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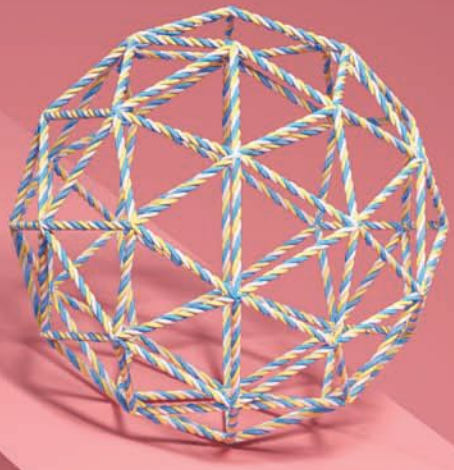

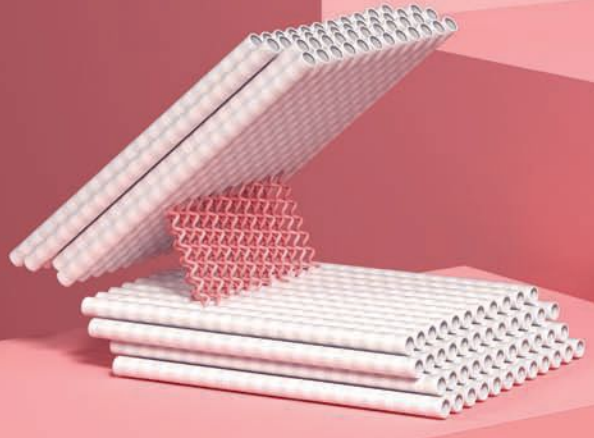

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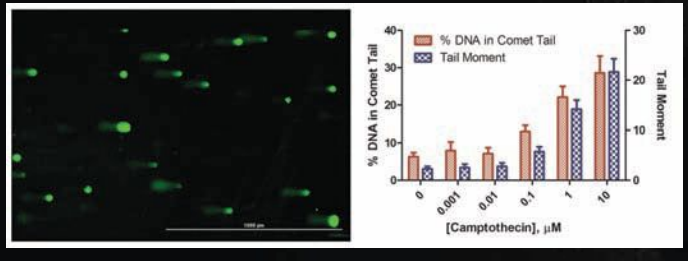

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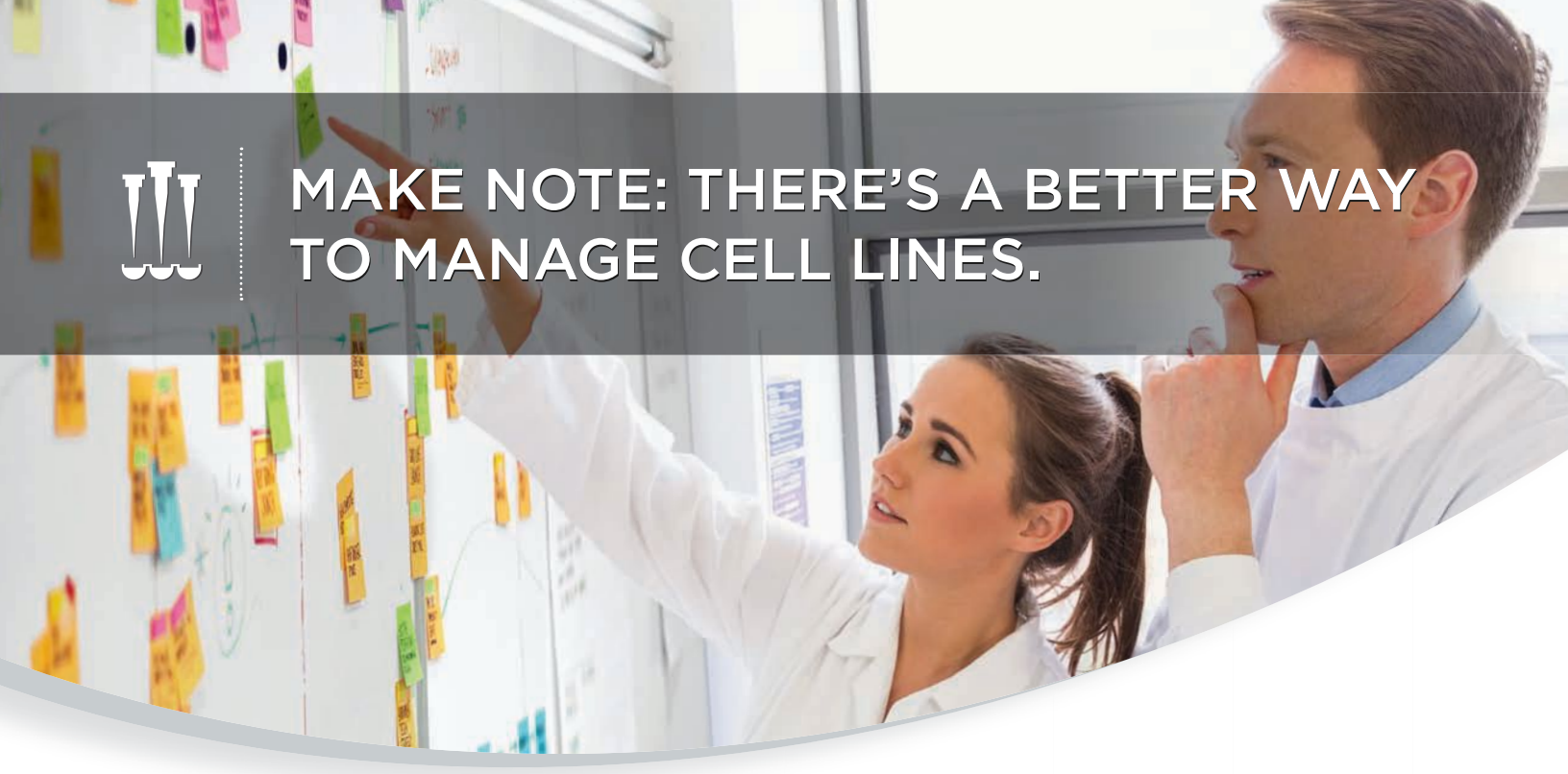
2017

TheScientist TOP 10 INNOVATIONS



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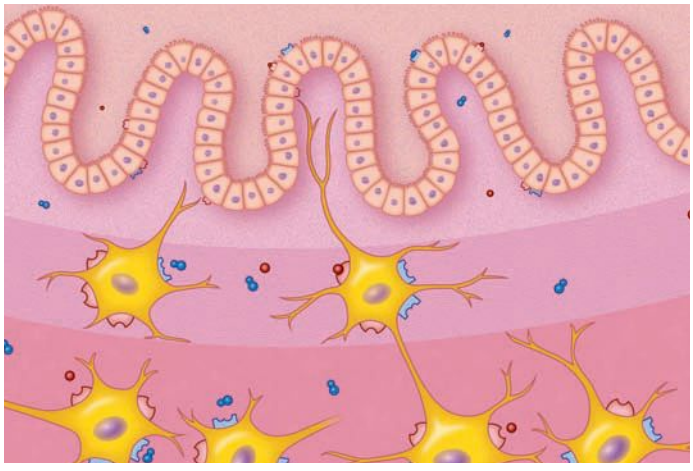

Features

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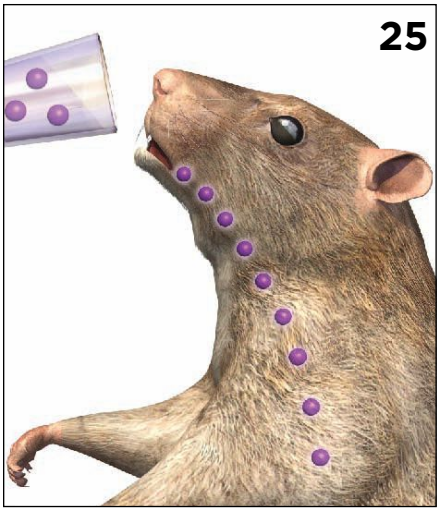

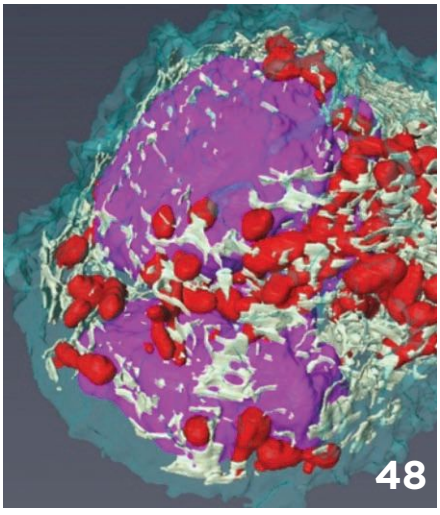

CORRECTIONS:
In "The Sports Bug" (*The Scientist*, June 2017), the accompanying photograph was incorrectly credited. The correct credit is Andrew Santoro.

The Scientist regrets the error.

Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Microbe Maven
Meet Scientist to Watch Emily Balskus, who studies the microbes that inhabit humans at Harvard University.

VIDEO
Lubchenco on Conservation
Marine ecologist Jane Lubchenco, a former NOAA administrator, discusses the importance of science in dealing with climate change.

VIDEO
DNA Origami
Will complex, folded synthetic DNA molecules one day serve as capsules to deliver drugs to cancer cells?

