice of research focus. Does a faculty candidate swim in the mainstream of their respective discipline or not? Does she select a topic that is challenging or go with a safe bet? Does he work on an easy problem or rather on a problem that is riskier and demands real exploration? I have found that the key difference between faculty at top universities and those at other institutions is not necessarily smarts or intellect, but the courage to work on consequential problems.

To lift a department's ranking, the faculty need to work on research problems that matter to society. And to embark upon an important line of research, one needs confidence in one's abilities and to be ready to pivot when things aren't going as planned. Maybe then, when interviewing a faculty candidate, hiring committees should use Kahneman's approach and assess traits that concern self-esteem, resilience, and the ability to accept failure. A psychologist could help formulate a few factual questions and a framework for scoring the responses.

Analyzing these types of data may help identify the best candidate during the faculty interview process: someone with substantial academic, social, and financial support plus a decent helping of confidence. With the Biden administration and bipartisan US Congress's efforts, substantial research funds are becoming available in the US and will address the financial piece of this puzzle. Additional focus on the selection of important research problems will be needed to improve the quality of the research enterprise, bolster the standing of individual institutions, and allow the US to continue competing internationally in various fields. ■

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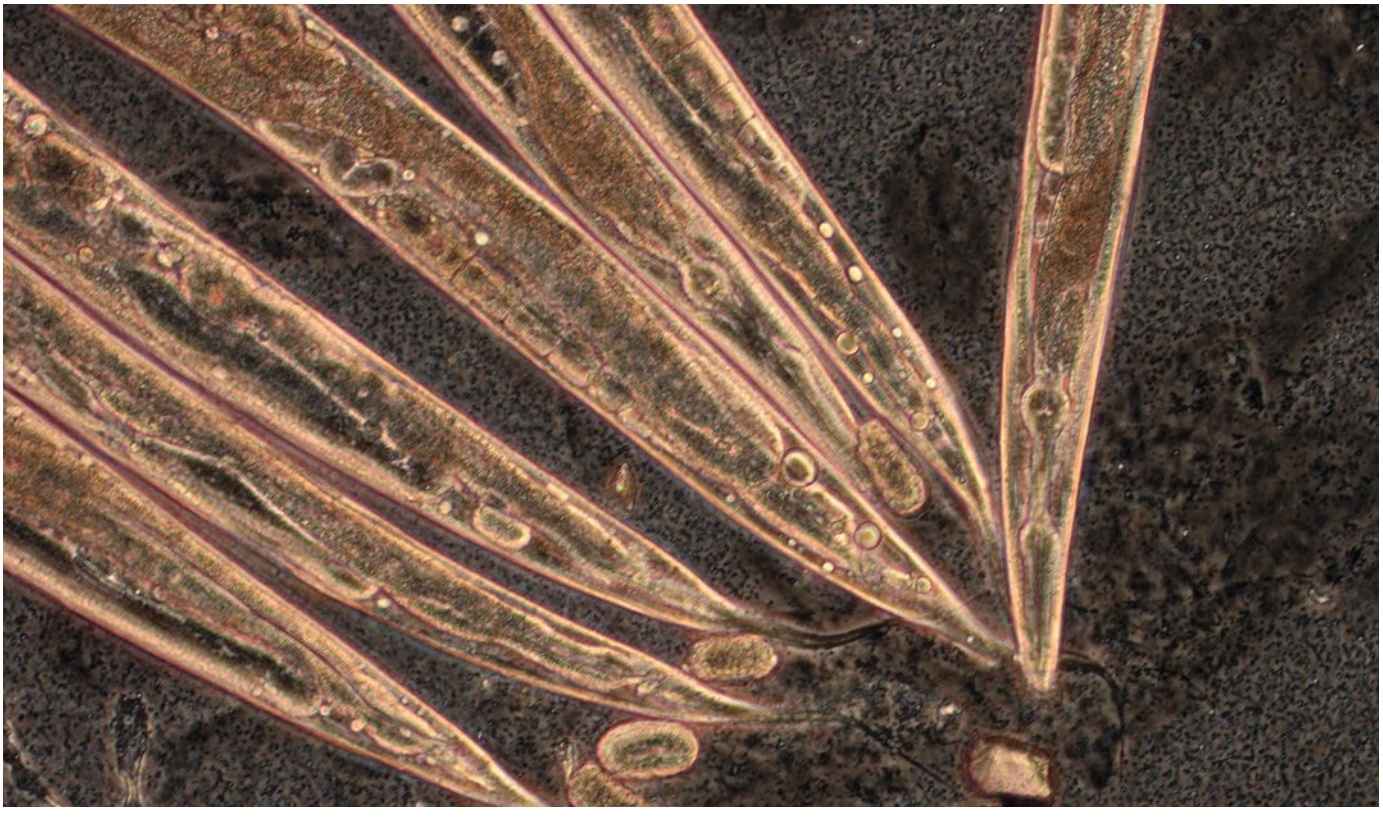
Notebook



MUSCLE CONTROL: Researchers pinpoint how *C. elegans* (pictured) manages to expel food from its mouth.

Worm Spit

Steve Sando was peering through a microscope at a miniscule worm squirming to escape a light when he made a surprising discovery. The type of worm he was observing, *Caenorhabditis elegans*, uses a muscular pump to swallow up tasty microbes from its surroundings. But when a worm was exposed to light, Sando noticed one day in early 2014, that suction reversed course—jetting liquid out of the worm's tiny, transparent throat. As he watched the little creature make this movement, the first thought that came to mind was, "Oh my god, it must be spitting," recalls Sando, then a doctoral student working under MIT molecular geneticist and neurobiologist Robert Horvitz. "I pulled my laptop out of the microscope and ran down the hall to show everyone in the lab."

That was the first of many worm spits that Sando would watch under a microscope over the next eight or so years. By recording several hundred worms and carefully sifting through countless hours of footage, Sando and his colleagues revealed that individual muscle cells in the *C. elegans* mouth are able to carry out two tasks simultaneously by contracting in different patterns at different ends of the cell. "Before this, the model was that the smallest controllable unit of muscles is a single muscle [cell]" Sando says, so this finding "changes how we think about how animals generate behavior."

The work that set the stage for Sando's study had been initiated several years earlier by another former graduate student in the lab, Nikhil Bhatla. Bhatla was interested in a mystery that had pestered the *C. elegans* community for many years: How do these organisms detect and escape light, despite lacking light-sensing molecules? Curiously, he discovered, light not only changed how the worms moved, but also made them stop eating. He also found that the proteins controlling the worms' ability to evade light were encoded by genes related to sequences encoding taste receptors in insects.

Through a series of experiments, Bhatla revealed that these genes also con-

trolled the worm's response to bad smells—specifically, to the odor of hydrogen peroxide, which is generated when light hits biological tissue. "His ultimate conclusion was that the worms kind of 'taste' the light based on these molecules," Sando says.

But solving that mystery raised more questions. Bhatla noticed that although *C. elegans* stopped eating in the presence of light, the muscles of its feeding tube, or pharynx, would briefly resume rapidly moving in a pump-like motion, just as if it was gobbling down a meal. Following this movement, the worms would occasionally blow bubbles out of their tube-like mouths.

Sando says he wanted to get a closer look at this strange behavior. He decided to slightly flatten the worms before viewing how they moved in response to light. The thinking was that "if we squish them a little bit, maybe that'll slow them down and help us see what the muscles are doing," he says. This adjustment did the trick—it worked so well, in fact, that Sando pinpointed muscle motions that appeared to be spitting behavior in the first batch of flattened worms he observed under a microscope. "It was one of those really cool eureka moments," Sando says.

After confirming that this behavior was indeed spitting—using tiny plastic beads to show that liquid was being expelled from the worms' mouths—Sando and his colleagues published that initial finding in 2015. They then spent years analyzing videos of slightly squished worms spitting in slow motion to try to pinpoint the exact muscle movements behind this behavior. Typically, when a worm eats, three muscle cells within the pharynx contract and relax rapidly to propel food into the body. The researchers found that when the worms spat, the front portion of each muscle cell contracted, holding the mouth open, while the back portion continued a pumping motion, expelling food from the worm's mouth.

Further experiments revealed that these movements were controlled by a single neuron within the pharynx. Using fluorescence imaging to measure levels of calcium ions, which help regulate the activity of neurons and muscles, the group observed that calcium levels stayed high at the front of a muscle cell and low at the back during spitting. Sando, now a postdoc at MIT, and the rest of the team published their latest results this July (*eLife*, 10:e59341).

"The finding of a compartmentalized signal in a muscle [cell] was completely unexpected to me," says Manuel Zimmer, a neurobiologist at the University of Vienna who was not involved in the work. This study reveals that, "even from a very elementary behavior like feeding, if you look at it carefully and quantitatively, you can actually learn a lot about basic neuronal mechanisms." Zimmer adds that he'd like to know why worms spit at all in response to the hydrogen peroxide generated by light—and whether such behavior might have any benefit to the organism's survival.

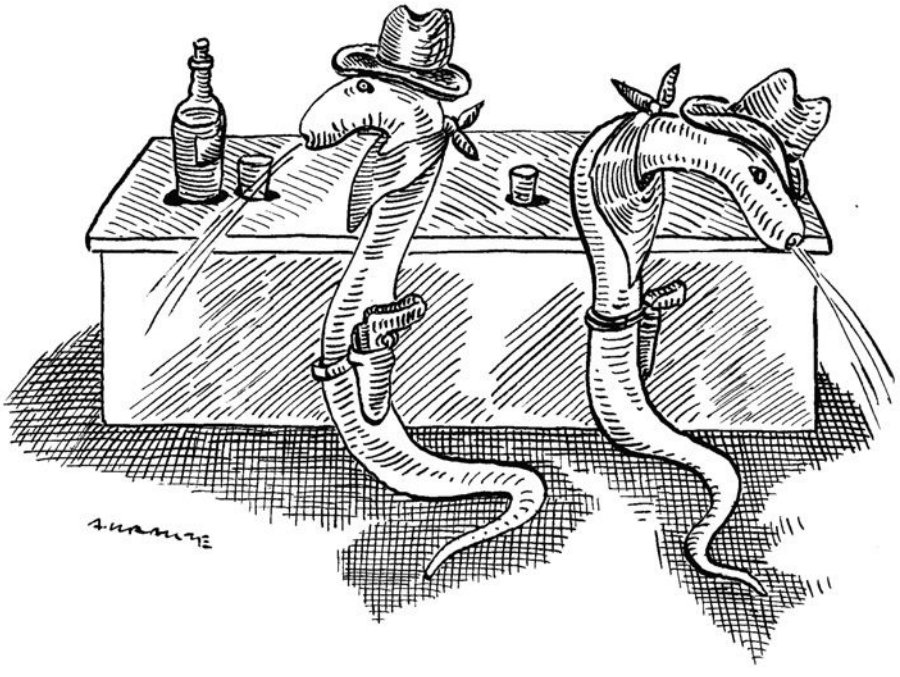
Before this, the model was that the smallest controllable unit of muscles is a single muscle [cell].

—Steve Sando, MIT

Whether or not this phenomenon of different, simultaneous actions by a single muscle cell exists in other, larger animals or is unique to *C. elegans* remains an open question. Aravinthan Samuel, a biophysicist at Harvard University who was not involved in the study, notes that in organisms with fewer cells, those cells tend to be more sophisticated—so it is unlikely that muscle cells in bigger organisms such as humans would split their actions in this way.

Broadly speaking, however, this study is a significant step toward understanding how neuronal circuits work, Samuel adds. To date, there has yet to be a complete neurophysiological model of even simple behaviors, he says, but "these guys have a big piece of that puzzle with this, and it's a step toward real models of real brains."

—Diana Kwon



ANDRZEJ KRAUZE

A Rare Success

Kelly Berthoud remembers the day in 2019 when her daughter Marley sprouted her first eyebrows. Already four years old at the time, Marley had been hairless for most of her life, one of several characteristics about the young girl that scientists and physicians had spent years working to explain. But with the family ensconced in the living room on that morning just before Christmas, each tiny blonde hair, now growing as the result of an experimental drug Marley had recently started taking, was a gift. "We were sitting on the couch and the sunshine was hitting her just right," Berthoud recalls. "I remember whipping my head around when I noticed."

Marley had in fact been born with silver hair, but shortly after, it had fallen out in thick clumps. Her head was also unusually large, and a neonatal MRI revealed that sometime before her birth she had suffered a brain hemorrhage, leading to the formation of cysts in the organ's white matter. As she grew, Marley started missing developmental milestones, including sitting up and learning to speak. The doctors and her parents were at a loss. It would take a chance meeting between three scientists to jump-start her case, but once Marley's team was assembled, it moved quickly. Within just two years, her symptoms would be diagnosed as a novel genetic disease, Bachmann-Bupp syndrome (BABS), and a promising therapy identified, representing one of the fastest turnaround times known for treating a rare disease.

The path to this treatment began in 2016, when Marley's doctors referred the family to one of the people the disease would eventually be named after, Caleb Bupp, a medical geneticist at the Helen DeVos Children's Hospital and the healthcare organization Spectrum Health, both in Michigan. Initially, Berthoud and her husband, both nurses, grappled with whether to pursue genetic testing, a fear Bupp often encounters in his work. Unearthing an unexpected risk factor or mutation "has implications for privacy, for insurability, for all sorts of things that people worry about with genetics," he says.

PATH TO TREATMENT: Marley, pictured here with her father, was diagnosed with the rare genetic condition Bachmann-Bupp syndrome a few years ago.

TAYLOR BALLEK, SPECTRUM HEALTH BEAT

After the couple decided to go ahead, the doctors first checked Marley's chromosomes for large duplications or deletions. Finding none, Bupp next turned to whole-exome sequencing, which scans all of the protein-coding regions in a genome. That analysis revealed two noteworthy findings—the first was a mutation that causes Smith-Lemli-Opitz syndrome, but Marley only had one copy of the disease-linked variant and the syndrome was recessive. The other mutation the team identified was in a gene called *ornithine decarboxylase 1* (*ODC1*), which codes for an enzyme called ODC that is involved in catalyzing the production of certain biomolecules.

Running the *ODC1* results through GeneMatcher, a database where clinicians share data on particular genes, revealed no known human diseases associated with Marley's mutation. "It was the kind of result that goes back in the drawer," Bupp says. But months later, Bupp attended a talk by Helen DeVos Children's Hospital pediatrician Surender Rajasekaran and Michigan State University cancer biologist André Bachmann, during which the pair talked about their work with polyamines, molecular derivatives of amino acids that are involved in multiple cellular processes. "Polyamines are not very well known, even though they're absolutely essential for so many things that the cell has to do," says Tracy Murray Stewart, a polyamine research scientist at Johns Hopkins Medicine—including roles in cell growth, survival, and proliferation. "Nothing happens without polyamines."

As it turns out, polyamine synthesis is mediated by ODC. Making the connection, Bupp struck up a collaboration with Bachmann and Rajasekaran to study a possible role for polyamines in Marley's case.

Because of their importance in normal cell function, disruptions in polyamine synthesis often manifest early in life as cancer, which is how Bachmann and Rajasekaran first came to study them. Marley doesn't have cancer, but her mutation, located on one end of *ODC1*, leads to a buildup of a truncated form of the enzyme that is much harder for the body to clear than the nor-

mal version, which is typically broken down in the cell within just 20 minutes of its production. "In Marley's case, because that end of the protein is missing, the mechanism to clear it is jammed," Bachmann says.

Marley grew not only eyebrows, but eyelashes and a full head of sandy-blonde hair.

Shortly after meeting, the team published an early account (*Am J Med Genet A*, 176:2548–53, 2018) of Marley's disease and started brainstorming ways to treat it. Bachmann had one idea: an ODC-inhibiting drug called difluoromethylornithine (DFMO). Initially approved in 1990 to treat African sleeping sickness, DFMO had since shown promise in clinical trials for pediatric neuroblastoma and colon cancer, while preclinical evidence from a 1996 mouse model that coincidentally mimicked BABS—the mice also accumulated ODC and had a similar phenotype, including silver hair that quickly fell out—demonstrated that DFMO reversed many of their symptoms. To test the drug against Marley's specific mutation, the researchers cultured some of the girl's skin cells and found that DFMO reduced ODC activity.