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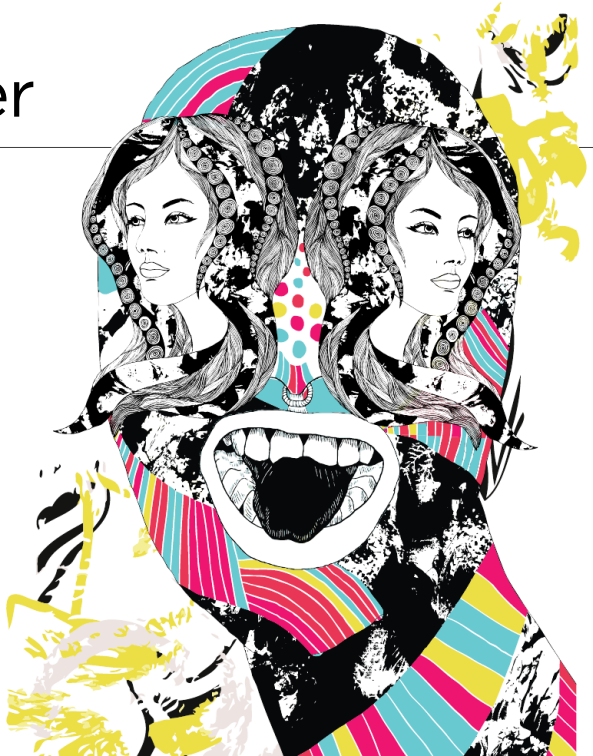
HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- The neurological effects of psychedelics
- Is there a connection between Alzheimer's and infection?
- Techniques for culturing stem cells
- The PARP inhibitor market
- Profile: Ronald Vale, UC San Francisco

AND MUCH MORE



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AMERICAN SOCIETY OF BUSINESS PUBLICATION EDITORS (AZBEE) 2017 • **March 2016 issue**—Print, Single Topic Coverage by a Team—National Gold and Northeast Regional Gold • **Modus Operandi**—Print, Regular Department—Northeast Regional Bronze • **Magazine of the Year,** More Than $3 Million Revenue—Honorable Mention

FOLIO AWARD S 2016 • **March 2016 issue**—Winner B-to-B Full Issue • **B-to-B News Coverage**—Honorable Mention

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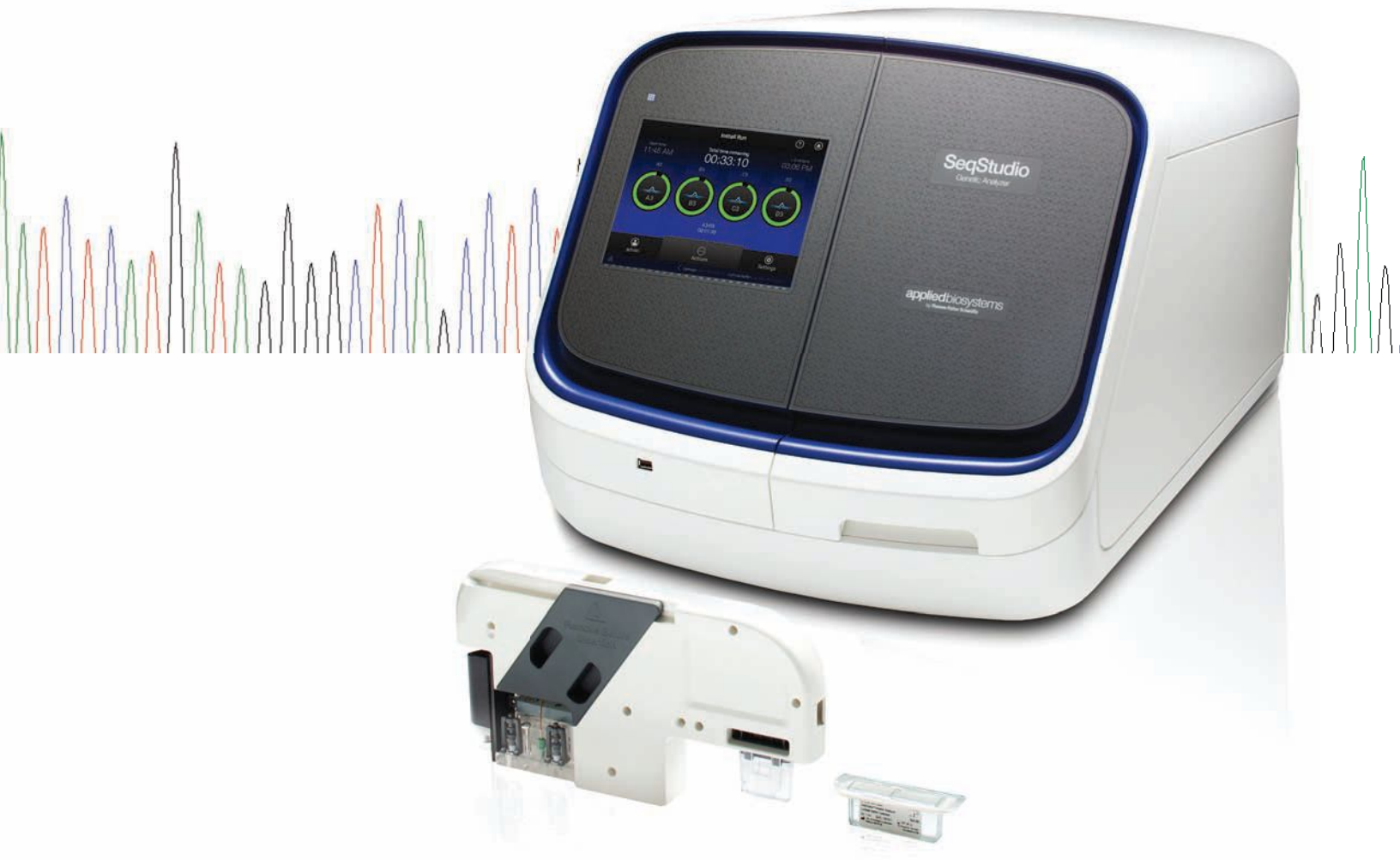

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Contributors

Arun Richard Chandrasekaran liked animals as a child growing up in India, where he earned an undergraduate degree in zoology from the American College in Madurai. After realizing his career options were limited to teaching, Chandrasekaran pivoted to nanoscience. "It was something new and different, and I just wanted to try it," he says. He studied three-dimensional DNA crystal structures for his master's degree at the University of Madras in Chennai. Chandrasekaran then moved to the U.S. to do a PhD in chemistry at New York University. There, he worked with DNA motifs that self-assemble into three-dimensional DNA crystals in the lab of Ned Seeman, who Chandrasekaran refers to as the father of DNA nanotechnology. Later, during a postdoc at the University at Albany, he worked on developing an assay for nucleic acid detection with Ken Halvorsen. Chandrasekaran now lives in Boston, where he's a senior scientist at Confer Health. On shifting fields and leaving academia for a startup, Chandrasekaran says his career diversity has kept him going. "Science is cool, and I do it for the fun," he says. In this issue, he reviews the design and building of nanoscale architectures using DNA (page 26).

As a child, **Emily Monosson** says she was always covered in sap. Her father would wash it off with gasoline, and she would resume climbing trees or cavorting in the field next door. "I loved to be outside," she says. Monosson would also play chemist with "all the nasty stuff from under the sink," a childhood foray into chemistry that eventually led to her passion for toxicology. "I sort of stumbled upon it," she says of her career. After completing an undergraduate degree in biology at Union College in New York, Monosson began a microbiology PhD program at Cornell University, though she was not set on the field. Quickly realizing her zest for microbiology was tepid at best, she switched her focus to toxicology after taking one class. "I was hooked, and realized that was what I wanted to do." Tapping into her love for the outdoors, she eventually settled on environmental toxicology. Her writing career, which is now her main focus, began after she had gotten her doctorate and become a mother, during a self-described career trough. Monosson edited and wrote essays for *Motherhood, the Elephant in the Laboratory: Women Scientists Speak Out*, and eventually wrote her first book, *Evolution in a Toxic World*, which quickly inspired her second, *Unnatural Selection: How We Are Changing Life, Gene by Gene*. Her most recent book, *Natural Defense*, centers on maintaining human and agricultural health while reducing dependence on traditional antibiotics and pesticides. Read her essay based the new book on page 63.

Aggie Mika never really considered a career in science until she was in college at Arizona State University. When her dad began having some health issues, she was faced with the language barrier posed by medical jargon. "I didn't understand anything," she says. "I took it upon myself to do a lot of research, and then really fell in love with medicine and biology." Mika switched her major from journalism to psychology/premed, thinking she'd become a doctor. But upon joining the behavioral neuroscience lab of ASU's Cheryl Conrad, she again switched gears—this time to focus on the research itself. In Conrad's lab, Mika studied how psychological stress can change the physical structure of hippocampal neurons. "I became obsessed with that," she says, and after obtaining her undergrad degree, Mika began a graduate program in a stress physiology lab at the University of Colorado, Boulder. Having focused entirely on neuroscience in college, however, she had to take a step back and teach herself some of the basics of human anatomy and molecular biology—an experience that whet her whistle for science communication. She started a blog with some fellow graduate students, and upon meeting a science writer at a seminar, realized she could focus her career on just the communication part of science. As *The Scientist*'s current intern, Mika has written about all things life science for both the print and online sides of the publication, and last month covered the American Society for Microbiology meeting in New Orleans. In this issue, check out her stories on fish migration (page 20), the evolution of bioluminescence in marine organisms (page 49), and new techniques for defining immune cell subtypes (page 49).

SWETA VANGAVETI; ROBERT SCHERZER; DANIEL SWANSON

Twists and Turns

New starring roles for nucleic acids

BY MARY BETH ABERLIN

Watching Broadway celebrate itself several weeks ago at the Tony Awards ceremony left me idly asking myself: If you were a theater critic awarding a Tony for lifetime achievement as a macromolecule and the nominees were DNA, RNA, and protein, which would you vote for?

For decades (more like centuries), proteins reaped the lion's share of awards. Then, in the middle of the 20th century, DNA stole the limelight. Biological impresarios Watson and Crick introduced its structure with a tongue-in-cheek understatement in their seminal 1953 *Nature* paper: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." That base pairing blew directors away at auditions, and the elegant and functional helical design brought down the house whenever DNA made an appearance on stage.

If you were a theater critic awarding a Tony for lifetime achievement as a macromolecule and the nominees were DNA, RNA, and protein, which would you vote for?

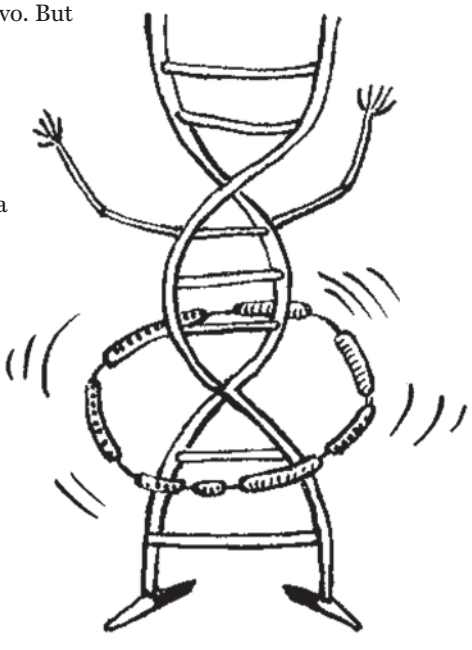
Putting that elegant structure to other uses has not escaped the notice of researchers who want to construct unique DNA edifices that have nothing to do with passing on genes. "Building with DNA," our cover story by Arun Richard Chandrasekaran (page 26), describes how three-dimensional nanostructures can be built from short segments of the inherently linear nucleic acid, taking advantage of both the stiffness of molecules with 15 or fewer helical pitches and the stickiness that can be engineered into the ends of DNA segments. What is so remarkable about these features is that exact links and nodes can be configured so that precise mixtures self-assemble to form desired shapes.

In the latest twist on designing DNA nanostructures, dubbed DNA origami, a long strand of specially designed single-stranded DNA is bent into its final form by adding short complementary oligonucleotide strands to precisely fold a long strand of nucleic acid. DNA architects are working on origami designs that orient enzymes in positions that are bioactive, sense changes in a cell's environment, detect single nucleotide polymorphisms, or deliver drugs directly to the therapeutic site. Definitely Tony-contender material.

But then there is RNA to consider. Originally, its various forms (mRNA, tRNA, and ribosomal RNA) seemed more or less relegated to ensemble roles involved in the choreography of protein production. But RNA has also played starring roles in the origin of life: various forms of the nucleic acid took center stage long before DNA arrived on the scene. Lately, though, it's making a stellar career comeback: genome sequencers and molecular biology sleuths seem to announce new RNA types and vital roles for them on a remarkably frequent basis. (See "The RNA Age: A Primer," *The Scientist,* May 11, 2017.)

Circular RNA is the latest form to set tongues wagging, as Catherine Offord reports in "Round and Riveting" (page 40). First considered mistakes or "noise," circRNAs are now suspected to be important cellular components. They consist of one or more protein-coding exons (and sometimes the odd intron) that form a circle. It turns out cells contain lots and lots of them, and some are even translated into proteins in vivo. But like any ingénue, circRNAs are still cloaked in mystery. One researcher interviewed says, "We're really just at the beginning of an exciting journey. It doesn't happen often in molecular biology that you find such a fundamentally new phenomenon."

Although they appear in every issue, DNA, RNA, and proteins elicit bravos in this July/August issue of *The Scientist.* And because they all play such interesting roles, I'd be a really lousy Tony judge . . . ■



ANDRZEJ KRAUZE

Editor-in-Chief
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INSIGHTS AMPLIFIED

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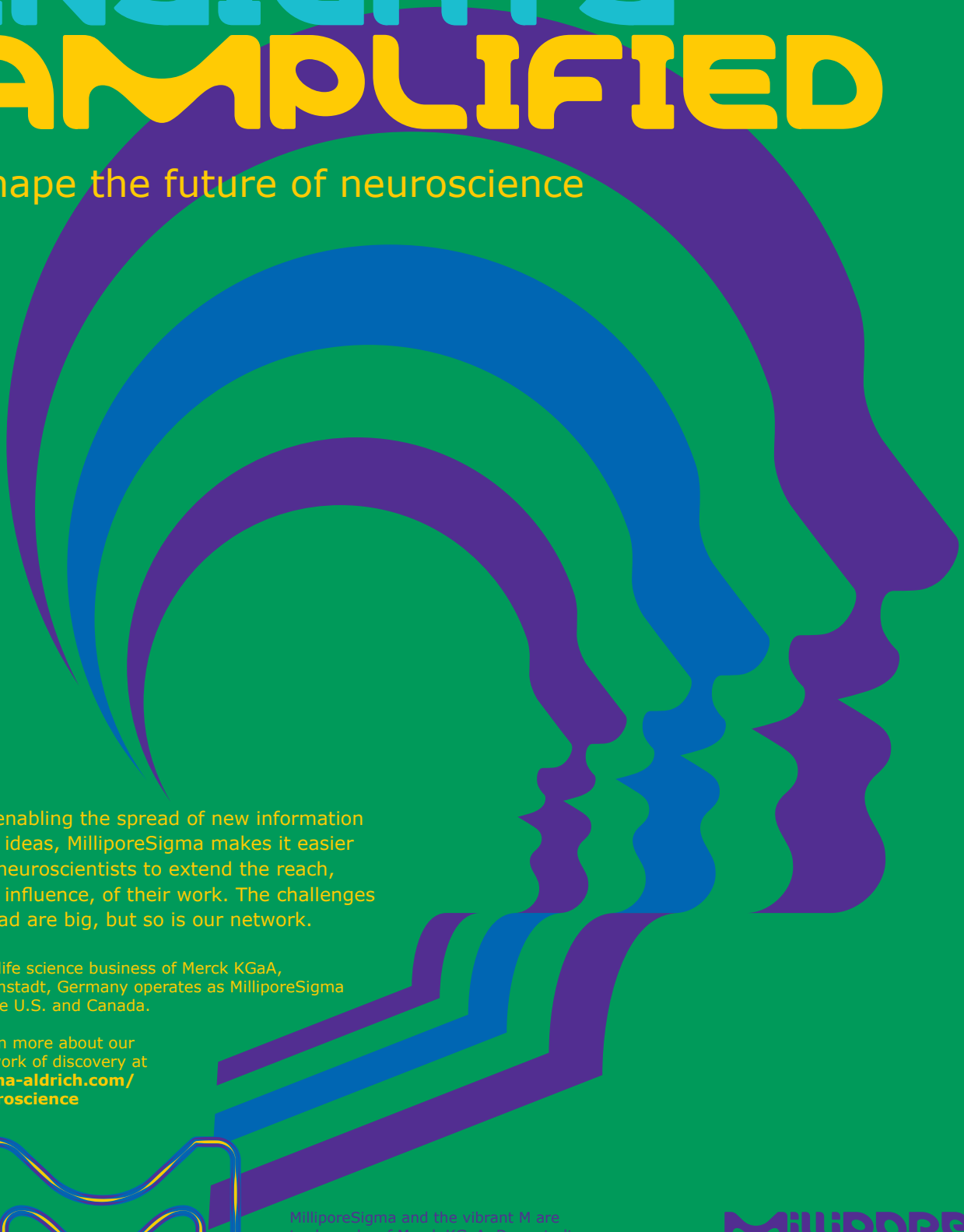

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Speaking of Science

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—Nobel laureate **Sydney Brenner**, quoted in *A Passion for Science* (1988)

Given the exploratory and, hence, unpredictable nature of fundamental discovery, basic science is generally not supported in the private sector—but it provides the critical foundation for advances in disease diagnosis, treatment, and prevention through future clinical applications.

—**Francis Collins**, Director of the National Institutes of Health, in testimony delivered before the Senate Appropriations Subcommittee on Labor, HHS, Education, and Related Agencies regarding the FY 2018 NIH budget (June 22)

A cut to NIH is not a cut to Washington bureaucracy—it is a cut to life-saving treatments and cures, affecting research performed all across the country.

—Senator **Roy Blunt** (R-MO), chairman of the Senate Appropriations Subcommittee on Labor, HHS, Education, and Related Agencies (June 22)

This idea that [the] science is just absolutely settled, and if you don't believe it's settled, then you're somehow or another a Neanderthal—that is so inappropriate, from my perspective. I think if you're going to be a wise, intellectually engaged person, being a skeptic about some of these issues is quite all right.

—Energy Secretary **Rick Perry**, on CNBC's *Squawk Box* talking about skepticism concerning the degree to which human have contributed climate change (June 19)

So far, the things that have shaped society—what we measure ourselves by—have been either religious rules about how to live a good life, or more earthly goals like getting rid of sickness, hunger, and war. . . . What would the world be like if we actually achieved those things?

—Technology magnate **Bill Gates,** in a review of *Homo Deus* by Yuval Noah Harari (*Gates Notes*, May 22)

We have known for a while about the negative consequences of advanced paternal age, but now we have shown that these children may also go on to have better educational and career prospects.