Even with no guarantee of success, the Berthouds agreed to try DFMO, and Marley began taking the drug on a compassionate use basis when she was four years old. A month later, she sprouted the unexpected eyebrows. Having known Marley for most of her life, Bupp recalls the day he heard about it as "one of the best days of my life."

Marley grew not only eyebrows, but eyelashes and a full head of sandy-blonde hair; the team documented these and other changes in a recent *eLife* publication (10:e67097, 2021). Within six months, she was able to sit up by herself, and today she's able to scoot around and trade high fives with her brothers. Last winter, she tried sledding. Post-treatment MRIs showed that myelination in the white matter of her brain had increased, signs that the drug, which she takes twice daily, might be spurring neurological improvements. The team also sent blood samples to a company called Metabolon that specializes in detecting biomarkers of rare diseases. Their tests, which involved comparing her samples to a reference cohort of almost 900 pediatric patients, confirmed that the drug normalized Marley's levels of a well-studied polyamine called

TURN AROUND: Marley, whose doctors identified a genetic mutation underlying her condition and repurposed a drug to treat it within just two years, with her family in 2018 (above) and with her physician Caleb Bupp in 2021 (below)

N-acetylputrescine, suggesting that her polyamine pathway had stabilized.

This quick turnaround—from diagnosis to administering a potential treatment in roughly 16 months—is "extraordinarily unusual," says Anne Pariser, the director of the Office of Rare Diseases Research at the National Institutes of Health. "There's really only a handful of cases like that." She attributes the speed to the ready availability of an off-the-shelf drug that came with detailed dosage and safety assessments in children, as well as a preexisting animal model that had responded positively to the therapy. "In this case, they had a body of evidence that they could rely upon."

Since Marley's diagnosis, the number of documented BABS cases has risen to at least nine, including four described in a 2018 study (*Am J Med Genet A*, 176:2554–60). It's still extremely infrequent, but according to Pariser, diagnoses of rare diseases tend to accumulate as evidence grows, and Bachmann also expects more to surface. "I'm sure in five or ten years, we'll know of maybe forty or fifty," he says. (He, Bupp, and Rajasekaran are named as coinventors on a patent application related to treating disorders caused by *ODC1* mutations.) Researchers have already learned a lot from the nine cases so far, including the fact that each patient has a unique mutation, all of which affect polyamine synthesis in different ways. Shortly after Marley began treatment, a seven-year-old boy diagnosed with BABS started taking DFMO; Bupp says that his mother has told him that her son is "getting better every day."

The success of the DFMO has spurred additional research into BABS and other rare diseases. Stewart of Johns Hopkins is using mice and patient cell lines to investigate DFMO as a treatment for Snyder-Robinson syndrome, another polyamine disorder. And Kwame Anyane-Yeboa, a geneticist at Columbia University Medical Center, is testing turmeric in an 18-year-old BABS patient. One of the spice's ingredients, curcumin, has been reported to reduce ODC activity in certain cell lines, although there are no data about its effectiveness in people. "If we could treat this [condition] through diet, that would probably be easier for parents and other people to use," Anyane-Yeboa tells *The Scientist*. He adds that while his patient has grown some hair since starting a dietary regimen of one teaspoon of turmeric per meal almost a year ago, "the question in our minds is whether the neurological part is reversible or not."

For Marley's part, she's busy playing catch-up. On a video call with *The Scientist* in August, Marley, now six years old, sat on her mother's lap, smiling and crying in equal measure. This, her mother says, is something the family is still adjusting to. Marley is now more than a year into her treatment—which will continue indefinitely. And while her quality of life has undoubtedly improved, the changes have also brought new behavioral problems. Even so, her daughter's journey "is my favorite story in life," Berthoud says. "There are so many patients out there with undiagnosed diseases that this doesn't happen to, and when I look back at how quickly it happened, it's absolutely amazing that everything lined up."

—Amanda Heidt

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An Early Start: The Impact of the Microbiome on Pediatric Development in the First Year of Life

Dirk Gevers, Ph.D., Global Head, Microbiome Solutions, World Without Disease Accelerator
Richard Insel, M.D, Global Head, Healthy Baby Initiative, World Without Disease Accelerator

The incidence and prevalence of pediatric immunity-related conditions—from food allergy to type 1 diabetes—has increased over recent decades.[1-3] Researchers, clinicians, and policymakers are trying to identify potential underlying causes for this phenomenon to prevent and intercept these diseases. In particular, the World Without Disease Accelerator (WWDA), an R&D group within Janssen Research and Development, LLC, one of the Janssen Pharmaceutical Companies of Johnson & Johnson, is pursuing science related to the infant gut microbiota, which is thought to play a key role in the development of pediatric allergic and autoimmune diseases.

Starting with a Single Step

The infant microbiome is shaped by external factors that begin in utero.[4] These factors include mode of delivery, maternal and pediatric diet composition (e.g., breast milk vs. cow's milk formula) and diversity, and antibiotic exposure.[5] Societal pressures may also impact microbiome health, as children born in industrialized nations present less species diversity in their gut flora.[6] The infant microbiome matures until roughly two to three years of age, after which the gut microbiota takes on adult properties.[4] Microbiome disruption or dysfunctional colonization during infancy is linked to immune dysregulation and increased risk of disease. Children born via cesarean section or who are not breast fed, for example, show elevated risks for asthma and allergic disease.[4]

The WWDA brings together research and external collaborations to discover and develop novel innovations aimed at promoting health and decreasing disease. The WWDA, through its Healthy Baby Initiative (HBI), is targeting three major questions surrounding the potential of pediatric microbiome modulation for preventive purposes.

How, What, and When?

The WWDA first aims to determine how certain microbial compositions or microbes of interest interact with the immune system to provide protection against allergic and autoimmune disorders. What specific microbial molecular patterns or metabolites are critical and what particular pathways are being activated? Most importantly, can these mechanisms and phenomena be targeted for decreasing risk

of childhood disease, such as atopic dermatitis and food allergy? For example, the introduction of specific beneficial microbes such as *Bifidobacterium infantis (B. infantis)* and/or glycans[7] directs major microbiome shifts at birth and weaning.[8] Researchers are investigating whether restoring the disappearing microbe *B. infantis*[7] in infants or replacing synthetic glycans can improve overall health and prevent disease by modulating the gut microbiota.

The next question is, what are the defining hallmarks of healthy immunoregulatory development in early life?[9] The gut microbiome trains the immune system to avoid deleterious responses to stimuli, but it is unclear what a healthy, trained immune system looks like. For example, researchers found that the microbiome promotes protective immune cell phenotypes that drive tolerance of food allergens. However, the precise processes and mechanisms driving protection, along with how and when they function, remains unclear.[10,11] Characterizing healthy immunoregulatory development will help identify what could be monitored to determine whether immune training-centered approaches are taking effect.

Finally, researchers are looking for timepoints in infant microbiome development that might serve as key interventional windows. The infant microbiome undergoes dramatic transformations at major developmental milestones, such as weaning and introduction of complementary food or with exposure to antibiotics.[12] A dysbiotic gut microbiome at these pivotal transitions may disproportionally contribute to future susceptibility to the development of allergy or autoimmunity. Researchers have observed this in mice, where inhibiting the typical murine intestinal microbiota-induced immune response upon weaning led to increased susceptibility to colitis, allergic inflammation, and cancer later in life.[13] Bestowing a healthy infant gut microbiome at these key windows may have the potential to abrogate the development of childhood disease.

Prevention, Interception, and Cure

The WWDA aims to help change healthcare from "diagnose and treat" to "prevention, interception, and cure." Through initiatives such as the HBI, the WWDA strives to better understand how early childhood offers a unique window for detecting disease risk and intervening appropriately. HBI aims to deliver ways to change the trajectory of pediatric health to ultimately give every child the healthiest start in life and hopefully prevent childhood and even adult disease.

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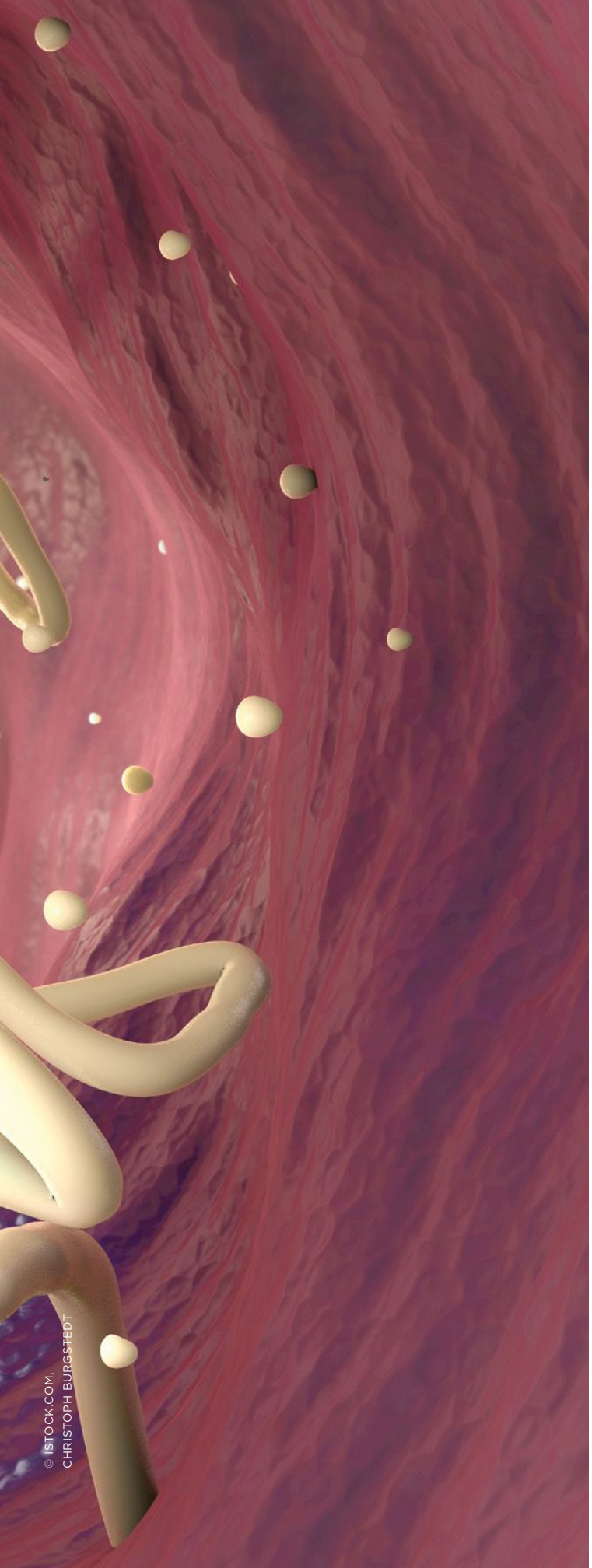
Return of the Worms

Immunologists and parasitologists are working to revive the idea that helminths, and more specifically the molecules they secrete, could help treat allergies and autoimmune disease.

BY CATHERINE OFFORD



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In the middle of 2020, Alex Loukas deliberately infected himself with intestinal worms. The procedure was pretty straightforward: he used a Band-Aid to press a few larvae of the New World hookworm (*Necator americanus*) gently onto his forearm, and waited for the microscopic critters to burrow on in. Although it wasn't painful, exactly, he describes a tingly feeling like "little tiny electric shocks as these guys go through your skin," he says. "It's intensely itchy for a number of days and then that resolves." Some people who undergo this process experience stomach discomfort when the worms arrive in the gut, where they will grow up to 1 cm long, but many "will then never have any other clue that they're infected."

There were several reasons that Loukas wanted the parasitic worms, or helminths, on board. For one thing, his research at James Cook University in Australia focuses on multiple aspects of *N. americanus* biology, and as obligate human parasites, these intestinal worms just don't grow very well outside of people. Rearing a few in his own gut and then collecting eggs via a bathroom visit would be a lot simpler than trying to maintain a population in the lab, Loukas explains. (Judging by how many eggs he's currently shedding—he estimates it's around 20,000 per day—his worms seem to be doing just fine.)

Loukas has also, in the course of his research, developed the view that infection with *N. americanus* and other intestinal helminths, which together are thought to inhabit at least 2 billion people worldwide, isn't always harmful. In fact, he argues, work by his group and others indicates that there could be some unique benefits to controlled, low-level infection with certain worm species, particularly for combating so-called Western diseases, including allergies, autoimmune disorders, and various other inflammation-related conditions. As an advocate for exploring helminth infection as a potential therapy against such conditions, Loukas realized he had to give it a go. "I'm sitting there telling the world how great this is," he recalls thinking. "I should probably experience it for myself."

Often referred to as immunoregulators, helminths secrete and excrete vast quantities of proteins and other molecules that influ-

ence the activity of the host immune system. It's a strategy born of necessity for a large, multicellular parasite that typically persists months or years in a single gut and, unlike a bacterium or virus, can't out-multiply its host's defenses, says Rick Maizels, an immunologist at the University of Glasgow and Loukas's former postdoc adviser. Helminths have been coevolving with humans for as long as humans have been around. Until a century or so ago, when improved hygiene and healthcare began to wipe out worm infections in industrialized countries around the world, "the whole human population would have had these parasites for most of their life," Maizels says. "They've had all the time in the world to adapt and to learn how best to live in the environment."

This intimate biological relationship forms the basis for the argument made by Maizels and others that helminths play a crucial role in keeping harmful immune responses in check—and that their loss in certain modern societies might account for some of the observed increases in autoimmune and inflammatory conditions. It's a controversial theory that some scientists have taken issue with. Parasitic diseases expert Peter Hotez of Baylor College of Medicine and colleagues have questioned whether the observed associations are causal, noting that research has found that many helminths can exacerbate and may even promote inflammatory conditions; a few years ago, Hotez referred to worm therapy as belonging in the category of "pseudoscience cult therapies." But although Loukas, Maizels, and others in the field agree that some helminth infections can be dangerous and require treatment, they posit that the manipulation of the immune system by more-benign species may in some cases be able to rein in immune responses that are potentially harmful to the host.

Previous attempts to convert this line of thinking into therapies for immune-related conditions have had mixed success. Despite a promising start in the early 2000s, subsequent clinical trials of helminth infection as a treatment for conditions including Crohn's disease, celiac disease, and asthma generally produced unimpressive results.[1] Unfazed, proponents of the approach are now coming at the problem from a different angle, one that places stronger emphasis on understanding the mechanisms underlying host/helminth interactions, and views both the worm and the individual compounds it secretes as potential therapeutics. "There's been disappointment over the results of the trials," says William Harnett, an immunologist of the University of Strathclyde in Scotland who is named as an inventor on patents covering the therapeutic use of some worm-derived molecules. But "I think people still believe that there are good immunological reasons for continuing to pursue this."

Keeping the immune system in check

P'ng Loke was a postdoc at the University of California, San Francisco, working on mouse models of helminth infection when he met the man who'd become his first human subject. It was 2006, and the 35-year-old man, diagnosed with ulcerative colitis a couple of years earlier, had taken an unusual approach to tackling his debilitating symptoms: having heard stories of helminths' possible anti-inflammatory effects, he'd traveled to Thailand and gotten his hands on more than a thousand whipworm (*Trichuris trichiura*) eggs, which he'd swallowed. This behavior isn't unheard of among people with severe inflammatory diseases, Loukas says, and there's a troubling black market for helminth eggs in many countries and online.

Remarkably, the man's ulcerative colitis seemed to be in remission. So Loke began studying the man's physiology, using colonoscopy images and intestinal biopsies, some of which had been collected prior to the egg-swallowing and others afterward. "We followed him for a few years and really characterized what was happening in his gut," says Loke, now with the National Institute of Allergy and Infectious Diseases in Maryland.

The researchers found that the man's colon, which had been inflamed prior to his worm infection in 2004, showed less damage in 2005, and there had been a reduction in the number of inflammatory cells known as neutrophils. This switch occurred more than once: after experiencing worsening symptoms in parallel with a decline in helminth eggs in his stool in 2008, the man reinfected himself, this time with 2,000 eggs, and his colon showed the same calming of symptoms following that infection too, Loke says. From additional analyses, the team also found that while his gut had been full of T helper cells producing the inflammatory cytokine IL-17 just prior to his swallowing more worm eggs in 2008, it now contained T helper cells producing IL-22, a cytokine involved in repairing the gut wall.[2] "It looked like worms were restoring the mucosal barrier."

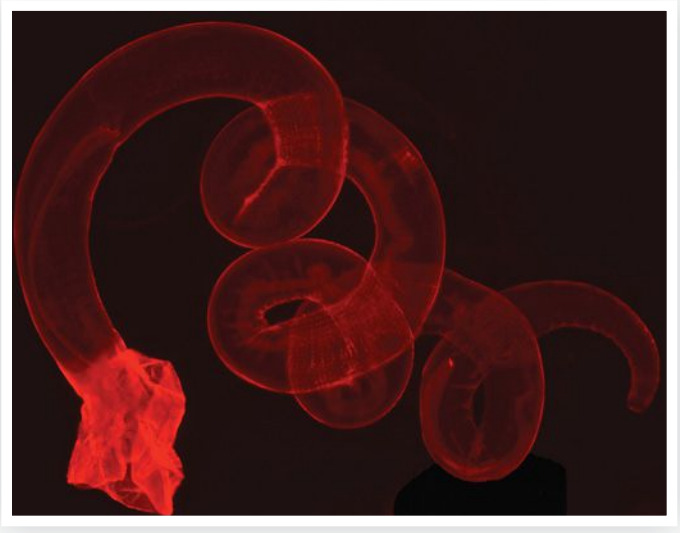
People still believe that there are good immunological reasons for continuing to pursue this.

—William Harnett, University of Strathclyde

The value of such one-off studies is limited from a therapeutics point of view. "They are just case reports," says Loke. "You don't really know how broadly applicable it is." But they do help researchers piece together the mechanisms by which helminths modify human biology, as do complementary studies on animals infected with worms.

Maizels has also been digging into these mechanisms over the last few decades, and has documented myriad ways in which helminths manipulate and evade host immunity. (See illustration on page 30.) In his view, intestinal worms essentially "decide that they're a transplant," he says. "They walk in and assimilate themselves as if they were a normal part of the body."

Several of the mechanisms Maizels has studied operate via regulatory T cells, or Tregs for short—specialized immune cells that typically dampen immune responses. Human studies, for example, have found higher levels of Tregs in the blood of people infected with *N. americanus* or the large roundworm *Ascaris lumbricoides* compared with worm-free controls. Maizels and others have also reported that helminth infection is associated with increased production of immunoglobulin G4 (IgG4), an



GUT RESIDENT: An adult male *Heligmosomoides polygyrus*, an intestinal parasite of rodents

antibody released by B cells that is associated with anti-inflammatory pathways. Levels of IgG4 typically fall in people whose helminth infections are eliminated with deworming drugs.

These kinds of studies help provide support for new clinical trials. Last year, scientists in the UK reported findings from a randomized controlled trial of *N. americanus* infection as a therapy for relapsing multiple sclerosis. As predicted, worm infection boosted Treg levels in people's blood, the researchers reported. There were also fewer relapses among hookworm-infected people than in the placebo group, though this finding wasn't statistically significant.[3]

Loukas's group, meanwhile, has been studying host-helminth interactions in type 2 diabetes, another condition associated with elevated inflammation. Earlier this year, he and his colleagues published data from a study of mice fed fatty or sugary diets: animals infected with the nematode *Nippostrongylus brasiliensis* had higher levels of anti-inflammatory cytokines such as IL-4 than uninfected controls and were protected from diabetes-like pathology.[4] Loukas and colleagues are now running a randomized controlled trial to assess safety and tolerability of hookworm infection in people who are obese and show insulin resistance or other symptoms of metabolic syndrome. (The team is using hot sauce to simulate the tingly feeling of burrowing larvae on the arms of people in the placebo group.)

Despite this progress, results from the latest handful of clinical trials haven't been hugely encouraging. A small randomized controlled trial of celiac patients published earlier this year by Loukas and colleagues, for example, failed to find a positive effect of hookworm infection on gluten tolerance when people consumed moderate amounts of the protein, although when given questionnaires about their experience, some helminth-positive people reported higher well-being and quality of life.[5] (Loukas tells *The Scientist* that researchers had trouble establishing stable infections in some participants, perhaps because worms fared badly on the trip from Australia to the New Zealand trial site.) An earlier, smaller trial led by the same group had suggested a beneficial effect of worm infection on gluten tolerance, but it wasn't placebo-controlled.

"I think that's the part that's really difficult," Loke says of the placebo effect in helminth therapy studies. "Before we started to do trials, I never really appreciated how strong the placebo effect can be." Moreover, he adds, the complexity of worm-host interactions makes it hard to know whether a negative trial result means a helminth therapy is completely ineffective, or just that the treatment only helps specific subpopulations of patients. One way to resolve this puzzle could be to learn more about variation in immune system responses to helminths, something that Loke is working on now. Another may be to take the worm, a multicellular animal with its own lifecycle and behavior, out of the equation.

Potential therapies in worm secretions

Around a decade ago, Loukas set out to determine what exactly the dog hookworm *Ancylostoma caninum* pumps into the gut of its host. Using some of the best available protein identification techniques to analyze secretions and excretions from *A. caninum* worms in culture, Loukas and colleagues identified more than 100 different proteins. When they revisited the same question a couple of years ago using more-sensitive technologies, they found 315 different proteins;[6] Loukas suspects newer methods would identify even more.

Deciphering how these proteins interact with the mammalian immune system is a mammoth task, and some research groups have decided to focus on characterizing the form and function of specific peptides that seem likely to have therapeutic properties. Harnett has worked particularly on ES-62, a glycoprotein secreted by the rat parasite *Acanthocheilonema viteae*, which typically inhabits tissue deep under the skin rather than the gut.

The immunomodulatory part of this protein—"the business end," as Harnett calls it—consists of several phosphorylcholine groups that the team has shown influence mammalian immune cells in vitro and in mice. "At the molecular biochemical level, it's interfering with the immune system cells' ability to produce inflammatory responses," he explains. This happens in "quite a range of cells," including macrophages, dendritic cells, and mast cells as well as B cells and T cells, and at least in some cases depends on toll-like receptor 4 (TLR4), a protein on these cells' surfaces.

The team has been testing the molecule in animal models of disease; last year, for example, the group reported that ES-62 extended "health- and lifespan" in some mice fed a high-calorie diet throughout their lives.[7] "We got some really interesting data from that," Harnett says, adding that although both male and female mice showed better health with worm treatment, only male mice lived longer, for reasons the team doesn't fully understand. While the results haven't all been good—a couple of years ago the researchers reported ES-62's failure to protect mice against type 1 diabetes, multiple sclerosis, and inflammatory bowel disease (IBD)—Harnett says the team is now

JANICE MURRAY, MAIZELS LABORATORY

involved in developing small-molecule drugs that could mimic ES-62 and serve as potential therapeutics. The University of Strathclyde recently secured a licensing agreement with the US-based company Vimelea Therapeutics ("vimelea" means "parasite" in Swahili), which will aim to develop drug candidates for cutaneous lupus, he says. "That's just taken off in the last few months."

Other groups have zeroed in on compounds secreted by *Heligmosomoides polygyrus bakeri*, an intestinal parasite of rodents. One international team recently found that mashed-up *H. polygyrus* larvae dampened the activity of various immune cell types in vitro. Using a series of assays including heat inactivation and chromatography to identify active ingredients in the mixture, researchers picked out the enzyme glutamate dehydrogenase as one protein that could be responsible for some of the worm juice's effects. Intranasal treatment with this molecule suppressed allergic airway inflammation in mice, the researchers report in their paper, and could perhaps be used as the basis for an anti-inflammatory therapeutic for asthma or related conditions in the future.[8]

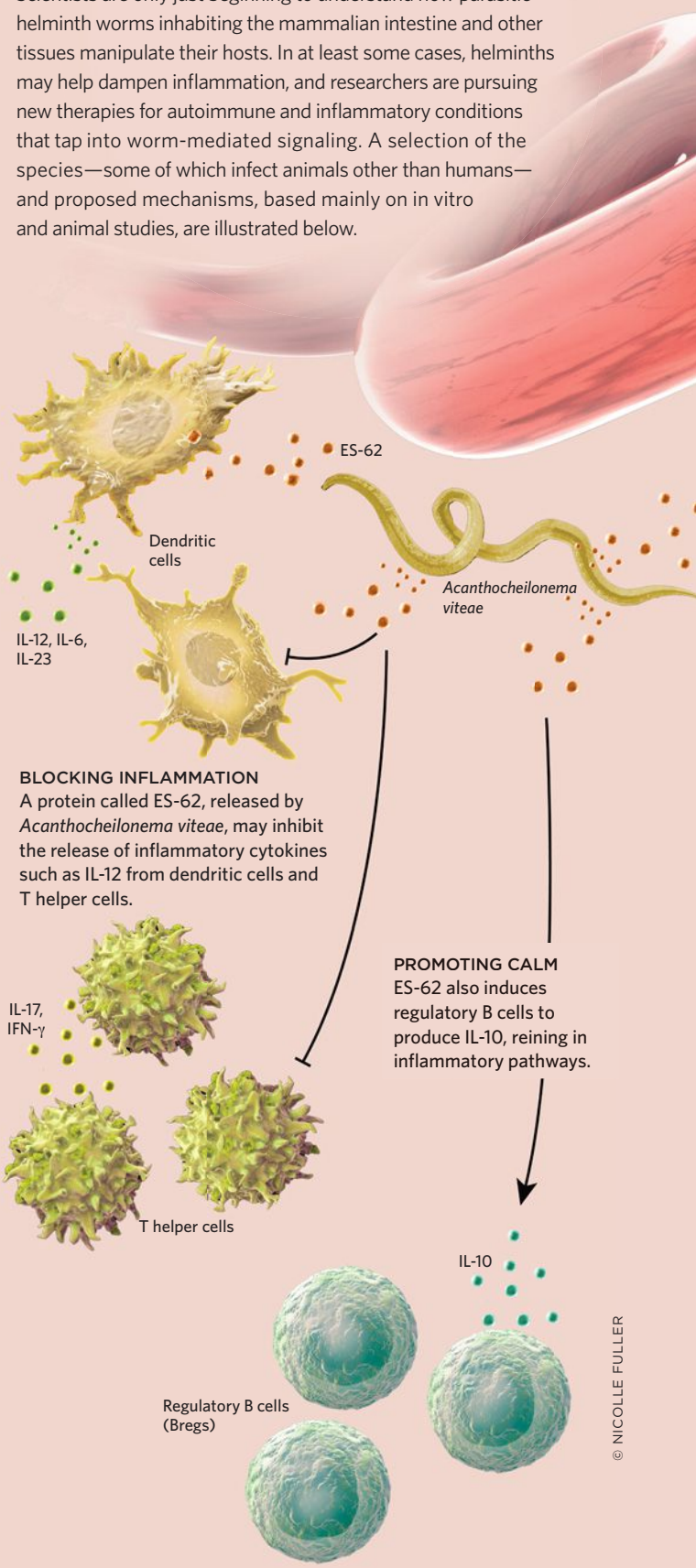
Additional *H. polygyrus* peptides include Hp-ARI, which blocks certain inflammatory pathways by neutralizing the cytokine IL-33, and Hp-TGM, which mimics the mammalian protein TGF-β and which Maizels and colleagues found to activate a pathway that upregulates Tregs. "That's turned into a really fascinating story," Maizels says, noting that the protein contains several mystery structures in addition to the TGF-β–mimicking part that the team thinks might be involved in determining where Hp-TGM goes. "So it has both an address and a message, if you like." The therapeutic potential of Hp-TGM is still unclear, however. Maizels and colleagues reported last year that it failed to prevent development of severe inflammation in a mouse model of multiple sclerosis when administered by injection into the animals' bellies.[9]

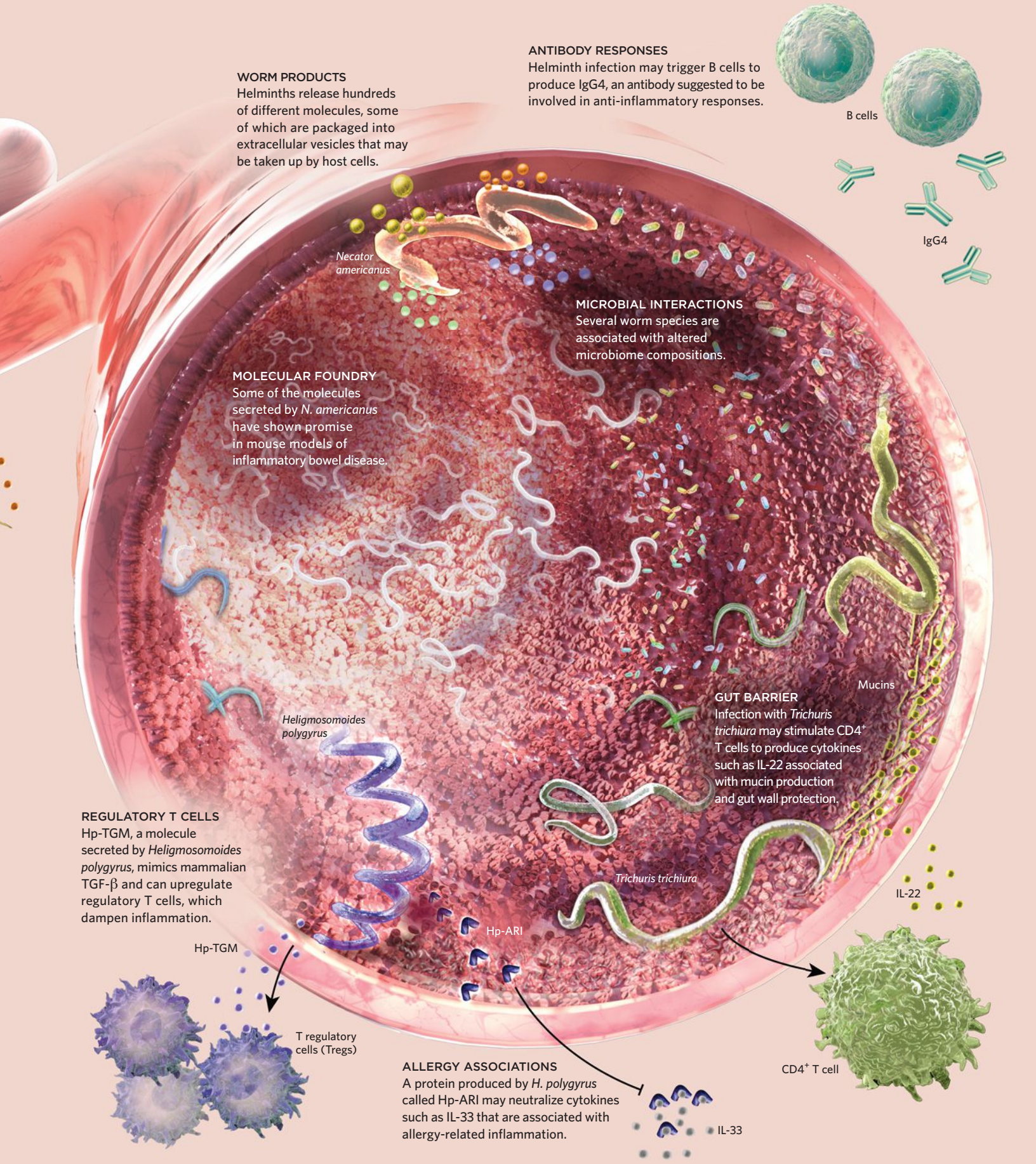
For groups less focused on specific molecules or mechanisms, there's also the brute force approach to identifying promising worm-derived drugs. Loukas is doing this for the secretome of *A. caninum*: his team recently made recombinant versions of all 100 or so proteins they identified a decade ago and have been systematically testing them in a mouse model of IBD. "There's a whole bunch of proteins that got a [check mark] in that screen, and some of them were things we might never have thought about beforehand," says Loukas, who recently cofounded the startup Macrobiome Therapeutics to further his helminth-therapy work. (A previous startup Loukas cofounded, Paragen Bio, closed down last year.) "Now we're trying to put together a preclinical program to assess those proteins in much greater depth," he says, "and see which ones really are potentially drug-like and which ones will not be suitable." He's continuing to document worm secretions, too—a recent study identified nearly 200 proteins from *N. americanus*.[10]

Harnett says researchers may well discover more interesting worm-derived compounds in the future. "Any parasitic worm that you look at secretes a number of anti-inflammatory molecules," he says. "Many species have yet to be examined, so it's possible that there's a lot of treasures we didn't come across yet."