—**Magdalena Janecka**, postdoctoral fellow at Mount Sinai School of Medicine, who recently coauthored a study that found older fathers are more likely to have "geekier" sons who have higher IQs than those born to younger fathers (*The Guardian*, June 20)

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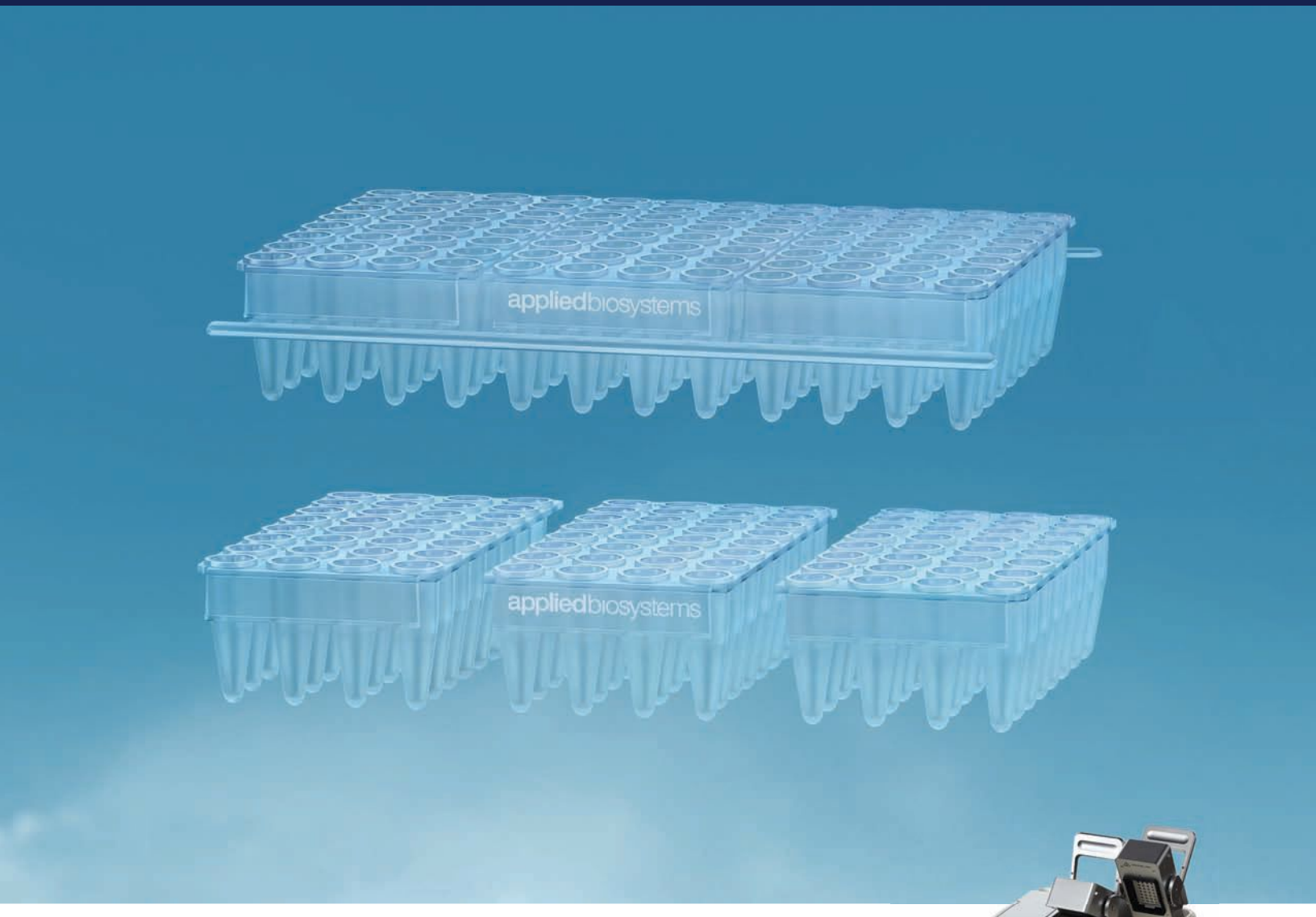

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Notebook

JULY/AUGUST 2017

Following Instincts

A mother rat's care for her pup reaches all the way into her offspring's DNA. A young rat that gets licked and groomed a lot early on in life exhibits diminished responses to stress thanks to epigenetic changes in the hippocampus, a brain region that helps transform emotional information into memory. Specifically, maternal solicitude reduces DNA methylation and changes the structure of DNA-packaging proteins, triggering an uptick in the recycling of the neurotransmitter serotonin and the upregulation of the glucocorticoid receptor. These changes make the nurtured rat's brain quicker to sense and tamp down the production of stress hormones in response to jarring experiences such as unexpected sound and light. That pup will likely grow into a calm adult, and two studies have shown that female rats who exhibit a dampened stress response are more likely to generously lick, groom, and nurse their own young.

Caring for pups is one example of what casual observers of behavior might call an animal's instinct—generally considered to be an innate, genetically encoded phenomenon. But could such epigenetic changes, when encoded as ancestral learning, also be at the root of maternal care and other seemingly instinctual behaviors we see across the animal kingdom?

WAGGLING THROUGH THE AGES: Are seemingly innate behaviors, such as the dance honeybees perform to direct hivemates to pollen sources, the result of epigenetic mechanisms informed by ancestral learning?

It's very difficult for us to come to terms with just how much of our behavior is set in stone.

—Lars Chittka
Queen Mary University of London

"We don't have a general theory for the mechanics of instinct as we do for learning, and this is something that has troubled me for a very long time," says University of Illinois entomologist Gene Robinson.

He studies social evolution in the Western honey bee and recently coauthored a perspective piece in *Science* together with neurobiologist Andrew Barron of Macquarie University in Sydney, Australia, suggesting methylation as a possible mechanism for the transgenerational transmission of instinctual behavior, rather than those behaviors being hardwired in the genome (356:26-27, 2017). Robinson and Barron suggest that instinctual traits, such as honey bees' well-known waggle dance or a bird's in-born ability to sing its species' songs, are the result of traits first learned by their ancestors and inherited across generations by the process of methylation. This differs from classical thoughts on animal learning, which say that if a behavior is learned, it is not innate, and will not be inherited.

Today, a fuzzy dichotomy exists in behavioral science circles, and instinct has become "the fixed and simple component of behavior."

—Andrew Barron, Macquarie University

Researchers first discovered methylation in the mid-20th century, and scientists now understand the epigenetic mechanism to be a key mediator of gene regulation during development. What exactly spurs changes in methylation, however, is unclear. As researchers seek to better understand this process, some animal behaviorists see it as just the mechanism that could answer long-standing questions in their field about the evolutionary relationship between instinct and classical learning.

Thanks in large part to the work of Columbia University neurobiologist Eric Kandel, animal behaviorists are steeped in the molecular mechanisms that underlie learning. "But we haven't discovered the mechanism for how instinct gets passed on," says Barron.

Starting in the 1930s, renowned psychologist B.F. Skinner, then a Harvard grad student, was influenced by Ivan Pavlov's now-famous notion of operant conditioning. As the founder of the school of psychological theory known as radical behaviorism, Skinner went on to define learning as a product of positive or negative reinforcement. He was sure that all animal behavior arose from learning. By the late 1960s, zoologist Jack Hailman argued that instincts do exist, but they are coupled with some learned elements. Today, a fuzzy dichotomy exists in behavioral science circles, and instinct has become "the fixed and simple component of behavior," says Barron.

Recent research has supported the idea that instinct might be deeply rooted in what are often considered learned behaviors. Another aspect of the serotonin-triggered epigenetic response in young rats to good mothering is changes in chromatin structure leading to higher expression of genes known to be linked to brain cell growth. The resulting increase in neural plasticity may be devoted, at least in part, to enshrining similarly nurturing behaviors in an offspring's behavioral repertoire.

These studies do suggest that an organism's experience can lead to changes in behavior that have a subsequent effect on their offspring, but tracking the further passage of inheritance over multiple generations is harder to tease out.

Robinson and Barron contend that natural selection can act on the epigenetic mechanisms, just as it does genetic traits. "At its core, this is still very Darwinian," says Barron.

Beloit College biologist Ken Yasukawa thinks that the concept of instinct resulting from epigenetically encoded learning in ancestors is not all that revolutionary. "I don't think this paper is as controversial as the authors think it is," he says. University of Nevada biologist Vladimir Pravosudov doesn't find much novelty in Robinson's and Barron's conclusions. "The main point is that these ideas were proposed a long time ago," he says, "like more than 100 years."

Pravosudov is referring to the late 1800s, when psychologist James Baldwin coined the term "organic selection." Within a population, this so-called Baldwin effect suggests that at least some individual variation exists in the ability to learn, giving certain individuals an adaptive advantage to access resources that others have not yet been able to exploit. This enhanced ability to learn about a certain feature in the environment could, theoretically, become instinctual by being cemented epigenetically over multiple generations, says Pravosudov. "It's actually shocking to me that they don't reference Baldwin because it's basically exactly what their article is about."

Even though he did not cite Baldwin, Barron freely acknowledges that what he and Robinson propose borrows from his ideas. "The history of our argument for learning and its interplay with evolution is very old and goes back to that concept," he says.

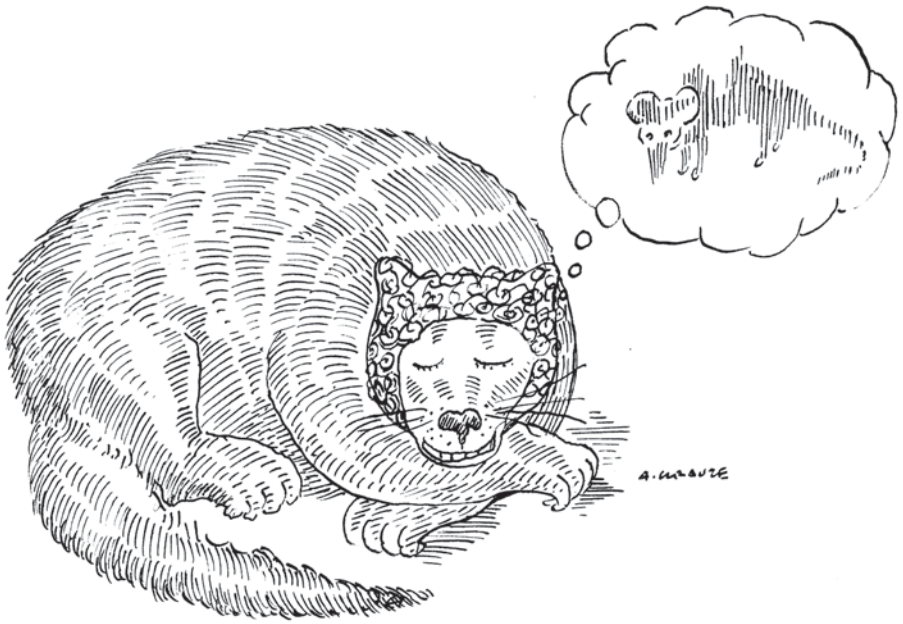
The framing of instinct as ancestral behaviors that were learned and encoded through epigenetic mechanisms, Queen Mary University of London ethologist Lars Chittka admits, will forever be seductive to citizens and scientists alike. "It's very difficult for us to come to terms with just how much of our behavior is set in stone."