GUT GUESTS

Scientists are only just beginning to understand how parasitic helminth worms inhabiting the mammalian intestine and other tissues manipulate their hosts. In at least some cases, helminths may help dampen inflammation, and researchers are pursuing new therapies for autoimmune and inflammatory conditions that tap into worm-mediated signaling. A selection of the species—some of which infect animals other than humans—and proposed mechanisms, based mainly on in vitro and animal studies, are illustrated below.




WORM PRODUCTS
Helminths release hundreds of different molecules, some of which are packaged into extracellular vesicles that may be taken up by host cells.
ANTIBODY RESPONSES
Helminth infection may trigger B cells to produce IgG4, an antibody suggested to be involved in anti-inflammatory responses.
B cells
IgG4
Necator americanus
MICROBIAL INTERACTIONS
Several worm species are associated with altered microbiome compositions.
MOLECULAR FOUNDRY
Some of the molecules secreted by N. americanus have shown promise in mouse models of inflammatory bowel disease.
Mucins
GUT BARRIER
Infection with Trichuris trichiura may stimulate CD4+ T cells to produce cytokines such as IL-22 associated with mucin production and gut wall protection.
Heligmosomoides polygyrus
Trichuris trichiura
REGULATORY T CELLS
Hp-TGM, a molecule secreted by Heligmosomoides polygyrus, mimics mammalian TGF-β and can upregulate regulatory T cells, which dampen inflammation.
Hp-TGM
Hp-ARI
IL-22
T regulatory cells (Tregs)
CD4+ T cell
ALLERGY ASSOCIATIONS
A protein produced by H. polygyrus called Hp-ARI may neutralize cytokines such as IL-33 that are associated with allergy-related inflammation.
IL-33

Indirect effects of helminth infection

While most researchers in this field have been focusing on direct interactions between worms and their hosts, several who spoke to *The Scientist* highlighted an additional dimension to their work, one that acknowledges the trillions of bacteria occupying the same space a parasitic worm calls home. Increasingly seen as a mediator of human health in its own right, with hypothesized effects on everything from intestinal inflammation and immune development to cancer progression and mental health, the gut microbiome could also be an important piece of a worm's relationship with its host.

Nicola Harris, an intestinal immunologist at Monash University in Australia, has been delving into this tripartite relationship for years now. Part of her work examines how worms react to the gut microbiota; some of the team's latest mouse data suggest that at least some worm species are "much, much happier without any bacteria around it at all," Harris says. Another facet concerns the microbiome's role in mediating helminth-host interactions—an issue with particular relevance for understanding the possible therapeutic effects of helminth infection. Indeed, work by several groups suggests that the microbiota seems to be required for some of the beneficial consequences of helminth infection. In one study, for example, Harris, Maizels, and colleagues reported that mice that were inoculated with *H. polygyrus* before being infected with a respiratory virus typically showed less lung inflammation than helminth-negative mice given the same virus, but this protective effect disappeared when the experiments were repeated with germ-free mice.[11]

One of the ways helminths might act on the host via the microbiota is by changing the overall composition of bacterial species in the gut—something that has frequently been linked to disease risk and health outcomes independent of helminth infection. Circumstantial evidence for this mechanism comes from observations of humans showing that worm-infected people have different microbiomes than uninfected people. For example, while Loke was working a few years ago in Malaysia, where he's originally from, he tells *The Scientist*, he found that infection with *Trichuris* species was linked to greater phylogenetic diversity of bacteria in the gut.[12] The results were interesting, Loke says, because parasitic infections—at least, harmful ones—are typically associated with *lower* bacterial diversity. In microbiome research, "there's kind of a general impression that more diversity is better," he says, adding that he and colleagues are now using metagenomics and metatranscriptomic techniques to further characterize the microbiomes of helminth-infected people.

Evidence for a potentially causal link comes from experimental work in animals and humans. Harris's group has found that infection with helminths such as *H. polygyrus* can completely remodel the gut microbiota in mice, for example. And in a clinical trial of celiac patients carried out several years ago, Loukas and colleagues reported that experimental infection with *N. americanus* led to a small but statistically significant increase in the number of bacterial species detectable in the human gut, though community structure and bacterial diversity seemed broadly unaltered.[13] Loke's group is currently studying the countereffect: what happens to microbiota composition when helminth-positive people take deworming drugs.

Worm infection may also favor the growth of specific types of bacteria over others, and in doing so, promote particular gut environments associated with disease or the absence of it. In 2016, for example, Loke and colleagues reported that mice that were genetically susceptible to developing Crohn's disease had a lower risk of developing intestinal inflammation if they were infected with *Trichuris muris*, and that this protective effect occurred via

COURTESY OF ALEX LOUKAS

Necator americanus

INSIDE AGENT

In addition to their potential as macrotherapeutics, helminths have caught the attention of organizations interested in developing ways to augment human biology. Alex Loukas and Paul Giacomin at James Cook University in Australia recently received funding to convert worms into "molecular foundries," Loukas tells *The Scientist*. The project, supported by Charles River Analytics as part of a contract with the US government's Defense Advanced Research Projects Agency (DARPA), aims to "take our worms, which have been shown to be safe and well-tolerated and can be genetically modified using techniques like CRISPR, and actually engineer them now to secrete therapeutic molecules that might combat bioterrorism agents like anthrax, or VX gas, or sarin gas," he explains. The modified helminths could be then "used to infect soldiers or medical first responders who are working in areas where there is a bioterrorism agent threat."

The research could have applications beyond the battlefield, Loukas says, noting that the funding will help the teams establish proper development and manufacturing protocols that could advance the area of helminth therapy more generally. In the long run, he adds, it might even be possible to engineer worms to release drugs to combat disease. Before, you just had the parasites "in the body producing their own goodies, but now we can engineer them to secrete foreign molecules," he says. "My goal one day is to have a worm that's genetically modified to secrete an anti-inflammatory monoclonal antibody into the gut that might cure inflammatory bowel disease, for example."

Increasingly seen as a mediator of human health in its own right, the gut microbiome could also be an important piece of a worm's relationship with its host.

the microbiota: helminth infection favored growth of bacteria in the *Clostridiales* order, which in turn kept a check on the inflammatory bacterial species *Bacteroides vulgatus*.[14] (Loukas's recent diabetes study also identified elevated abundance of *Clostridiales* in mice with *N. brasiliensis* infection, although it wasn't clear if this aided in preventing disease.)

Researchers including Harris suggest that this kind of microbiome involvement could help explain not only the putative health benefits of worm infection, but also the effects of worm-derived molecules. She highlights a 2019 study from Harnett's group reporting that ES-62 protected mice from rheumatoid arthritis, an autoimmune disease that causes gradual bone erosion and that has previously been associated with disrupted gut microbiota. Monitoring the composition of the gut microbiome, the researchers also found that ES-62 treatment promoted growth of certain *Clostridiales* bacteria that produce butyrate, a metabolite previously shown to promote bone formation and prevent bone loss in mice.[15]

Causation couldn't be established from the study, but Harris posed a question in a *Nature* perspective article accompanying the study's publication: "Could interactions between gut parasites, such as helminths, and gut microbiota be the key to normalizing an unbalanced microbiome and preventing arthritis?" Relevant mechanisms could run both ways, she says—in some cases, the host immune response to helminths may alter the gut microbiota; in others, helminth-secreted products may alter microbiome composition directly, and subsequently affect host biology.

Harnett says his group is delving further into this topic, adding that it's possible that microbiome effects could help explain why ES-62 hasn't proven to be very effective for some conditions such as type 1 diabetes. Loke notes that the microbiome could also contribute to variation among people and should be considered when trying to suss out who might benefit from particular helminth or helminth-derived therapies.

This growing appreciation of microbes' involvement in helminth-host interactions is a reminder of the complexity of the body's biological community—and how much more there is to learn before worms or their derivatives can be widely deployed as therapeutic tools. The relatively recent discovery that worms secrete some of their proteins within extracellular vesicles that are taken up wholesale by host cells, for example, represents a previously unappreciated way for worms and hosts to communicate. Loukas also highlights what he says are intriguing findings about helminths' effects on host brain chemistry, with a handful of small studies linking helminth infection with serotonin levels in mice. "It could well be that worms manipulate brain chemistry to make people . . . have a greater sense of well-being than an uninfected person," he says. "That could be an evolutionary strategy: that the worms want you to feel good so that your life isn't affected," and you can transmit infection to others.

Loukas speculates that such phenomena could even offer a possible explanation (other than placebo effects) for why many people report feeling so much better when infected, whether or not their disease improves clinically. Following one of the team's celiac trials, some patients "had what a celiac pathologist would call fully blown disease, but these people didn't feel unwell," Loukas says. When trials end and participants are offered a deworming drug, many refuse it, he adds. "A number of people refer to [the worms] as their families."

And what about Loukas—would he kill off his parasites? "No!" he says. "I'm over fifty now and I felt like my knuckles were starting to feel slightly arthritic, and I thought 'Oh, I wonder if the worms will do anything for them.'" Acknowledging that it's just an anecdote, not a scientific insight, he says he thinks his knuckles have been feeling slightly better. "I don't know if it's due to the worms," he says. "But I'm not getting rid of them in a hurry." ■

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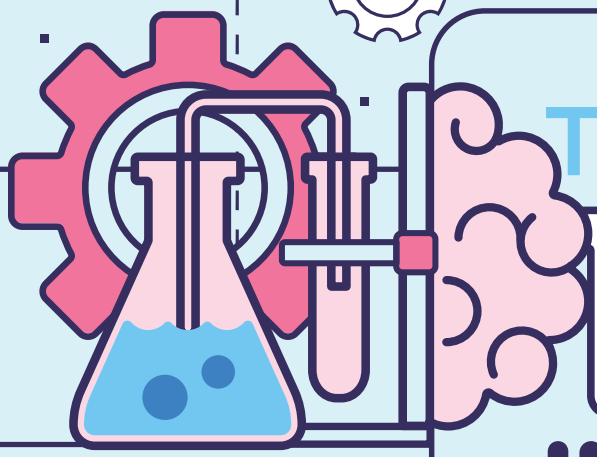


TheScientist 2021 TOP 10 INNOVATIONS

Biomedical innovation has rallied to address the COVID-19 pandemic while continuing to tackle broader research challenges.

BY *THE SCIENTIST* STAFF

With the COVID-19 pandemic dragging toward a most unwelcome third year, it's not surprising that the biomedical community has continued to focus on diagnosing and treating the disease. The list of this year's Top 10 Innovations winners reflects these shared goals with a couple of products that can help researchers better understand the biological realities of SARS-CoV-2 infections, interrogating cells neighboring those infected with the virus, for example, and the immune system's reaction to it over time.

But 2021's innovation landscape also includes laboratory and clinical products that provide a more expansive view on biology. The winners of this year's competition include an implantable miniscope that can track activity in the brains of freely moving organisms; a microfluidic device that aims to recapitulate whole-organism physiology; and a few products that build on the emerging trend toward characterizing individual cells, with the added components of spatial information or multi-omics.

Since the last installment of our Top 10 innovations, the world has witnessed the successful deployment of multiple COVID-19 vaccines, and those are, in their own right, truly awe-inspiring innovations. In a way, it's heartening that scientific advances have continued to occur in spaces outside of the crucial coronavirus focus. It suggests that the global biomedical apparatus is robust enough to address a pressing and pointed concern while not losing ground in fields not directly related to that crisis.

Here are the breakthroughs and advances that, thanks to the careful consideration of our panel of independent judges, have won a spot in our annual Top 10 Innovations competition.

1

Inscopix nVue™ System

Weighing in at two grams, the nVue™ System is about the size of a Lego brick. This "miniaturized microscope" relies on red and green fluorescent indicators targeted to neurons to trace calcium ion influx and, in turn, the activity of two different neuronal populations in freely moving animals, according to Alice Stamatakis, director of applications at Inscopix, the company that makes the nVue. Thus far, researchers have mounted nVue on the heads of rodents, birds, and monkeys.

The miniscope offers another advantage, Stamatakis adds: longitudinal deep-brain imaging, wherein the same cells can be analyzed over multiple imaging sessions. Two-photon microscopy also allows simultaneous imaging of two neuronal populations, but it is mostly limited to the brain cortex and requires animals to be constrained by the head, compromising the study of behavior, she says. "[nVue] is going to give neuroscientists an unprecedented view into how these different brain signals communicate and talk with each other during naturalistic behaviors." The system's built-in data acquisition and processing software helps complete the picture.

Beyond basic biology, the dual miniscope can aid translational research for neuropsychiatric and neurodegenerative conditions, such as anxiety or Alzheimer's disease. Kelly Tan, a neurologist at the University of Basel, Switzerland, uses the nVue system to study communication between neuronal populations in a mouse model of Parkinson's disease. "It's been a breakthrough for circuit neuroscience," Tan, who highlighted the dual miniscope's merit in a video and webinar for Inscopix, tells *The Scientist*.

In addition to tracking two neuronal populations, researchers can use the miniscope to juxtapose fluorescence signals from calcium influx in neurons and plasma in blood. This allows for analysis of the relationship between neuronal activity and vascular dynamics, including capillary diameter and red blood cell velocity, in the brain. Stamatakis says that Inscopix is now working to layer electrophysiology recordings and enhanced behavioral analyses into the miniscope.

Inscopix declined to provide a price for the system, explaining that the cost varies regionally.

WILEY: *The innovation is in what it allows researchers to do, which is to follow two activities in the brains of freely behaving animals over time.*

INSCOPIX

THE JUDGES

PHILIP HOCKBERGER
Associate professor of neuroscience in the Feinberg School of Medicine at Northwestern University. He is recognized internationally for his leadership role in research core facilities and for promoting the careers of core scientists.

KIM KAMDAR
Managing partner at Domain Associates, a health care–focused venture fund that creates and invests in biopharma, device, and diagnostics companies. She began her career as a scientist and pursued drug discovery research at Novartis for nine years.

H. STEVEN WILEY
Senior research scientist and laboratory fellow at Pacific Northwest National Laboratory. He published some of the earliest computer models of receptor regulation and is known for developing a variety of quantitative biochemical and optical assays as a basis for validating computational models of cell processes.

2 CN Bio PhysioMimix™ OOC Multi-Organ Microphysiological System

CN Bio released the PhysioMimix™ OOC Multi-Organ Microphysiological System in March 2021 after about 10 years of research and development through a collaboration between the Defense Advanced Research Projects Agency (DARPA) and MIT. A microfluidic organ-on-a-chip platform undergirds the PhysioMimix™ Multi-Organ System and allows scientists to connect individual organ-on-a-chip models—for example, a liver model with a gut or lung model also developed by CN Bio—for disease research and drug development, explains company CEO David Hughes.

Each chip contains millions of organ-specific human cells that can be connected in a multi-well plate format. The system mimics biological conditions by allowing media recirculation to different organ culture compartments, says Hughes; this "creates data that's more predictive of human response" compared to insights gleaned from animal models. He adds the product is geared toward "providing more-accurate, human-relevant information to researchers in the pharmaceutical industry."

Martin Trapecar, a Johns Hopkins immunologist and bioengineer, uses this system in his lab to study the effects of autoimmune and autoinflammatory diseases on gut and liver tissue. He says that the product presents a more realistic model to develop regenerative and personalized therapies and "eliminates a lot of the problems with studying immunology. . . . The other benefit is it gives me very granular insight into how tissue-tissue and tissue-immune interactions inform the behavior of the whole system." According to a company announcement, CN Bio considers this technology a milestone toward an eventual "body-on-a-chip" system.

The company declined to share the price of the system.

HOCKBERGER: *"[T]his product improves on the original system (launched in 2018) to enable a wider user base. . . . Cool!"*

© CN BIO

Vizgen MERSCOPE 3

One of two spatial genomics tools in this year's Top 10, Vizgen's MERSCOPE™ is the only single-cell spatial genomics instrument currently available for purchase. Designed to conduct and analyze multiplex error resistant fluorescence in situ hybridization (MERFISH) experiments, the platform detects RNA transcripts from hundreds of genes across intact tissue and returns imaging and expression data at subcellular resolution.

The product was developed as "a new sort of research tool that gives people this unprecedented view into biological systems," says Vizgen cofounder and director of technology and partnerships George Emanuel. "You know exactly where each transcript is with 100-nanometer accuracy."

The Salk Institute's Pallav Kosuri, who is using MERSCOPE™ for detailed cardiac tissue imaging, says it's useful to work directly with the instrument, adding that while sample prep is laborious, the analysis is fully automated by MERSCOPE™. "Everything has worked really smoothly," says Kosuri, who did his postdoc in the Harvard University lab where the technology was developed but was not involved in the work. When he's needed technical support, "the company has been really good at dedicating time and effort to troubleshoot with us."

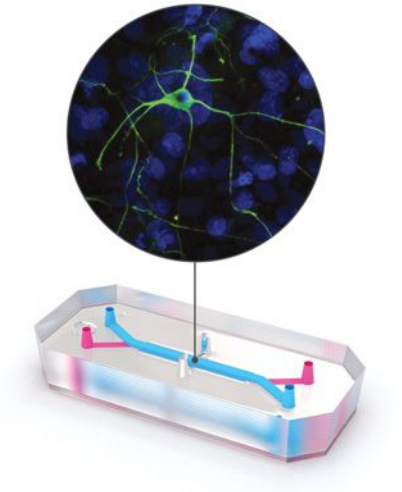
One $300,000 purchase includes the automated instrumentation, plus data visualization software and other infrastructure needed to run MERFISH experiments; reagents and probes for researchers' genes of interest cost extra. The first units were shipped in August of this year.

Kosuri says Vizgen can price the platform so high because currently, they "are the only ones doing this." But it's prohibitively expensive for many labs. "As an investigator, it's super steep."

KAMDAR: *"MERSCOPE is the first commercially available high-plex, single-cell spatial genomics platform for spatially profiling gene expression across whole tissues and resolving individual transcripts with nanometer-scale resolution. The coordination of gene expression and spatial profiling opens new windows in the precise architecture of a cell.*

Emulate Brain-Chip 4

The blood-brain barrier (BBB) poses a challenge for the development of drugs that target the brain. Layers of cells that line the blood vessels of the brain evolved to help keep out toxins or other molecules that could potentially harm this vital organ, but they also block the passage of most therapeutics. With a decade-long history of developing organ-on-a-chip models, biotechnology company Emulate set out to create one that could accurately model this barrier and the structures on either side of it.



VIZGEN; EMULATE INC.

"This is our most complex and most adventurous chip because it not only has the endothelial cells, it has astrocytes, pericytes, microglia, and neurons," says Lorna Ewart, the company's executive vice president of science.

The Brain-Chip, which was released in December 2020, consists of two channels embedded in flexible rubber polymer. One channel is lined with stem cell–derived endothelial cells, representing blood vessel walls, and the other is lined with neurons and glia. Midway along the chip, the two channels come into contact. As fluid moves through the "blood vessel" channel, scientists can study how molecules interact with and move to the other channel—effectively crossing the BBB—and how they affect structures there.

The Brain-Chip can model both healthy and unhealthy neurological states. At Cedars Sinai in Los Angeles, developmental biologist Michael Workman and colleagues have been using patient-derived stem cell lines to create models of neurodegenerative diseases such as Parkinson's on the Brain-Chip. "Each one of these chips is like a little patient avatar," he says. "We do have a large interest in that personalized health and precision medicine approach, and see these microfluidic chips as a way to push more towards that."

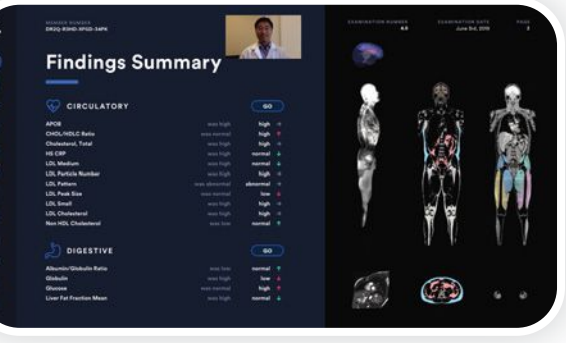
Emulate declined to provide a price for Brain-Chip, as it depends on end users' requirements.

WILEY: *"Very sophisticated organ-on-a-chip for brain research, providing a new and powerful approach for investigating mechanisms of neuroinflammation and blood-brain barrier function."*

Q Bio
Gemini and Bio Mark I

The Gemini platform and Mark I scanner by Q Bio were introduced in April 2021 as a way to monitor patient health more comprehensively than has previously been possible in healthcare. Although it is not yet widely available, the company is rolling it out with a limited number of patients and doctors as part of a pilot program.

The Mark I prototype scans the entire body with the patient sitting, standing, or lying down, using magnetic resonance imaging (MRI), which creates high quality images without radiation—unlike X-rays, computer tomography (CT), or positron emission tomography (PET). A scan with Mark I takes only about 15 minutes, compared with traditional full-body scans that can take more than an hour. The imaging information is uploaded to the Gemini platform, along with medical records, genetics data, and traditionally acquired tests of blood, urine, saliva, and vital signs. Putting all these data together, the platform creates a "digital twin" of the patient's anatomical structures, vital signs, and body chemistry.



By cataloging these data, small changes can be compared over time, and mathematical models could predict problems before they occur, says Jeffrey Kaditz, founder and CEO of Q Bio. This could allow doctors to efficiently triage patients' needs based on annual scans. Currently, an annual patient membership costs $3,495 and includes a scan and consultation. Q Bio has not yet applied for FDA approval, and the company does not accept health insurance.

"Our aim is to bring a sea change in how health care is delivered on a large scale," William Stanford, chief medical and scientific officer for the Beverly Hills Institute for Precision Medicine, says via email, adding that Gemini is the "perfect adjunct" to the facility's multi-omic data collection efforts. The approach can be a bit cumbersome, he notes, as his patients must fly from Los Angeles to northern California, then drive to Q Bio's facility in Redwood City for the scan—all of which takes around eight hours, round trip. The results come back two weeks later and can be sent to the patient's primary doctor.

WILEY: *"This is a software-hardware platform to create a digital representation of a patient that can be stored and analyzed over time. The very fast (<15 min) whole-body scanner is key. This is truly a groundbreaking innovation in developing a digital framework for understanding human physiology and aging."*

10x Genomics
Chromium X

Chromium X is 10x Genomics's newest instrument for single-cell analysis. Users load cells in suspension and add reagents and a partitioning oil into the microfluidic chip, which goes into the benchtop instrument. The resulting droplets, or Gel Bead-In-Emulsions (GEMs), each contain a single cell, a single barcoded Gel Bead, and a reagent, and are ready to be sequenced and used in assays offered by 10x Genomics, including gene expression analysis, epigenetic profiling, and immune cell profiling. Each GEM carries a unique barcode, allowing the user to later link results back to a single cell.

The Chromium X is the latest in a long line of products from 10x Genomics that have won top spots as Top 10 Innovations. In 2019, the firm's single-cell, droplet-based sequencing system made the Top 10, and in 2020, the Chromium Single Cell Multiome ATAC + Gene Expression assay won a spot. Chromium X improves on previous products because of its flexibility, says Jens Durruthy Durruthy, associate director of product management–single cell at the company. "Chromium X is scalable and can be used both for low-throughput assays, with hundreds of cells, as well as for high-throughput assays with up to one million cells."

Sisi Chen, director of the Single-Cell Profiling and Engineering Center at Caltech, notes in an email to *The Scientist* that the high-throughput capability of the Chromium X is essential to her research. She uses the Chromium X, which was launched in July 2021, to explore how therapeutic compounds influence the human immune system, and the system allows her to simultaneously stimulate 1 million immune cells, each with one of up to 100 different therapeutics, and track their responses. "We want to profile the [immune] system across hundreds to thousands of different unique conditions," says Chen.

In the US, Chromium X is available from $100,000. With high-throughput assays, users can get the cost per run down to 2 cents per cell.

HOCKBERGER: *"10X Genomics is back with a new high-scale, high-resolution version of its flagship instrument, Chromium. The latest product democratizes access to high throughput, single-cell analysis of gene expression and immune profiling by offering it at an affordable price. Well done!"*

Q BIO; 10X GENOMICS

The Native Antigen Company
SARS-CoV-2 Neutralization Assay Development Kits

A few years ago, the World Health Organization added "Disease X" to its short list of emerging diseases—a placeholder for unknown pathogens with pandemic potential. Researchers at The Native Antigen Company, a UK-based group that designs reagents for infectious disease research, speculated in November 2019 that one candidate might be a coronavirus, one that would likely arise in Asia and spread from animals. Then came SARS-CoV-2.

By spring 2021, The Native Antigen Company had developed a coronavirus neutralization assay to determine a serum sample's level of antibodies that bind, and therefore neutralize, the virus. The assay uses synthetic versions of the SARS-CoV-2 spike protein's receptor binding domain and its target, the mammalian ACE-2 receptor, and pairs them with an ELISA-based platform that quantifies the neutralizing ability of the antibodies via a color change, explains the company's Commercial Director, Andrew Lane. Researchers can use this tool to probe how patients respond to infection and to study vaccine efficacy, among other applications, he adds.

The kit doesn't require live virus and is speedy compared to methods that use benign, engineered viruses called pseudoviruses, according to Lane. Since the first kit was released in April 2021, the company has produced assays for five variants. "It's a bit like a plug-and-play system for us. We can make kits with different variants quite quickly," Lane says. Each kit analyzes 960 samples and costs $2,728.

"Overall, we're very happy with its response," says Matthew Edmans, a postdoctoral researcher at the University of Oxford who is using the assay to study how patients on immunomodulatory drugs respond to SARS-CoV-2 infection. Edmans also uses pseudoviruses, but agrees they can be "quite complicated," while The Native Antigen Company's assay "is just a lot faster and more straightforward to run."

KAMDAR: *"Needed to assess the protection and longevity of patient immunity to emerging variants. This is all done without the need for BSL3 facilities, thereby providing a safer alternative for these critical public health questions."*

Mission Bio
Tapestri Single-cell Multi-omics Solution

Mission Bio's Tapestri Single-cell Multi-omics Solution, launched in October 2020, is a process for single-cell analysis that allows users to consider DNA sequence and proteomic information simultaneously—an innovation on traditional setups that required separate systems to analyze nucleic acids and proteins, which could therefore not be correlated at the single-cell level. Mission Bio's Tapestri Precision Genomics Platform earned a spot in *The Scientist*'s Top 10 Innovations of 2018 and was the first high-throughput instrument for single-cell DNA sequencing sample prep. "We were then able to add other analytes, such as proteins, subsequently," says Adam Sciambi, Mission Bio's cofounder and senior director of technology & systems.

With the Tapestri Single-cell Multi-omics Solution, assays for DNA and protein are combined in a single integrated workflow that can analyze up to 10,000 cells at a time. The Tapestri instrument uses microfluidics to capture individual cells in droplets that contain both the reagents for DNA sequencing and antibodies for tagging cell-surface proteins, plus a barcoding bead. "The result of our platform is every piece of DNA comes out labeled as telling you which droplet it came from," says Sciambi. The DNA is then sequenced using next-generation sequencing, and the cells' surface proteins are characterized. Mission Bio declined to disclose the cost of the platform.

Molecular biologist Jan Cools of the VIB-KU Leuven Center for Cancer Biology in Belgium has used the Tapestri platform to investigate mutations underlying acute lymphoblastic leukemia (ALL). He is now planning to use Mission Bio's Tapestri Single-cell Multi-omics Solution to obtain additional information on cell-surface markers, a setup he says will be especially useful for studying a different type of blood cancer, acute myeloid leukemia (AML). In AML, some leukemia cells are known to be more stem cell–like, while others are more differentiated, a difference that could be captured by looking at cell surface markers and sequencing data, Cools says.

KAMDAR: *"Tapestri is the only commercialized multiomics platform capable of analyzing DNA and protein simultaneously from the same sample at single-cell resolution. The real power is the ability to generate correlation data between the genome, transcriptome and the proteome"*

THE NATIVE ANTIGEN COMPANY; MISSION BIO

9 Cardea Bio
CRISPR-SNP-Chip

Cardea Bio's CRISPR-SNP-Chip is the first device capable of detecting single base differences in DNA without generating millions of copies of the DNA first. "We can do DNA tests without the need of a DNA lab," explains Cardea CEO Michael Heltzen.

The latest of Cardea's biological processing units, or BPUs (analogous to the CPUs that underlie computer technologies), the chip is an updated version of the company's CRISPR-Chip™, which already allowed for rapid, amplification-free detection of large, disease-associated sequence variants and transgene insertion success, among other applications, says Keck Graduate Institute biomedical engineer and Cardea Chief Scientific Officer Kiana Aran. She explains that both versions are composed of a CRISPR-Cas system tethered to a graphene transistor. When the Cas enzyme's guide RNA binds to the correct sequence, it pulls the DNA closer to the transistor. Because DNA is positively charged, this generates an electronic signal in the semiconductive graphene that can be digitally read. "You let the biology do what it's good at, and then you sense it with our sensor," says Aran. "We use the power of biology as technology."

Giving the chip the ability to detect single nucleotide polymorphisms (SNPs) involved replacing the Cas enzyme with a more sensitive version and upgrading the data analysis, Aran notes. In an April paper, the team demonstrated the updated chip's ability to detect SNPs that underlie sickle cell anemia and amyotrophic lateral sclerosis (ALS), though the potential applications are bounded only by creativity, the authors write. Its most immediate use is for quality control of gene editing for medicinal or agricultural purposes, Aran says.

Those interested in using the chips can apply for Cardea's partnership program. While the exact cost depends on the application, Heltzen notes that the price per chip has dropped to tens of dollars from the thousands they were a few years ago.

HOCKBERGER: *"Another game changer for clinical diagnosis."*

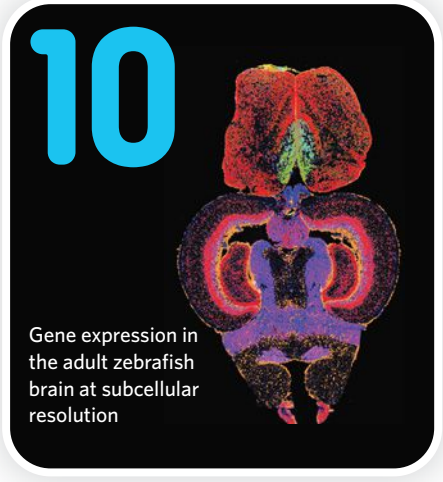
10 Resolve Biosciences
Molecular Cartography™ Single-Cell Spatial Analysis Service

Spatial biology addresses how cells function in the context of tissues. While single-cell sequencing methods have permitted many advances in this field, they lack the resolution to provide 3D data at subcellular scales, and involve destroying the tissue sample. Resolve Biosciences's Molecular Cartography™ Single-Cell Spatial Analysis Service, the second spatial genomics tool in this year's Top 10, instead offers fluorescence in situ hybridization (FISH) to create high-resolution images of what genes are expressed—down to the subcellular level.

The mail-in service, launched June 2 in North America and Europe, detects individual RNA transcripts inside intact tissues. "We can interrogate pretty much any tissue you can put on the slide," says company CEO and cofounder Jason Gammack, adding that the platform can analyze 24 samples simultaneously. Costs depend on project specifications, but most customers can generate sample data for around 4,000 euros, he says. That includes a meeting with a customer technology adviser to define project scope and a sample prep kit in a return-mail box. Researchers receive a summary report and data on their chosen genes—the platform lets researchers visualize up to 100—in about four weeks; the adviser also helps researchers interpret the data.

Jean-Christophe Marine of the VIB-KU Leuven Center for Cancer Biology has used the service through an initiative at his institution that supports early access to new technologies. "We are very satisfied by the data," says Marine, who studies intratumor heterogeneity in melanoma. "[The] vast majority of the probes worked, and . . . you have a nice resolution." The service is well-priced, he adds, although his team has only been able to analyze mouse samples due to restrictions on mailing human samples.

In the future, Resolve Biosciences plans to make the whole Molecular Cartography™ platform available for researchers to operate themselves. The company will expand what types of molecules can be imaged, too, Gammack says, with proteins up next. "We're actively developing that chemistry right now."