—Becca Cudmore

Riding the Waves

In 1959, two French scientists, Michel Jouvet and François Michel, recorded strange patterns of neural activity in the brainstem of sleeping cats. The brain waves seemed remarkably synced to rapid eye movement (REM) sleep, which University of Chicago researchers had connected with dreaming six years earlier. These new brain activity patterns seemed as though they might also correspond with dreaming.

In the 1960s, Jouvet and collaborators showed that cats with a lesion introduced into that same brainstem area—the pons—exhibited odd behavior. Cats displayed REMs as though they were asleep, while reacting to nonexistent prey or predators, pouncing, or hiding. Humans can also experience REMs while dream-



ANDRZEJ KRAUZE

ing, hallucinating, or even recalling deeply emotional memories while awake. But do humans also exhibit the same patterns of neural activity—dubbed PGO waves?

The waves are so named because they are generated in a part of the brain stem called the pons, and propagate to the lateral geniculate nuclei of the brain—relay stations in the thalamus for incoming visual information—and then to the occipital lobe, where most visual processing takes place. Studies have suggested that this neural pathway is crucial for functions ranging from basic ones such as the control of eye muscle movements to more-complex phenomena, including visual experiences during dreams and in hallucinations, memory consolidation, and even psychotic behavior. Researchers have recently proposed that a common thread shared by these phenomena is the overriding of retinal visual input by internally created visual experiences (*Front Hum Neuro*, doi.org/10.3389/fnhum.2017.00089, 2017).

Studying PGO waves and their connection with sleep, REMs, and visual experiences requires monitoring deep-brain activity using electrodes poked directly into the deepest parts near the center of the brain, a procedure that rarely passes ethical muster in human research (and then only in the course of necessary neurosurgery). Cats, which have most often served as subjects in PGO wave studies, can't report experiential aspects of dreams, imagery, or hallu-

cinations required for a complete understanding of the waves' potential role in creating such internally generated visual phenomena.

Even with these limitations, there have been a few studies of PGO waves in humans. Neuroscientist Andrew Lim and colleagues at the University of Toronto recorded PGO patterns in a human in 2007 (*Sleep*, 30:823-27). The researchers capitalized on a rare opportunity to record the waves in a 67-year-old patient with Parkinson's disease who was participating in a study of deep brain stimulation as a treatment method. "We were only directly able to record from the pons, and so only recorded the P component of PGO waves," Lim says.

A 2009 study used similar methods to record PGO waves in a larger cohort of participants. Researchers in Spain showed consistent PGO-like patterns in the brains of 12 patients with Parkinson's disease (*Sleep*, 32:1117-26). "PGO waves, if identified the same way that they were in animals by seminal researchers, can only be recorded in humans with highly invasive methods: opening a hole in the skull and implanting sensors inside the brain," says Julio Fernandez-Mendoza, first author of that study who is now at Penn State University. "These patients with implanted deep-brain stimulation electrodes opened an opportunity."

Even with strong evidence that human brains generate PGO waves, the function of these signals remains mysterious. "It is very likely that PGO waves are the electrical brain activity necessary to stimulate the visual processing area of the brain, and that other visual experiences such as hallucinations may have a similar brain substrate," suggests Fernandez-Mendoza. "I think that this type of deep-brain activity is part of the missing ingredients that could explain the associations found between REM sleep and cognitive and emotional functions."

PGO waves have been linked with dreaming since they were first recorded in the 1950s. Harvard University professor emeritus Allan Hobson is a central figure in current dream theory. He suggested in a landmark 1977 paper that the study of dreaming was within the realm of neuroscience and a subject for objective investigation—contravening Freudian psychoanalytic theory, which Hobson declared unscientific.

Hobson believes PGO waves provide a key bridge from subjective investigation of dreams to their neuroscientific study, and that this opportunity should motivate the waves' investigation. "[PGO waves] are probably not just conveying visual information; they are conveying the construction of an internal model of the world inside your head," he says. "There has to be an internal mechanism for generating such a model. I think PGO waves are a good candidate for such a mechanism."

Recent developments in noninvasive scanning methods—especially those utilizing magnetoencephalography (MEG)—might help researchers peer deep into the brains of sleeping humans to begin to untangle the role of PGO waves. MEG has both the temporal and spatial resolution for this task. But the use of MEG to investigate PGO waves has been impeded by the extensive computational requirements needed to pick out PGO patterns and localize them accurately. "In principle, MEG is capable of detecting deep magnetic fields that are inaccessible to noninvasive methods such as scalp EEG," says Lim.

Fernandez-Mendoza predicts that "if the field is able to develop high-resolution, noninvasive methods able to record these electrical neurophysiological signals, that will completely change the way sleep is currently being studied, and PGO waves will start to be almost a standard in human sleep research."

—Philip Jaekl

I think that this type of deep-brain activity is part of the missing ingredients that could explain the associations found between REM sleep and cognitive and emotional functions.

—Julio Fernandez-Mendoza
Pennsylvania State University

Reanimating Research

After a decade studying microscopic marine life, biologist Nina Lundholm decided in 2011 that it was time to bring back the dead. She had first become fascinated with phytoplankton when she was a PhD student at the University of Copenhagen, and much of her research since then has focused on how their populations respond to short-term ecological changes.

Studying variation between modern species led her to wonder how these tiny organisms changed over longer stretches of time. "Because [marine phytoplankton] make up the basis of the food web, it is important to see how they respond to changes in the environment and the climate," says Lundholm, who is now an associate professor at the Natural History Museum of Denmark.

Marine phytoplankton, which include a diverse array of photosynthetic organisms, are responsible for roughly 48 percent of photosynthesis globally, meaning that "they generate every other breath of oxygen," explains Tatiana Rynearson, a University of Rhode Island oceanographer who was not involved in the study.

Some phytoplankton produce algal blooms that can be used to study the impact of short-term environmental changes, and paleoecologists collect dead phytoplankton from ocean sediments to study longer-term climatic changes, recorded in the chemical structure of their microscopic skeletons.

But not everything in the sediments is dead. Some phytoplankton species form cysts that have been shown to lie dormant for up to a century. "It's a good strategy to make a cyst, go to the sediment at the bottom, and hang out until conditions improve," says Rynearson. "Significant time periods [can pass] where conditions aren't suitable for growth."

As well as helping phytoplankton species survive hard times, their cysts serve

HARD-CORE BIOLOGY: One of the sediment cores from which Nina Lundholm pulls slumbering phytoplankton to reanimate and study

MARIANNE ELLEGAARD, UNIVERSITY OF COPENHAGEN

as time capsules for scientists to crack open. The idea of resurrecting these dormant cells to study past organisms directly, known as resurrection ecology, isn't new—similar techniques have been used to revive dormant moss and crustaceans. But applying the method to marine ecosystems has proved more challenging.

In 2011, Anna Godhe at the University of Gothenburg and colleagues succeeded in resurrecting dormant cysts of marine phytoplankton called diatoms (*PNAS*, 108:4252-57). They found that the genetic structure of the diatom *Skeletonema marinoi* had remained relatively unchanged for more than 100 years, but that populations inhabiting a fjord were genetically different from open-water diatoms.

Since then, Lundholm, Godhe, and their colleagues have been applying the same technique to another type of phytoplankton: dinoflagellates. Whereas diatoms are at the base of ocean food webs, dinoflagellates sit in the middle, feeding on algae and other organisms as well as producing food via photosynthesis themselves.

But reanimating dinoflagellate cysts after nearly 100 years is no mean feat. In order to estimate the age of each section of a sediment core, and to collect cysts for resurrection, the team had to slice the cores into sections. But doing so exposes them to oxygen, triggering a rapid degeneration process. "We discovered that we had to keep them complete until we wanted to isolate the resting stages," Lundholm says.

Her new line of research hasn't made her very popular with her colleagues in the lab, Lundholm admits: "The cores smell like rotten eggs all over the department."

Lundholm selected *Pentapharsodinium dalei* for her latest study because "it had a reasonably high germination percentage, and it germinated the furthest back in time." Even for *P. dalei* the resurrection success rate remained relatively low—between 5 percent and 65 percent depending on the age of the sediment. "Dinoflagellates are challenging to work with," Anke Kremp, a researcher at the Finnish Environment Institute in Helsinki who was not involved in the study, writes in an email. "They are difficult to cultivate and have large and peculiar genomes." Lundholm's team also developed new genetic markers that would allow them to study the population structure of the resurrected plankton (*J Appl Phycol*, 26:417-20, 2014).

Despite these challenges, Lundholm and her team were able to revive 193 clonal strains of the dinoflagellate from sediment cores collected in Koljö Fjord in Sweden (*Ecol and Evo*, 7:3132-42, 2017). The cysts they reanimated had been deposited between about 1922 and 2006.

The researchers used DNA microsatellite analysis to identify two subpopulations, which alternated in frequency during the 84-year record. One was common in older sediments but became relatively rare between 1960 and 1985, and is currently experiencing a population revival.

These fluctuations correlate with cyclical changes in the ocean environment in Koljö Fjord, explains Lundholm. Both the oldest and the most recent sediments

were formed during summer phases of the North Atlantic Oscillation (NAO), which generates warmer, less-salty waters in the fjord. The second subpopulation became common only in the cool, saline waters of the winter NAO, between 1960 and 1980.

These fluctuating subpopulations maintain high genetic diversity in the fjord, which may help the dinoflagellates cope with dynamic environmental conditions. "We see that they respond to the environment, but overall still are quite resilient," says Lundholm.