Gene expression in the adult zebrafish brain at subcellular resolution

KAMDAR: *"The technology has potential to help researchers better understand human brain development, cell type evolution, and how the SARS-CoV-2 infection affects neighboring cells over time."*

FRANCISCA DANIELA ACUNA HINRICHSEN AND EMRE YAKSI, NORWEGIAN UNIVERSITY OF SCIENCE AND TECHNOLOGY, AND ASTHA GUPTA, RESOLVE BIOSCIENCES; CARDEABIO.COM

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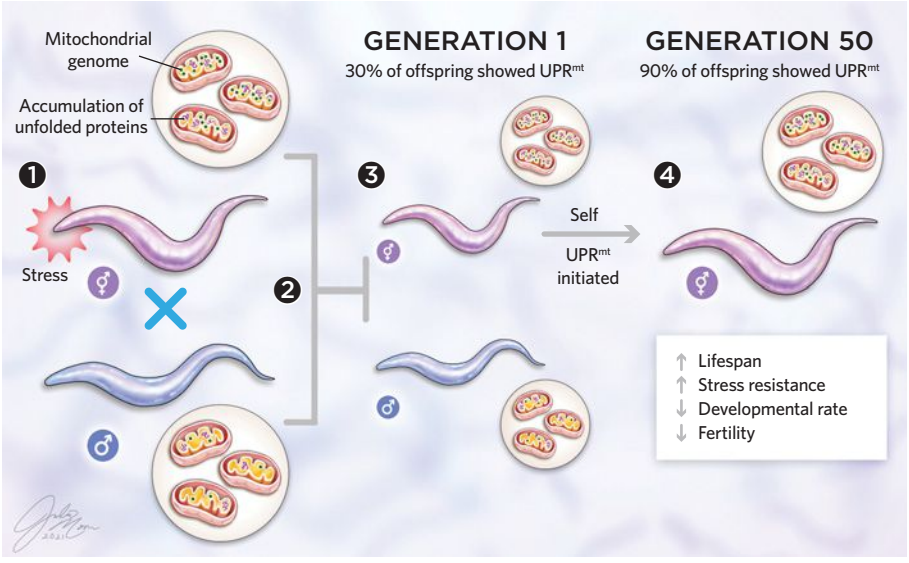
The Literature

CELL BIOLOGY

Mitochondrial Memories

THE PAPER

Q. Zhang et al., "The memory of neuronal mitochondrial stress is inherited transgenerationally via elevated mitochondrial DNA levels," *Nat Cell Biol*, 23:870–80, 2021.



INHERITED STRESS: Exposing neurons of hermaphroditic *C. elegans* to high levels of either the Huntington's disease–causing protein Q40 or the ligand Wnt triggers a stress pathway, the mitochondrial unfolded protein response (UPR^{mt}), in many of their cells, including their oocytes. The UPR^{mt} involves elevated copy numbers of the organelle's genome and an accumulation of unfolded proteins ❶. When researchers bred these animals with wild-type males that had not been stressed ❷, they found that about 30 percent of the offspring continued to carry a "memory" of that stress, as evidenced by the UPR^{mt} in their tissues ❸. This transgenerational inheritance, the researchers found, was mediated by Wnt. Hermaphroditic offspring with the strongest stress responses were allowed to self-fertilize until up to 90 percent of offspring showed the UPR^{mt} inherited from the experience of their ancestors—a "memory" passed down for as many as 50 generations ❹. Worms with this stress-primed phenotype had increased resistance to other stressors such as heat and pathogens, and lived longer, but they grew more slowly and were less fertile than controls.

Under stress, mitochondria rapidly increase their copy number—that is, the number of mitochondrial genomes in each organelle—as part of a process called the mitochondrial unfolded protein response, or UPR^{mt}. This process prompts the upregulation of certain stress response genes in the cell's nucleus. Now, a team has found that roundworms that experienced mitochondrial stress pass on a "memory" of that stress to their descendants by propagating the elevated copy number through the germline.

Chinese Academy of Sciences geneticist Ye Tian and her colleagues had previously found that a signaling molecule called Wnt is involved in the neuronal response to mitochondrial stress. Working with *C. elegans*, the team created transgenic worm lines that expressed the Huntington's disease–causing protein Q40 in their neurons, which then started secreting Wnt, initiating the UPR^{mt} in not only the animals' brains, but their intestines too. Tian also noticed that some worms that had not experienced stress themselves, but whose ancestors had, continued to exhibit the stress response in the intestine, she says.

To study whether mitochondrial stress might be sending a signal from the brain to the germline, thereby passing down a "memory" of the stress through the generations, the team exposed the neurons of hermaphroditic worms to either Q40 or Wnt, then bred those worms with wild-type males that hadn't experienced stress. In the first generation, about 30 percent of the offspring, which were hermaphroditic, retained the activation of the UPR^{mt} in their intestine, muscle cells, and oocytes.

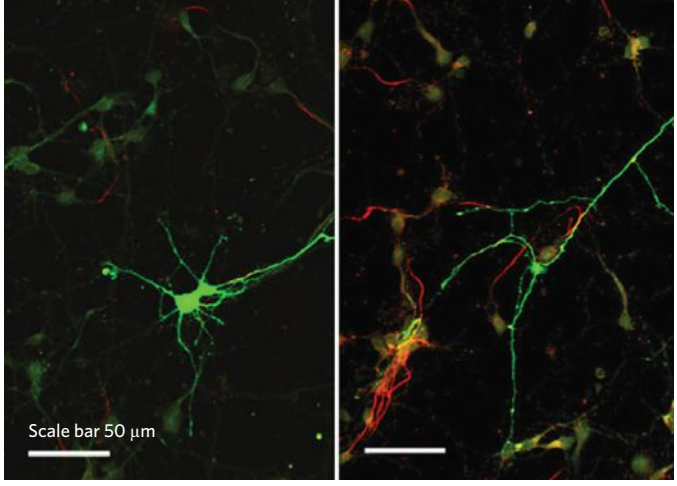
The researchers then chose the individuals with the strongest response and allowed them to self-fertilize until roughly 90 percent of the worms showed the high copy number and stress response phenotype, which was retained for more than 50 generations. In other experiments, the team confirmed that the Wnt pathway is necessary for the inheritance of this stress "memory."

The stress-primed phenotype was a fitness tradeoff: the descendants of stressed worms lived longer and had improved resistance to heat and pathogens, but they also grew more slowly, were slower to reach sexual maturity, and produced fewer offspring than worms with less mitochondrial DNA. "You cannot have all the advantages," Tian says. "In our case, their mitochondria are under stress, so they develop a little bit slower."

"They did a nice job of sorting this out, and it's as good as it gets in terms of methodology," says Cole Haynes, a cell biologist at the University of Massachusetts Chan Medical School who was not involved in the work. "The findings are sort of remarkable. . . . Neurons telling the germline to make more mitoDNA that will affect the next generation is pretty wild."

—Amanda Heidt

FISH PHYLOGENY: The man-of-war fish (*Nomeus gronovii*), a species of medusafish, near the tentacles of a siphonophore.



APP-LESS: An APP-knockout neuron (right) shows extended axonal and reduced dendritic growth compared with a normal mouse neuron (left).

EVOLUTION

Medusafish Morphology

THE PAPER

M.N.L. Pastana et al., "Comprehensive phenotypic phylogenetic analysis supports the monophyly of stromateiform fishes (Teleostei: Percomorphacea)," *Zool J Linnean Soc,* doi:10.1093/zoolinnean/zlab058, 2021.

Fishes such as driftfishes, butterfishes, and barrelfishes—traditionally grouped as medusafishes (suborder Stromateoidei)—share a gizzard-like "pharyngeal sac" lined with tooth-like projections that grind up food. But despite their shared morphology, recent molecular studies have placed them into multiple groups rather than one evolutionary lineage. "Conflicts between morphology and DNA-based hypotheses are particularly striking for this group, and their resolution represents one of the biggest challenges of the systematics of bony fishes," says Murilo Pastana, an ichthyologist at the National Museum of Natural History.

To determine whether the 15 genera of medusafishes are in fact closely related, Pastana and his colleagues conducted the largest morphological study of the group to date, examining more than 200 characteristics. Through dissection, staining, and imaging, they detailed the internal and external structures of more than 20 species.

The team found that, in addition to pharyngeal sacs, medusafishes share a system of canals and pores that supply their skin with mucus. This mucus may protect young individuals, which frequently hide near stinging animals like jellyfishes, says Pastana. His data suggest that, despite the molecular contradictions, medusafishes do share a common ancestor, one that possessed a pharyngeal sac, skin mucus, and 11 other features, including 18 pectoral-fin rays and a unique nerve branching pattern.

Dahiana Arcila, an ichthyologist at the University of Oklahoma who studies phylogeny using molecular tools and wasn't involved in the study, explains that some of the conflicting evidence stems from the taxon's history. "Their rapid evolution after the end-Cretaceous extinction makes their relationships particularly challenging to sort out," she says. Both Pastana and Arcila agree that, although their approaches differ, the combination of morphological and molecular data will ultimately solidify scientists' understanding of fish diversity.

—Devin A. Reese

NEUROSCIENCE

Hidden Function

THE PAPER

T. Liu et al., "The amyloid precursor protein is a conserved Wnt receptor," *eLife*, 10:e69199, 2021.

Amyloid precursor protein, which generates amyloid-β when broken down, has long been associated with Alzheimer's disease. But its normal function in the brain has remained relatively mysterious. Over the past decade, Bassem Hassan of the Paris Brain Institute and others have found hints that the protein (APP) is part of a complex involved in Wnt signaling—an evolutionarily conserved pathway that regulates animal development—as well as in synaptic plasticity and adult neurogenesis.

Studying human APP and the *Drosophila* homolog APPL in vitro, Hassan's team now reports that these membrane proteins bind directly to two types of Wnt peptides, Wnt3a and Wnt5a, in a way that regulates intracellular APP levels: Wnt3a increases APP's stability and enhances its persistence, while Wnt5a promotes its breakdown. "It looks like they're acting opposite to one another," Bassem says, adding that APP seems to be "kind of a calibrator of Wnt signaling."

In additional experiments, cultured mouse neurons lacking functional APP showed unusual development, including greater axonal but reduced dendritic growth. In neurons that did contain APP—and in particular, a cysteine-rich domain that the researchers found is required for the Wnt peptides to bind the protein—the team could tweak those growth patterns by altering the relative amounts of Wnt3a and Wnt5a. While it's unknown if this role in regulating neuronal growth is conserved in humans, the findings point to Wnt signaling as a potential factor in neurodegenerative diseases, Bassem adds.

Christina Elliott, a molecular neuroscientist at the University of Glasgow who was not involved in the study, says it'd be interesting to see how APP-Wnt interactions work in other cell types and agrees the work could inform Alzheimer's research. "This paper suggests the possibility that perhaps we actually should be looking at the biology of APP itself," she says. "Perhaps amyloid-β is not as important as we think."

—Catherine Offord

LINDA IANNIELLO; *ELIFE*, 10:E69199, 2021

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TheScientist

Brooke Gardner: Probing Peroxisomes

Assistant Professor, Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara

BY CHLOE TENN

Brooke Gardner recalls embarking on road trips, a favorite family activity, while growing up in Northern California. Her father, an oceanographer with the United States Geological Survey, would stop the car to point out rock strata and recite the scientific names of plants to his wife, a library and IT budget director at Stanford University, and their children. "Through both of them, I was exposed to higher education, academia . . . and that kind of scientific approach to the world," says Gardner.

Gardner's love of travel and interest in foreign relations first prompted her to enroll at Middlebury College in Vermont as a language major. However, she retained a fascination with science from her childhood. "I had to choose . . . my freshman year whether I wanted to continue to take intensive Italian or intensive organic chemistry, and I chose organic chemistry," she recalls. From there, Gardner developed a particular interest in the inner workings of the cell.

After graduating with a degree in biochemistry in 2006, Gardner began a PhD program, also in biochemistry, at the University of California (UC), San Francisco, working under molecular biologist Peter Walter. Her doctoral research focused on the stress-induced unfolded protein response in the endoplasmic reticulum (*Science*, 333:1891–94, 2011), but the work inspired her interest in the versatility of other cell organelles, particularly the mysterious peroxisome, an organelle that is involved in cell metabolism, cell signaling, and the reduction of damaging reactive oxygen species.

The peroxisome derives its name from the fact that many of the reactions catalyzed by the organelle's proteins, such as the breakdown of fatty acids and amino acids, form hydrogen peroxide as a byproduct. Individual peroxisomes are created by the cell using 37 so-called pex proteins, which can be modified, removed, or created from scratch. "I got really interested in the peroxisome because it's a place where cells can make a completely new organelle according to what they need," Brooke writes in an email to *The Scientist*. "It seemed like an area where there were a lot of open questions and where I could try to make an impact."

Gardner next joined biochemist Andreas Martin's lab at UC Berkeley in 2013 as a postdoc, studying motor proteins that play a role in the formation of peroxisomes. Specifically, she studied the structure and function of the proteins Pex1 and Pex6, which are required for peroxisome biogenesis. In one study, Gardner's group found that the two proteins assemble to form a motor, one that is often mutated in Zellweger spectrum disorders—human disorders characterized by defects in peroxisome biogenesis (*J Mol Biol*, 427:1375–88, 2015). In a follow-up study, the team showed that this motor complex also unfolds another protein, Pex15, which allows the complex to import other proteins from the surrounding cell matrix, among other functions (*Nat Commun*, 9:135, 2018).

Martin describes Gardner as "very fun to do science with," adding that, while in his lab, "she really made major contributions to our understanding of the unfolded protein response and how cells deal with or sense protein folding stress."

In 2019, Gardner joined the faculty at UC Santa Barbara (UCSB), where she continues to study the creation, growth, propagation, and specialization of peroxisomes and the ways in which their dysfunction can lead to disease. This past May, Gardner was named a Searle Scholar, and she is using the $300,000 award to tackle some of her "wilder" ideas in the lab, she says. Her team has already conducted a large screen looking for novel genes affecting peroxisome functioning in human cells and is beginning to analyze the results. "It's incredibly exciting, but I think it's also going to be pushing us into directions that we weren't necessarily anticipating," Gardner says.

Meghan Morrissey, a current colleague of Gardner's at UCSB, tells *The Scientist* that she feels lucky to have started her own lab next door. Calling Gardner an exceptional biochemist, Morrissey adds that "one of her main strengths is how incredibly rigorous she is." Morrissey co-teaches a class with Gardner and says that "Brooke always steps up and takes care of any gaps. . . . It's just very nice to work with someone so reliable." ■

BROOKE GARDNER

The RNA Run

RNA editing has been in DNA editing's shadow for nearly a decade, but recent investments in the tech could bring it into the limelight.

BY CHRISTIE WILCOX

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Rett syndrome seems to appear out of nowhere. Infants with the rare neurological disorder grow and develop normally at first, but then—generally between 6 and 18 months of age—they suddenly regress. Toddlers forget their words, lose the ability to crawl or walk, develop involuntary hand movements and sometimes seizures, and can even struggle to eat or breathe. The children, predominantly girls, "are very sick," explains Gail Mandel, a molecular neurobiologist at Oregon Health & Science University who has studied the condition for more than a decade.

The symptoms stem from loss-of-function mutations in a gene near the tip of the X chromosome that codes for methyl CpG binding protein 2 (MeCP2). This protein is a transcription factor, and it's especially abundant in the central nervous system, where it helps to ensure that particular genes are switched off at the right time during development. "We know a lot about how this protein works," Mandel explains. "We know how it binds to the genome; we know how it represses genes from being expressed; we know where it is in the nervous system. But what we don't know is why mutations that eliminate its function give rise to this neurological disease." That lack of essential knowledge, particularly about how MeCP2 dysfunction affects the expression of other proteins, has hindered attempts at identifying effective drug targets. So, while conducting basic research into that mystery, she says, "I decided to try to think of a way to fix the disease by fixing the mutations in the gene."

In other genetic diseases, gene therapies have proven successful—and indeed, Mandel gave that idea a go at first. Through multiple preclinical studies, she and her colleagues developed a viral vector designed to slip a healthy copy of the *MECP2* gene into cells and get them to make functional MeCP2. But even people with mutations in *MECP2* often produce some functional protein, and may even produce normal amounts in some cells. Too much MeCP2 is as bad as too little, so an extra copy of the gene could end up doing more harm than good, says Mandel. "That means that the therapeutic window is very small," she says.

The gene therapy she helped develop is still being pursued clinically, but what Mandel says she really wants is a way to repair the mutation without increasing the overall abundance of the protein. So she decided to try correcting the messenger RNA (mRNA) for the protein, using an enzyme that can switch out certain mutated bases in transcripts with the correct one. In a paper published in 2020, she and her colleagues successfully used this approach to restore MeCP2 function in live mice with a specific mutation in *MECP2*.

Mandel sees so much potential in RNA editing that she cofounded VICO Therapeutics, a biotech startup centered around the technology. In 2018, the Rett Syndrome Research Trust awarded nearly $6 million in grants to researchers, including Mandel, pursuing RNA editing–based therapeutics for the condition. Still, Mandel's 2020 paper was only the second to report successfully employing RNA editing therapeutically in vivo, and there has yet to be a clinical trial to test the approach in humans. In that way, RNA editing-based therapeutics "haven't even passed the first hurdle yet," says Mandel, "but the technology is moving super fast."

In addition to VICO, there are at least seven companies worldwide with proprietary platforms for developing RNA editing–based therapies, although none have yet published preclinical data evaluating those platforms' efficacy in vivo. Many were already pursuing other nucleic acid therapies, and have recently added RNA editing to their portfolios. Wave Life Sciences, for instance, has developed several RNA-binding proteins, which regulate the translation of mRNAs, that are being tested in clinical trials, and Beam Therapeutics is known for its DNA base editing platforms; both Massachusetts-based companies are now also pursuing RNA editors.

Increasingly, investment firms and big pharmaceutical companies are taking note of the technology. In August, for instance, Seattle-based Shape Therapeutics signed a $3 billion deal with the pharmaceutical giant Roche, which Shape's vice president and head of research David Huss says is evidence that RNA editing is taking off.

"I think that industry and the larger biopharma companies are really starting to notice that this is an important wave of the future," he tells *The Scientist*.

Keay Nakae, a senior healthcare research analyst for the investment bank Chardan, agrees. "From the activity of these announced deals and financings and collaborations, you can see that the interest is heating up."

Safety first

Editing RNA isn't all that different from DNA base-editing techniques, which typically use Cas9 or other enzymes attached to a CRISPR guide RNA to replace one nucleotide with another. In the case of RNA editing, the enzymes being used in research are predominantly adenosine deaminases acting on RNA (ADARs), which have multiple functions in humans and many other animals, including ensuring that the cell's own RNA molecules, which can form double-stranded structures reminiscent of viral genomes, don't get destroyed by antiviral defenses on the lookout for foreign genetic material. To do this, ADARs flag double-stranded RNAs coming out of the nucleus by converting some of the adenosine (A) bases to inosines (Is), which are read by the cell's translation machinery as guanosines (Gs).

Researchers have capitalized on this enzyme activity to edit RNA. In the early 2010s, for example, RNA biologist Joshua Rosenthal's team, then at the University of Puerto Rico, and Thorsten Stafforst's group at the University of Tübingen in Germany independently combined the editing domain of ADAR with another protein that can attach to a guide RNA, which both provided an attachment point for the protein machinery and transformed the target sequence into a double-stranded RNA ready for ADAR editing (see graphic on next page). These engineered ADARs and their guide RNAs could be introduced into cells via viral vectors or other means.

Stafforst and Rosenthal published their work in late 2012 and 2013, respectively—just in time to be eclipsed by CRISPR, notes Rosenthal, now at the Marine Biological Laboratory in Massachusetts. "But then, it gradually started dawning on people that, well, if you can change information in DNA, you can change information in RNA as well. . . . So we started getting attention to the site-directed editing of RNA."

Despite the technologies' similarities, researchers involved in RNA editing say it holds numerous advantages over DNA editing. Unlike DNA, RNA molecules are transient, lasting only days to weeks in a cell. Even if off-target editing occurs, the edited information doesn't last forever, so any potential harm is limited in scope, says Rosenthal, a cofounder of RNA editing–based therapeutics company Korro Bio. "RNA editing [falls] somewhere between small molecules, which have a very short duration effect, and CRISPR, which has an almost permanent effect," he says. "You've got to screen carefully these things and look for off-targets—do your due diligence and animal testing and all that. But I think, taken all together in the balance, you'd have to say it's highly likely that it's much safer to make off-target edits in RNA than DNA."

> It gradually started dawning on people that, well, if you can change information in DNA, you can change information in RNA as well.
>
> —Joshua Rosenthal, Marine Biological Laboratory

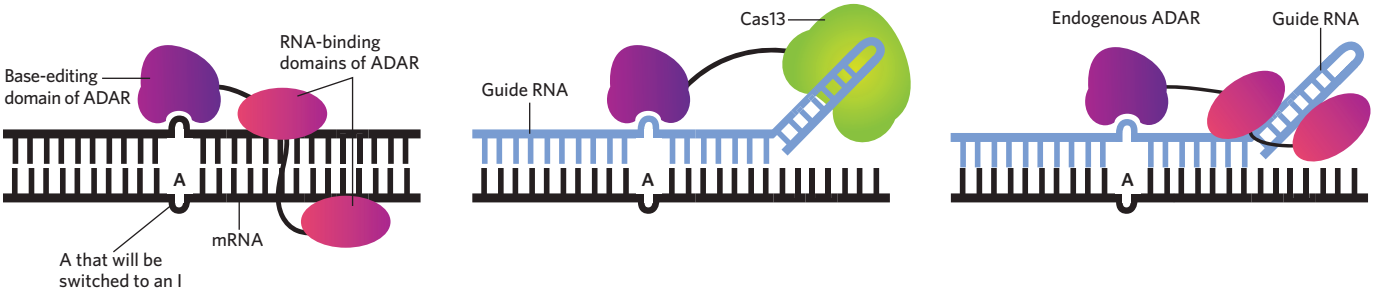
In addition to its safety advantages, RNA editing could lend itself to a wider variety of clinical applications than DNA editing, Huss says. With CRISPR, "every DNA change will be incorporated into 100 percent of the RNA transcripts that come off of that DNA." ADARs, on the other hand, typically only edit a fraction of those mRNAs. "You have certain diseases where you may want 100 percent of a change, but you have other ones where you may only want 50 percent, or 70 percent." One application could be altering mRNA to prevent translation or adjust the structure of tau protein, which helps stabilize microtubules in neurons but is also often associated with neurodegenerative diseases, Huss says. Altering every tau mRNA could cause pathologies, but altering some just might be beneficial.

Partial RNA editing could also prove sufficient for some diseases such as Hurler syndrome, a condition associated with severe skeletal abnormalities, cognitive impairments, and other health problems stemming from a lack of the activity of α-L-iduronidase, an enzyme that helps break down large sugars. About 40 percent of cases are caused by a G-to-A point mutation that creates an improper stop codon. In a recent mouse study, correcting just 30 percent to 40 percent of the mutant mRNAs led to a 60-fold increase in enzyme activity, a researcher with the company EdiGene reported in a 2020 conference presentation.

Bringing the tech up to speed

Efforts in the last few years have focused on increasing RNA editing's specificity and efficacy. For instance, Beam Therapeutics is developing the REPAIR™ (RNA Editing for Programmable A to I Replacement) system, which consists of a human ADAR enzyme attached to an inactivated Cas13 enzyme that binds to a guide RNA also introduced into the cell. Initial in vitro tests found that the combo enzyme made very few off-target edits. In a 2020 paper, researchers reported that the REPAIR™ system corrected a mutation that causes cystic fibrosis—specifically, a premature stop codon in the gene coding for cystic fibrosis transmembrane conductance regulator protein. The editing system was able to restore production of full-length protein in multiple cell lines, though the authors noted that the efficiency was low, so further refinements are needed.

RNA EDITING APPROACHES: Endogenous adenosine enzymes acting on RNA (ADARs) edit genetic material in the cell by attaching to naturally occurring double-stranded RNAs, including mRNAs, and switching out A bases with I bases (left). Therapeutic RNA editing platforms based on this mechanism fall into one of two categories: either they use engineered enzymes, which generally consist of the editing part of the ADAR enzyme attached to another protein such as Cas13 that boosts specificity, alongside a guide RNA that targets the enzyme to the desired location (middle); or they consist of a guide RNA alone, which recruits an endogenous ADAR to edit the target sequence (right).



Other companies, such as San Diego–based Locanabio, are also coupling Cas enzymes with ADAR to improve targeting. But there could be disadvantages to using Cas and other bacterially derived proteins, according to Erez Levanon, a computational biologist with Bar-Ilan University in Israel and former consultant for the San Diego company ADARx Pharmaceuticals, which is developing ADAR-based RNA-targeting therapies. Compared to ADARs, which aren't foreign to human cells, Cas enzymes are more likely to set off immune reactions, he says.

Some groups are tweaking ADARs directly to get the same specificity benefit without using Cas. University of California, San Francisco, biologist Leanna Monteleone and her colleagues, for instance, have developed a unique ADAR that only edits As when there's no base attached to the ribose backbone across from them in double-stranded RNA—a situation that is extremely rare in naturally occurring RNA, but that can be engineered into a guide RNA. In vitro testing showed that this so-called "bump-hole" strategy led to fewer off-target edits, says Monteleone, who has applied for a patent on the technology.

Other teams are exploring the possibility of using a person's own ADAR enzymes, rather than delivering them alongside a guide RNA. "You can deliver only a guide RNA molecule, and recruit the endogenous wild-type ADAR that's already present inside the cell, and do that at very high efficiency and specificity," says Huss—something first demonstrated back in 1995, and something that a few companies, including Shape, are exploring. "That kind of approach is beautiful," Rosenthal says. "In therapeutics, the simpler the system, the better, in general." Nakae agrees, adding that the elegance of a system that uses a guide RNA alone to recruit ADARs already present in cells is "one of the reasons why ADAR, I think, is attracting attention."

Strides are being made on generating guide RNAs, too. Shape Therapeutics, for example, has invested heavily in designing guides, making use of machine-learning techniques and high-throughput screening, says Huss. Such approaches could open up the possibility of editing "any adenosine in the transcriptome"—and with it, the ability to manipulate proteins in novel ways, he adds. Altering A residues involved in splicing reactions, for instance, could promote exon skipping or alternative splicing. RNA editing could also tamp down protein production by converting AUG start codons to GUGs, blocking translation initiation. It may even become trivial to tweak proteins at will—to add or remove a phosphorylation site, for instance, or alter protein cleavage points.

> Now that biotech companies are involved . . . I'm hopeful that things are going to accelerate with RNA editing quickly.
>
> —Gail Mandel, Oregon Health & Science University

Experts in the field are quick to note that the utility of RNA editing extends far beyond the genetic diseases that have been the focus of biotech interest until now. "The much more nuanced and I think powerful approaches to this down the line are going to be really manipulating systems," Rosenthal says. His group is looking into the possibility of editing neuronal RNAs to temporarily dull pain, for instance, as an alternative to opiates.

Huss says that one of the big challenges in the near term will be not putting the cart before the horse. "I think

THE SCIENTIST STAFF

the challenge for us is: there's all these possibilities—how do we focus in on the things that we think will give us the highest probability of success and also prove the technology before we expand it out into many different indications?"

Much to prove

It remains to be seen whether the tech can make good on its potential. Levanon says he expects that, among successes, "there will be many disappointments" in the coming years, especially when it comes to designing efficient guide RNAs. He notes that the bases near the target site on an mRNA, as well as the structure of the guide RNA itself, can tweak the efficiency of ADAR activity, and says that researchers will need to understand more about why natural ADARs edit when and where they do before they can truly manipulate them with precision.

There's also the obvious limitation that ADARs only edit As into Is. That's one of twelve possible manipulations one might want to make, notes Levanon; if another base is desired in a given spot, ADARs can't help. There are enzymes that edit cytosines (Cs) into uracils (Us)—the RNA version of thymine (T)—and these are now being explored, but that technology is years behind, and as of yet, enzymes that make other conversions either aren't known or aren't well characterized. While turning an A into an I (read as a G) may be helpful in many cases—in more than half of Rett Syndrome cases, for instance, Mandel estimates—that's not going to be the case for every genetic disease.

With research at such an early stage, the challenge of drug delivery still looms. "It's that next step of: Can I deliver the payload to the target tissue? Can I deliver it at a high enough concentration? And can I deliver it so that you have a sustained expression?" that may slow progress toward the clinic, says Huss.

At least in terms of regulatory hurdles on the road to market, "a lot of that ground has already been plowed" by other RNA-based technologies, Nakae says. "I think it'll actually be an easier path [to approval] than what some of those other modalities faced. But that said, they'll still have to have the preclinical data that shows that it's safe before it's allowed to go forward." Among companies working on the technology, Nakae says, there are no obvious frontrunners yet.

Nevertheless, "now that biotech companies are involved . . . I'm hopeful that things are going to accelerate with RNA editing quickly," says Mandel. And she has reason to hope; in May 2021, the Rett Syndrome Research Trust's press office wrote that RNA editing therapeutics funded by the trust are expected to hit clinical trials by the end of 2024. "It's certainly an exciting time," she says. ■

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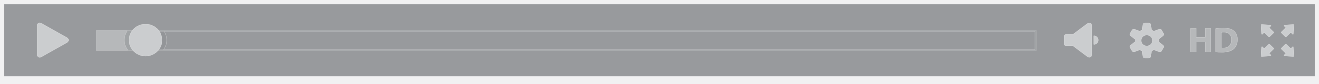

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- Persistent inflammatory signatures in long COVID

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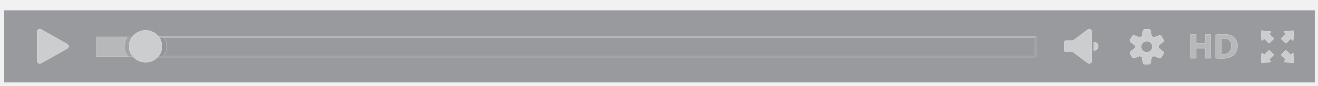
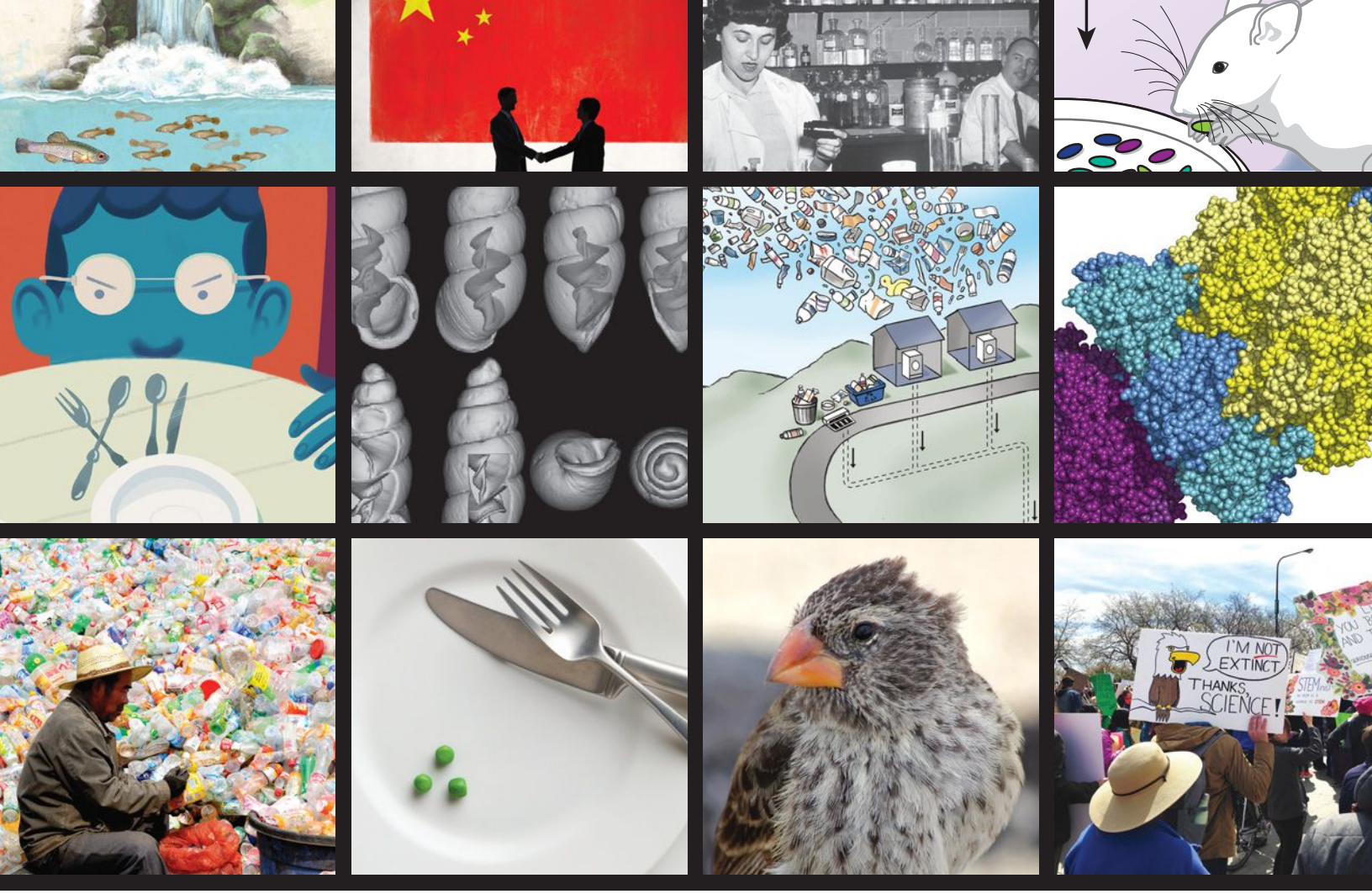
JÖRG BANTIN, PHD
Regional Specialist, ddPCR
Bio-Rad, Germany