The study "adds much-needed, very long-term data so we can start to think about climate-level responses in phytoplankton," Rynearson notes.

"This approach is so interesting because populations and individuals can be compared that have experienced past conditions. There aren't many systems present in nature that allow such direct tracing of evolution," Kremp says, adding that "resurrection ecology of plankton is still in an early stage, and the author team is a pioneer here."

While she praises the Lundholm team's research, resurrection ecology scientist Luisa Orsini of the University of Birmingham in the U.K. says that laboratory experiments may be required to confirm that there is a causal relationship between environmental changes due to the NAO and changes in dinoflagellate subpopulations. "Other environmental variables may drive changes in dinoflagellate subpopulations," she says.

Replicating the dinoflagellate study in other species and in different regions of the globe will add to researchers' understanding of how climate influences biodiversity. But there is only so much scientists can learn from single-species approaches. "The next step would be to go higher in complexity and study how communities evolve through time," says Orsini.

—Claire Asher

Fishing for DNA

When investigating the different forms of marine life inhabiting New York City's various bodies of water, Mark Stoeckle, a biologist at Rockefeller University, says "a bucket, like what you would use when you're painting your house," along with a rope and a few empty bottles will do the trick. Using these simple tools, he and his team collected water samples from the lower Hudson and East Rivers to obtain traces of DNA that had been sloughed off by various fish species.

"We threw the bucket in the water, hauled it up, [and] poured the samples into some rinsed-out juice bottles," says Stoeckle. They then took those water-filled containers back to the lab and analyzed the DNA.

Bits of free-floating DNA shed by fish and other living organisms are known as environmental DNA, or eDNA. When researchers analyze eDNA and compare it against a reference library of known genetic sequences, it can paint a comprehensive picture of what organisms presently inhabit a given environment. "Most people only see fish when you reel them up on your fishing line," says Stoeckle, a self-described lifelong naturalist. "This is another way of learning about them."

Stoeckle recently published the data he collected from the lower Hudson Estuary and East River in the journal *PLOS ONE* (12: e0175186, 2017). This study was the first to characterize marine fish migration using eDNA. In order to capture the passage of migratory fish, Stoeckle and his team—consisting of one postdoc and one high school student—gathered one-liter water samples from the same locations once a week for six months. Additionally, he and his team sequenced DNA from 18 species of fish, enhancing the library of reference sequences. The goal was to identify as many species as possible from their water samples, and compare their data to known fish migration patterns.

The researchers amplified a specific region of a ribosomal RNA gene in mitochondria—a useful molecular signature that is distinct in each species—in order to identify which species the floating bits of eDNA belonged to. Beginning in early April, they detected a dramatic increase in eDNA for many of the fish sampled, including Atlantic menhaden (*Brevoortia tyrannus*), tautog (*Tautoga onitis*), and cunner (*Tautogolabrus adspersus*), among others. "This method is new, and you have to have some way to benchmark your results," says Stoeckle. Indeed, their findings, demonstrating a large multispecies migration into New York City waterways beginning in the spring, were corroborated by prior work.

Stoeckle also unearthed some surprises: for example, the strong springtime increase in the population of Atlantic menhaden, a major food source for larger species such as humpback whales. The menhaden's presence could explain recent whale sightings in the East and Hudson rivers. Oyster toadfish (*Opsanus tau*) were also unexpectedly common in the Hudson and especially in the East River.

This no-frills, noninvasive method of ecological assessment can potentially improve protocols for aquatic biologists from a variety of disciplines, especially those who rely on traditional collection techniques such as boats, nets, and electrofishers—equipment that delivers an electric current to stun fish, enabling capture. Compared with such methods, eDNA is "less expensive and less damaging to the environment," says Stoeckle.

Elizabeth Alter, an evolutionary genomicist at the City University of New York, agrees. She notes that methods such as electrofishing can be disruptive to habitats and may not be able to capture the teeming diversity of ocean life, in particular. And nets meant for larger animals might not capture smaller ones, and vice versa.

In Alter's own work comparing eDNA with traditional methods, she notes rocky habitats tend to give off strong DNA signals, but are weak spots for electrofishing, because animals can find numerous hiding places. In such environments, eDNA can capture hidden organisms. "[It] can often uncover things that are cryptic or very rare," she says.

"The hope is that this method will not replace [traditional methods] completely, but will be able to do much more accurate, dense sampling than we do now," says Stoeckle.

But if the use of eDNA for characterizing aquatic ecological dynamics is to become more widespread, researchers first have to determine if the technique can answer the type of questions that they're asking. Prior to Stoeckle's study, it wasn't clear whether scientists could actually track migrating fish in marine environments. In theory, it would only work if eDNA is present when the fish are present and gone when they are absent. Jesse Ausubel, director of the Program for the Human Environment at Rockefeller University, says their project was successful because of the goldilocks effect: eDNA sticks around for just the right amount of time. "The really neat thing is that it seems to last a day or two, which is just right for the kind of questions that we're trying to answer," he says.

Alter is further validating eDNA to see if it can also characterize the abundance, not just the presence or absence, of species. Specifically, she's investigating how dams in the Bronx River affect the density of eel species, in collaboration with researchers at Queens College and the Wildlife Conservation Society.

Other researchers in the Northeast are catching on. Jennifer Miksis-Olds, a biological oceanographer at the University of New Hampshire, learned about eDNA from Stoeckle just this year, and is interested in pairing it with acoustic methods to both detect the presence of organisms and to identify them. "With multiple frequencies and sophisticated acoustic systems, you can get at much more information, like shape and composition of the animals, but you still don't know what species they are," she says. eDNA would provide an additional layer of information. Miksis-Olds says she'll bring back water samples from an upcoming cruise to the Outer Continental Shelf for Stoeckle to analyze for eDNA.

Most people only see fish when you reel them up on your fishing line. This is another way of learning about them.

—Mark Stoeckle, Rockefeller University

Thomas Noji, Chief of the Ecosystems and Aquaculture Division at the National Oceanic and Atmospheric Administration's Northeast Fisheries Science Center, is also excited about the potential of eDNA. As head of the lab that investigates ways to improve commercial aquaculture, Noji is interested in using eDNA to study how different caging methods for farmed oysters affect surrounding biodiversity. "It's very cool—we can get a quick overview of what fish are there," Noji says.

Encouraged by the falling costs of DNA sequencing, the rise of eDNA research has also been facilitated by an ever-expanding portfolio of reference genomes. Ausubel helped organize the Census of Marine Life, a 10-year endeavor aimed at taking genetic stock of Earth's marine life. This herculean task resulted in a much-improved reference library containing the genetic sequences for different species of fish and other aquatic organisms, according to Ausubel. "Scientists had known for some time that there is this loose DNA, the eDNA, floating around in water, but it wasn't really much good to sift it out because you had nothing to compare it to," he says.

Despite these recent advances, using eDNA as an ecological tool is still a relatively new strategy. "There aren't really standards for a lot of the protocols," Alter says. Standardization is important, to prevent cross-contaminating the samples and to allow for data comparisons between groups. "We're all developing the methods as we answer the questions, which is always a bit of a challenge."

Stoeckle is optimistic. "Ten years from now, my guess is this will be the main way that we analyze fish populations in the ocean," he says.

Currently, he is collecting eDNA from the Lower New York Harbor off Coney Island in order to assess the presence of dolphins, whales, and sharks. Stoeckle calls it the subway project; he gets on the southbound Q train and rides the 26 stops to his destination. "I've had some interesting conversations," says Stoeckle, admitting that he's been questioned by curious passersby—and one local policeman—during his sample-collecting expeditions around the city. But that hasn't stopped him from collecting samples from Central Park, or Coney Island's New York Aquarium. "It's a new technique; we're at the beginning of learning what it can do," he says. "I think it's going to give us a much better appreciation of nature . . . even in an urban environment such as New York City." —Aggie Mika

eDNA FISHERMEN: Sam Chin (left) and Nathan Morris (center), PhD students in Elizabeth Alter's lab, collecting water samples for eDNA analysis with Mark Stoeckle (right) at the New York Aquarium

JESSE AUSUBEL

Widening the Web

Taxonomic skew introduced by the domination of model organisms and charismatic megafauna in the literature is a disservice to the life sciences.

BY MALCOLM F. ROSENTHAL AND MAYDIANNE C.B. ANDRADE

KEN JONES

CHARISMATIC MINIFAUNA: Andrade's lab studies animal behavior in some of biology's less celebrated subjects. (Left)Texas widow harlequin spider; (top right) jumping spider; (bottom right) Western black widow

As researchers working to understand animal behavior, we have studied only a small subset of the more than 1.5 million described animal species. This is unavoidable, as there are many more species than scientists. But the animals we work with are our windows into nature, and it is increasingly clear that our field revolves around animal subjects that simply do not reflect the diversity of the natural world.

Studies in related fields such as ecology and conservation have repeatedly found that research effort is skewed towards warm-blooded vertebrates (birds and mammals) and against cold-blooded vertebrates and invertebrates in general. Our well-documented preferences for what we consider to be attractive or charismatic creatures may be limiting our contributions to a broad understanding of nature.

To test whether these skews exist in animal behavior research, we assigned taxonomic information to the 4,076 research articles published in the journal *Animal Behaviour* from 2000 to 2015 and compiled a data set that combined this with citation metrics for each article. By comparing this data set to actual species numbers, we were able to quantify the direction and magnitude of taxonomic skew in published papers.

Our findings can be summarized in two major points:

First: The warm-blooded vertebrate skew was intense. Almost 85 percent of described species are arthropods, but more than 70 percent of publications were on vertebrates. Birds and mammals alone accounted for well over 50 percent of publications, despite representing less than 2 percent of all animal species.

Second: In a world where citations are used to measure impact, publishing on understudied systems comes at a cost to the researcher. Publications on vertebrates received more citations on average than arthropod papers. They were also far more likely to be "blockbuster" publications with more than 100 citations.

How do we resolve this problem? First, we have to agree that there is one.

There is no law that says we must invest as much effort studying dung beetles as we do studying chimpanzees. Some would argue that biologists' work is intended to generate knowledge that

can benefit humanity, and that it makes sense to focus on taxa that are best suited to addressing human-relevant questions. So does it matter that there are five times as many publications per year on primates (fewer than 500 species total), as there are on beetles (with a staggering 240,000 described species), as our study shows? We think it does.

For theorists, a narrow taxonomic focus does a disservice to all branches of the animal sciences whose goals are to understand the broad processes and patterns of the natural world. When we limit ourselves to a narrow subset of life, we generate narrow answers to broad questions. For pragmatists, a narrow focus makes it challenging to apply insights from animal behavior research to real-world problems. For example, insects are a critical part of most terrestrial food webs. If we study only a handful, how do we predict how changes in climate will affect their dispersal, or their foraging behavior, or the welfare of the vertebrates that depend on them as food? We are not saying that the intensity of study should *match* biodiversity. We are saying that it is critical to try to learn more about a broader range of organisms.

Truly resolving this issue will require an understanding of its causes. For example, might human preferences for charismatic species affect what we consider to be important or broadly relevant science? Previous work shows that papers on non-model organisms have more broadly framed introductions, suggesting that the bar for relevance is higher when the taxon is less appealing.

Our demonstration of uneven citation patterns is also consistent with this. We must ask some uncomfortable questions: Do papers that differ only in their study subjects get treated differently by reviewers or editors? Do grants? Do job applications? We can speculate, but these questions can, and should, be answered empirically. Luckily, we are members of a community that is equipped to do exactly that.

In the meantime, we suggest three ways to engage with this issue:

Be aware of your own potential bias when reviewing grants or papers: Ask yourself honestly whether your assessment of the quality and relevance of the work is a result of the quality of the science or of the taxon under study.

Be proactive when citing other publications: Publications on birds and mammals are more likely to cite within their taxon than are publications on non-model systems. In addition to reading broadly, make systematic efforts to consider the relevance of studies asking similar questions in other taxa.

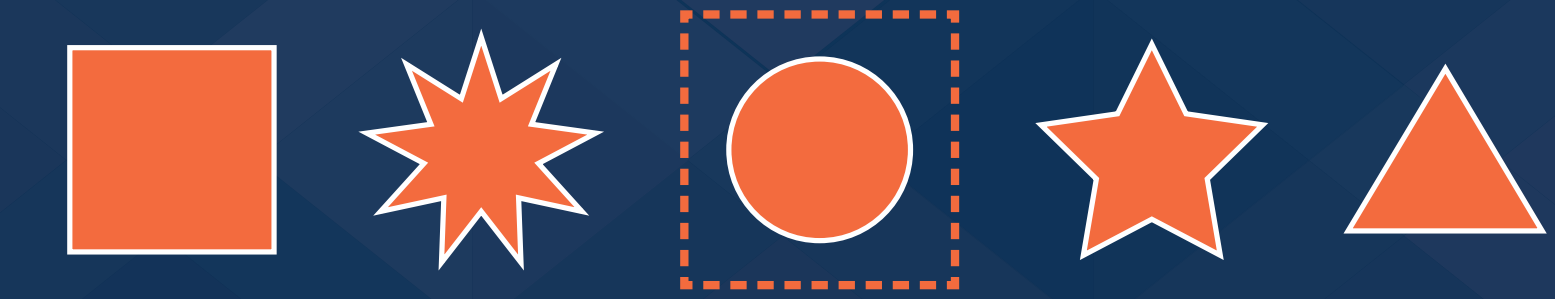
Keep the conversation going: How big a problem is all of this? How can we determine the causes underlying these patterns? Should established researchers consider branching out taxonomically? What can my department do; what can my journal do?

We have strong evidence that the taxonomic research skew exists, and that it is severe. We have some hypotheses about causes. We have the data needed to answer some of the most pressing questions. Now, all we need is the will to explore the issue further. ■

Malcolm F. Rosenthal is a postdoc in Damian Elias's lab at the University of California, Berkeley, and formerly a researcher in the lab of Maydianne C.B. Andrade, an evolutionary ecologist at the University of Toronto, Scarborough. A version of this story was published at the-scientist.com on May 30, 2017.

Does it matter that there are five times as many publications per year on primates as there are on beetles?

ONE WORKFLOW DOES NOT FIT ALL



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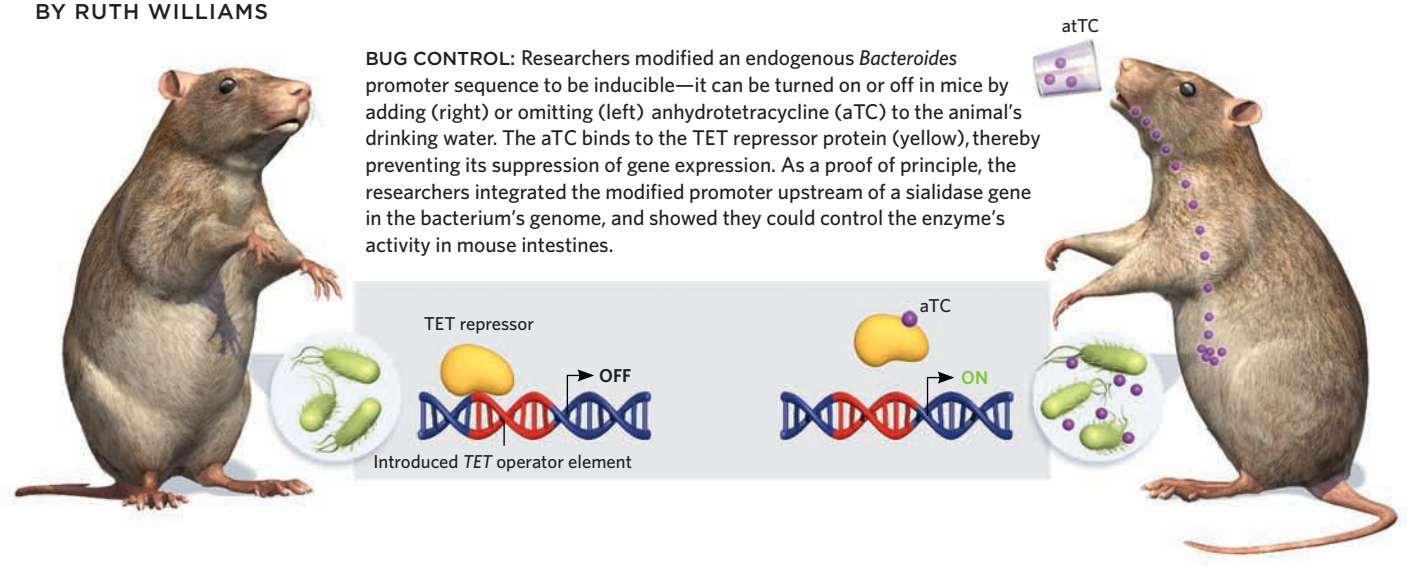
Microbiota Manipulations

Two research teams develop tools for tinkering with a bacterial genus prominent in human guts.

BY RUTH WILLIAMS



BUG CONTROL: Researchers modified an endogenous *Bacteroides* promoter sequence to be inducible—it can be turned on or off in mice by adding (right) or omitting (left) anhydrotetracycline (aTC) to the animal's drinking water. The aTC binds to the TET repressor protein (yellow), thereby preventing its suppression of gene expression. As a proof of principle, the researchers integrated the modified promoter upstream of a sialidase gene in the bacterium's genome, and showed they could control the enzyme's activity in mouse intestines.

The past decade has seen a surge in microbiome research and, with it, a greater appreciation of the relationships between resident microbes and their hosts. But the focus is shifting, says microbiologist and immunologist Justin Sonnenburg of Stanford University. A major goal of the field, at least in terms of human research, he says, is "to leverage our gut microbes so they can perform tasks," such as deliver drugs or take physiological measurements.

But engineering gut bacteria is not straightforward, primarily because researchers have relatively little experience with the species that live in and on our bodies, says gastroenterologist Suzanne Devkota of Cedars-Sinai Medical Center in Los Angeles. "There are 1,001 ways to genetically manipulate *E. coli*," she says, "but that's not particularly useful in the human context because [*E. coli*] is not a dominant member of our gut bacteria."

Now, two independent research teams led by Sonnenburg and by Andrew Goodman of Yale University have created tools to manipulate *Bacteroides* species, which represent about half of the bacteria that make up the gut microbiome, and have shown that they work in mouse guts.

Goodman's team modified an endogenous *Bacteroides* promoter sequence to be inducible—it could be turned on or off in mice by adding or omitting an effector molecule in the animals' drinking water (see illustration). Sonnenburg's system, on the other hand, used a viral promoter that upregulated gene expression to 70-fold higher than the type of promoter used by Goodman's team. The viral promoter had the power to drive levels of fluorescent protein expression high enough to visualize bacteria in the mouse gut—a previously impossible feat. Unlike Goodman's system, however, it was not inducible in vivo.

The two systems are "really quite complementary" says Devkota. Both provide "a way to gain insights into our native gut microbiota," she adds, which "is a big step forward." (*Cell,* 169:547-58, 2017; *Cell*, 169:538-46, 2017) ■

AT A GLANCE

BACTEROIDES GENE-EXPRESSION TOOLS	SYSTEM COMPONENTS	HOW IT WORKS	GENE CONTROL	APPLICATIONS
Goodman system (illustrated)	Bacteroides 16S rRNA promoter modified to contain an anhydrotetracycline (aTC)-responsive *TET* operator element	Inserting the modified promoter upstream of endogenous *Bacteroides* genes enables inducible gene expression.	From fully off to fully on, with dose-dependent expression in between	Investigating the phenotypic effects of switching gut bacterial genes on or off Future: Delivery of drugs or other factors with temporal control
Sonnenburg system	A set of powerful promoter sequences adapted from a bacteriophage promoter and fluorescent protein coding sequences	Constructs containing the promoters and fluorescent proteins are integrated into *Bacteroides* genomes. Each species produces a different fluorescent hue for identification.	A set of promoters drives a range of bacterial expression levels up to extremely high levels.	Distinguishing and localizing specific *Bacteroides* species in the gut Future: Delivery of drugs, or other factors needed at high doses

BUILDING WITH DNA

The versatility of geometric shapes made from the nucleic acid are proving useful in a wide variety of fields, from molecular computation to biology to medicine.