EDDY VAN COLLENBURG
Market Development Specialist, ddPCR
Bio-Rad, Netherlands





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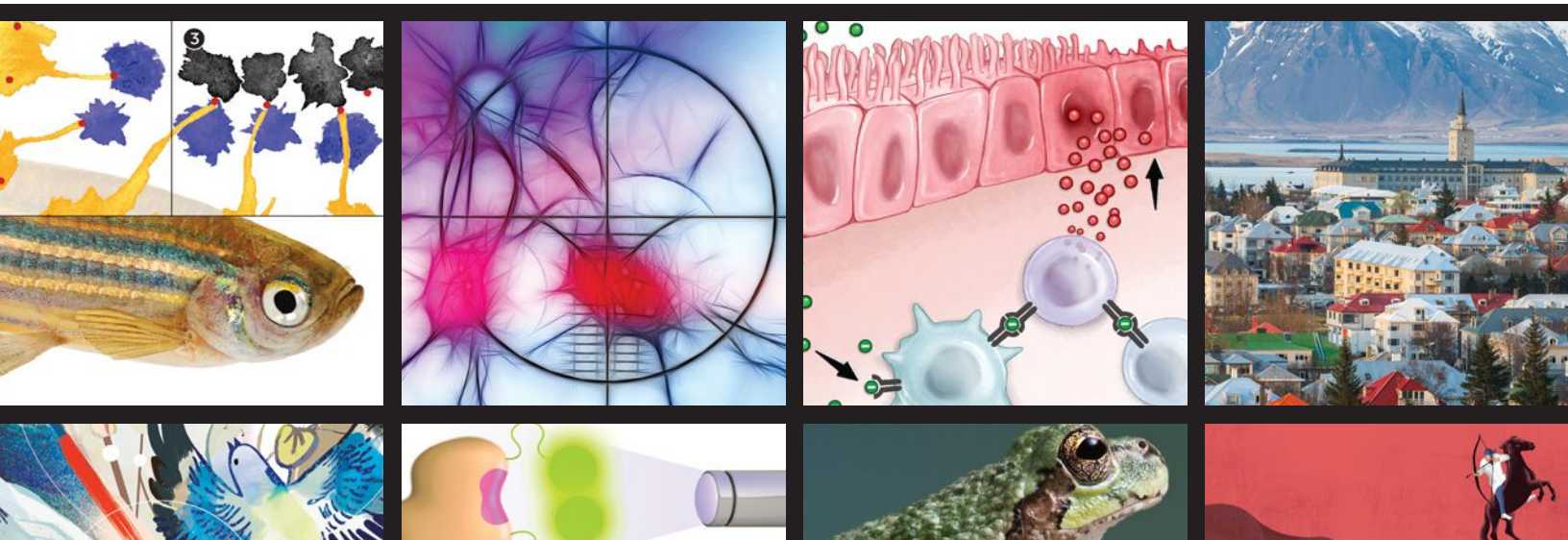

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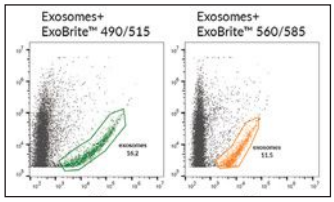

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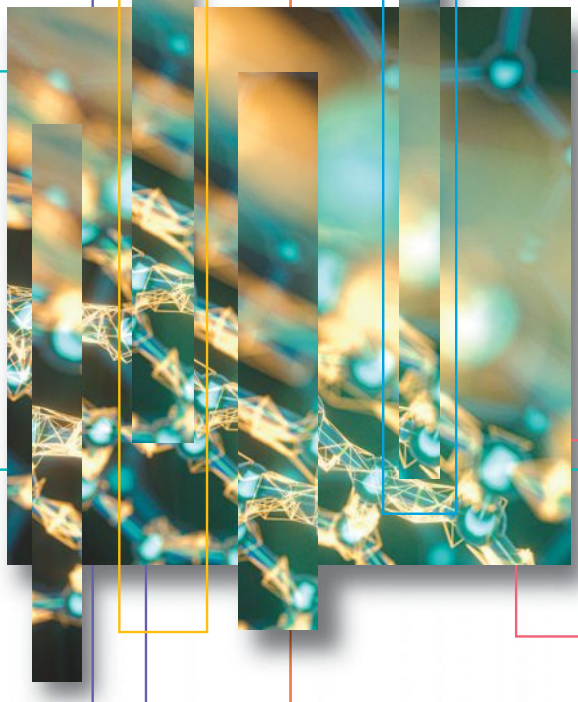

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Bridging the Intellectual Divide

Why society needs to break down the barriers between scientific and humanistic thinking

BY MARCELO GLEISER

In a 1959 lecture, the British physicist and novelist C. P. Snow admonished his Cambridge colleagues that "the intellectual life of the whole of western society is increasingly being split into two polar groups." Snow was referring to the divide between scholars of science and the humanities, complaining that "literary intellectuals" and "physical scientists" didn't understand or respect each other. Fast-forward 62 years and the situation has only gotten worse, aggravated by further entrenchment, a decrease in the number of students majoring in the humanities, and a series of global challenges that call for a creative confluence of different types of knowledge.

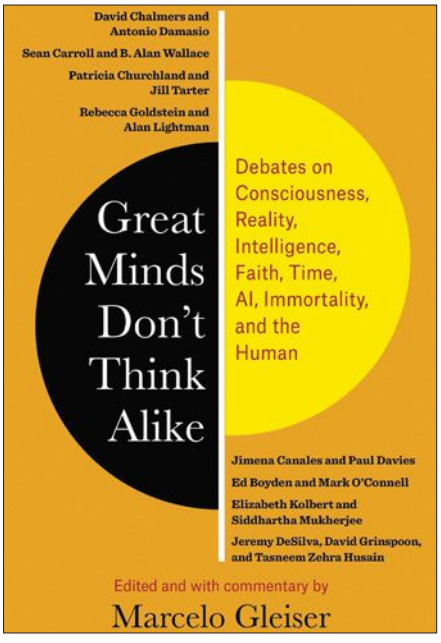
In my new book, *Great Minds Don't Think Alike*, I document conversations I had with leading thinkers who bridge the gap between the sciences and the humanities.

The meteoric growth of many fields of scientific knowledge in the past three centuries has led to a model of niche-knowledge. In the sciences, we are trained to be technically adept in a specific subfield; those who dare to cross fields before tenure are usually punished (read: refused tenure). The same hyper-specialization model has percolated through the humanities. Just as typical laser physicists don't understand astrophysicists, scholars of Classics don't research Edmund Husserl or Jorge Luis Borges. There are, of course, exceptions, but in both arenas, they are rare. While this focus is required for success in academia, it clashes with the intellectual openness necessary to learn from other fields of inquiry.

This entrenchment of knowledge influences our worldview and the way we teach. Snow's lament was meant as a wake-up call, an invitation, as yet unheeded, to promote intellectual openness and curiosity. To want to learn from another person, even one with interests far removed from your field of research or with different political or cultural viewpoints, is essential if we are to face the daunting challenges that threaten civilization. And for this to happen, the sciences and the humanities must be open to each other.

Thankfully, the barriers that separate science and the humanities are crumbling. Essential questions, once mostly the province of the humanities, are now part of scientific research. Conversely, science and its uses cannot be separated from moral choices. There is light and there is shadow in every new technology. The nature of free will, the nature of reality, the nature of consciousness, the future of humanity in an increasingly technological world, our future in space, our cosmic loneliness, the limits of scientific knowledge—such issues and many others cross disciplinary boundaries. To look at any of them from a one-sided perspective—either scientific or humanistic—is like looking through a window with the blinds down. With such questions at the forefront, we have the unprecedented opportunity to bring the sciences and the humanities into constructive engagement, and to reposition them as complementary and interdependent facets of human knowledge.

For example, with CRISPR and other genetic engineering technologies, we are now at the threshold of being able to modify the human genome in ways that benefit or vex future generations. Jennifer Doudna, who shared the 2020 Chemistry Nobel Prize with Emmanuelle Charpentier for the discovery of CRISPR, has stated that she's grown increasingly uneasy with the potential ethical repercussions of genome editing, citing questions of access, eugenics, privilege, and the difficulty in global regulation. Add to this the fast-growing capabilities of machine-learning and other technologies, and we see how pushing forward a scientific agenda based mostly on commercial interests and absent any humanistic consideration can turn promising technologies into existential risks. Unadvised utopian scenarios of science as a cure for all evils can quickly turn dystopian.



Columbia University Press, February 2022

But this is the world we live in, the world that future generations will inherit. *Great Minds Don't Think Alike* is a collection of conversations in which I had the privilege of unpacking some of these thorny, modern issues with leading scientists and humanists from various fields. They were part of a larger experiment, the Institute for Cross-Disciplinary Engagement at Dartmouth, created to bring down the cross-disciplinary barriers between practitioners of science and the humanities, through conversations, fellowships, and workshops. To my surprise, we witnessed great support from our guests and diverse audiences for this recalibration. Let us give voice to a need for new avenues of communication and bring down the walls that stop us from learning openly from one another. ■

Marcelo Gleiser is the Appleton Professor of Natural Philosophy at Dartmouth College. He is also the 2019 Templeton Prize Laureate.

Presidential Pox, 1863

BY ANNIE MELCHOR

Seven score and 18 years ago, Abraham Lincoln delivered a brief but consequential speech in Gettysburg, Pennsylvania, the site of the bloodiest battlefield of the American Civil War, where thousands of soldiers had died.

Lincoln was known for his general air of melancholy and bouts of severe depression, but the night after his November 19, 1863 address, he was plagued by something more. According to contemporary accounts, the president's weakness and dizziness from the day before had worsened into a high fever and a severe headache. A few days later, he developed a rash all over his body, followed by blisters. Although the diagnosis was a mild case of smallpox—suggesting he had preexisting immunity—Lincoln was ordered to quarantine and didn't resume official duties for almost a month. A more recent analysis suggests Lincoln's case may have been more severe, and some researchers speculate that his doctor may have intentionally softened the diagnosis to avoid stirring panic in the war-torn nation.

Lincoln survived, of course, and seemed to make a full recovery before his assassination less than two years later. His valet, however, died of smallpox shortly after the president's recovery. William Johnson, a free Black man who had accompanied the president to Gettysburg, was most likely the one caring for Lincoln, and experts think Johnson probably caught the virus from the president. Lincoln paid off Johnson's debts and had him buried at Arlington National Cemetery.

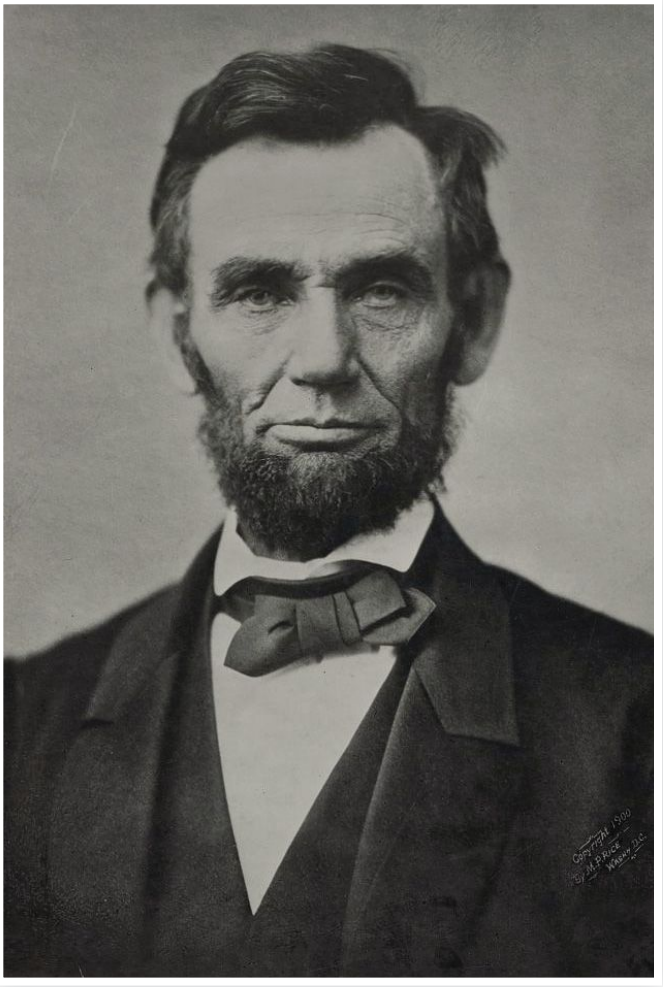
No one alive today knows if Lincoln had been immunized against smallpox. In 1796, Edward Jenner showed that vaccination with cowpox also protected against smallpox, but a standardized smallpox vaccine didn't exist in Lincoln's time, says University of Rhode Island medical historian Andrea Rusnock. Rather, immunity was often passed along "through [the] arm-to-arm vaccination of children," she says.

Healthcare workers would make a small incision in a child's arm to introduce scabs or fluid drained from smallpox pustules from an immunized child. Repeating that process—which caused pustules in the newly immunized child but not full-blown smallpox—kept the vaccine strain alive in a community. But without organized infrastructure to track immunizations and to continuously harvest the virus from newly inoculated children, the vaccine strain could peter out—and often did, says Rusnock, leaving the community vulnerable unless they got samples elsewhere, often through the mail.

Additionally, routine smallpox vaccination was uncommon outside of large cities, she says. Growing up in the rural town of Springfield, Illinois, Lincoln probably wouldn't have been vaccinated as a child unless there had been a major outbreak.

While quarantining and encouraging patients to get fresh air reduced deaths and spread, the mortality rate for the unvaccinated was still roughly 30 percent. According to Rusnock, smallpox was "an equal opportunity disease," killing prince and pauper alike, and she adds that crowded wartime conditions and disrupted supply chains likely contributed to additional outbreaks.

"It's important to remember that smallpox was incredibly frightening, because one out of three people [wasn't] going to survive," says Rusnock. "For Lincoln to have smallpox and then recover—it's such a precarious moment in our nation's history." ■



A DORMANT DISEASE: Abraham Lincoln sat for this portrait by Alexander Gardner on November 8, 1863, less than two weeks before he gave his famous Gettysburg Address. Shortly after, the president was diagnosed with smallpox. Because the incubation period for the disease is between 10-14 days, Lincoln could have conceivably been infected at the time the photo was taken.

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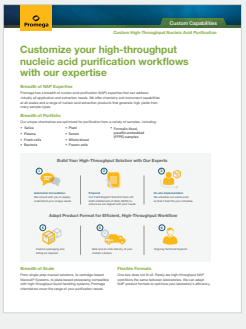

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