BY ARUN RICHARD CHANDRASEKARAN

DNA—the biological information-storage unit and the mechanism by which traits are passed on from generation to generation—is more than just an essential molecule of life. In the chemical sense, the nucleic acid has properties that make it useful for nonbiological applications. Researchers are now using DNA to store massive amounts of data, for example, including books and images, a Shakespearean sonnet, and even a computer operating system, with data encoded in the molecule's nucleotide sequences. At an even more fundamental level, DNA is a critical building block of nanoscale shapes and structures. Researchers have created myriad nanoscale objects and devices using the nucleic acid, with applications in biosensing, drug delivery, biomolecular analysis, and molecular computation, to name but a few. DNA provides a highly specific route to building nanostructures. While the field is still addressing how to scale up into the micrometer range, it is possible to imagine a future with DNA-based computer chips performing calculations and DNA nanobots delivering personalized medicine to target sites in the human body.

DNA as bricks and mortar

The four canonical nucleobases—adenine, guanine, thymine, and cytosine—are inherently programmable, as adenine always pairs with thymine, and guanine with cytosine. Just as these bases encode the biological instructions for building and maintaining an organism, so too do they form the basic code of designing shapes using DNA. Two strands that have complementary sequences of these nucleotides can bind to each other to form a double helix structure with a diameter of about 2 nanometers, and a single turn of DNA (a helical pitch) measuring about 3.4–3.6 nm. This helix is quite stiff within lengths of 15 helical pitches, or about 50 nm (its "persistence length"), allowing DNA to be used as a rigid construction material.

While entire chromosomes are composed of tightly coiled DNA, the double helix itself is inherently linear, extending in only one direction. For it to be useful for construction in two or three dimensions, branched DNA junctions are created by the reciprocal exchange of strands. This phenomenon occurs in nature—for example, during formation of the Holliday junction, an intermediate in genetic recombination. Synthetic DNA sequences can be designed to pair in certain ways, resulting in branched junctions with helices that extend in more than one direction. DNA can also hold other bits of DNA together: sticky ends, or short, single-stranded overhangs at the tips of a helical nucleic acid, can be designed to bind to one another by proper sequence complementarity. Such sticky ends act

as structural glue to bind DNA motifs or complexes, resulting in hierarchical self-assembly of these macromolecules into larger arrays and larger complex architectures. (See illustration below.)

Crystallographer Ned Seeman, now at New York University, first proposed the idea of using DNA to build molecular scaffolds in the early 1980s.[1] Seeman, then at the State University of New York at Albany, grew frustrated trying to crystallize molecules the traditional way, by exploring a range of experimental conditions by trial and error. He hypothesized that branched DNA could be used to build a framework to solve this crystallization problem by holding the molecule of interest in a defined spatial position, effectively crystallizing it. In 1983, he designed DNA sequences that formed stable, immobile, branched junctions containing four arms, which would lay the foundation for designing higher-order DNA structures and arrays, including a designed three-dimensional DNA crystal.[2] (See "3-D Seer," *The Scientist*, August 2011.)

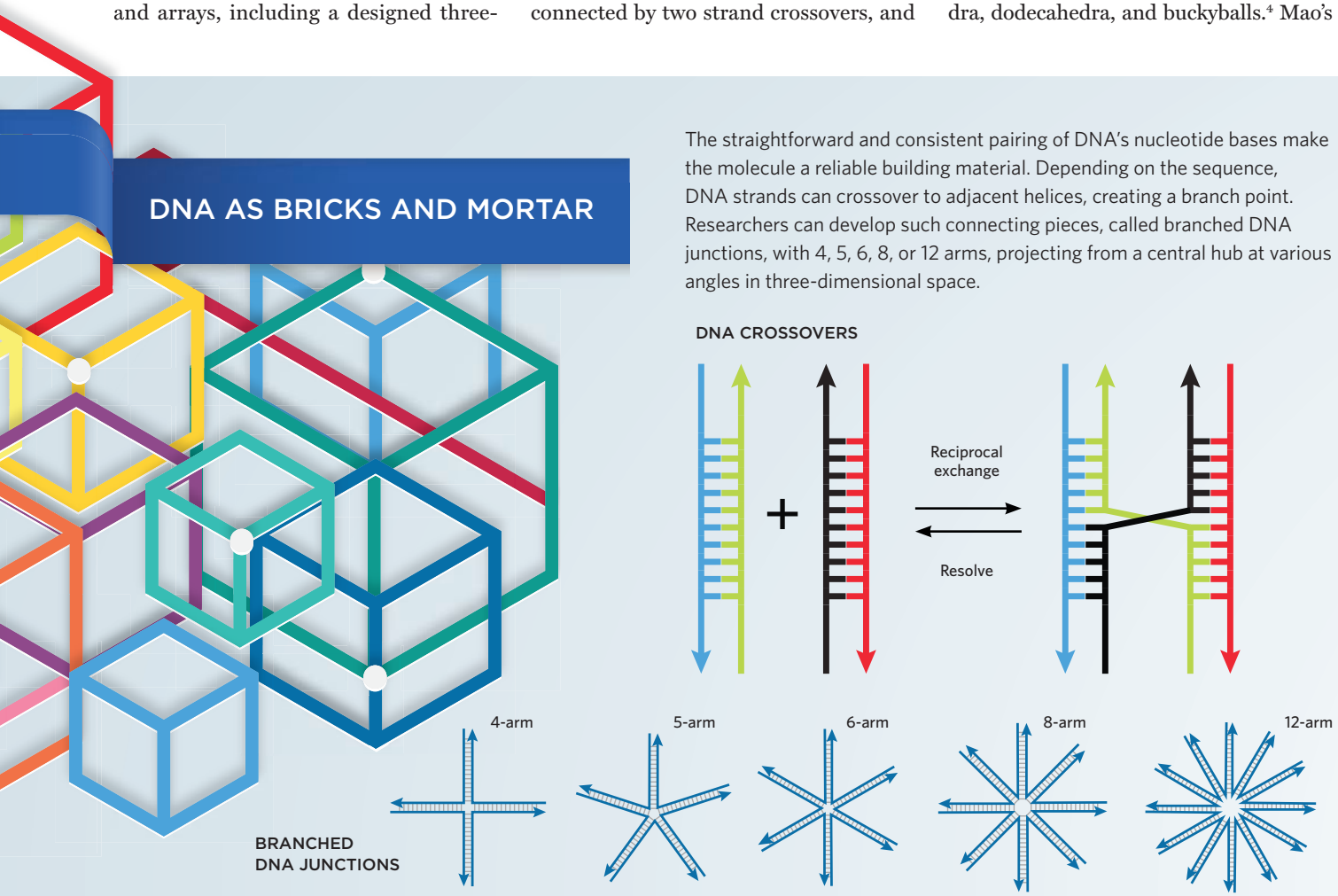
Researchers have created myriad nanoscale objects and devices, with applications in biosensing, drug delivery, biomolecular analysis, and molecular computation, to name but a few.

The study of DNA nanotechnology has expanded greatly in the past 35 years. Building upon the original four-arm DNA junction, Seeman's group upped the number of arms possible in a single branched junction to 12. To achieve more robust assembly, Seeman's group also designed a DNA motif called the double crossover, which contains two helical domains connected by two strand crossovers, and used these "tiles" to assemble the first two-dimensional crystalline lattice made out of DNA.[3] Another example is the triple crossover motif, which contains three adjacent helices, each joined to its neighboring domain twice, which has also been used to assemble two-dimensional arrays. Such periodic arrays became a useful framework for hosting other molecules, as Seeman had originally intended, as well as in molecular computation and to study distance-dependent interactions between proteins.

Researchers have also used DNA to create nanoscale objects. In 1991, for example, Seeman's group produced the first three-dimensional object made from DNA: a cube with double-helical edges. In 1994, the team made a truncated DNA octahedron with 14 faces. More recently, Chengde Mao's group at Purdue University designed a three-point-star DNA motif and used it to assemble tetrahedra, dodecahedra, and buckyballs.[4] Mao's

DNA AS BRICKS AND MORTAR

The straightforward and consistent pairing of DNA's nucleotide bases make the molecule a reliable building material. Depending on the sequence, DNA strands can crossover to adjacent helices, creating a branch point. Researchers can develop such connecting pieces, called branched DNA junctions, with 4, 5, 6, 8, or 12 arms, projecting from a central hub at various angles in three-dimensional space.



team also designed a five-point-star motif to create an icosahedron.

In all of these cases, researchers must create precise stoichiometric mixtures of purified DNA strands. The structures form as the mixtures are cooled, triggering the strands to hybridize to complementary regions of other strands—a process known as hierarchical self-assembly. This strategy is used to create a variety of nanoscale structures, with applications in sensing biological molecules, bioimaging, and drug delivery.

More recently, researchers have devised other techniques for building DNA-based architectures. Peng Yin's group at Harvard University's Wyss Institute developed alternate strategies to create large DNA nanostructures. In one approach, the researchers designed single-stranded DNA tiles that can attach to one another, with a full set of tiles forming a rectangular "molecular canvas." They rendered desired two-dimensional shapes by selecting a subset of these single-stranded tiles, each of which acted as a pixel on the canvas. The investigators subsequently expanded on this strategy to create three-dimensional shapes and arrays using DNA bricks. But today, a majority of DNA nanostructures are based on yet another approach, known as DNA origami.

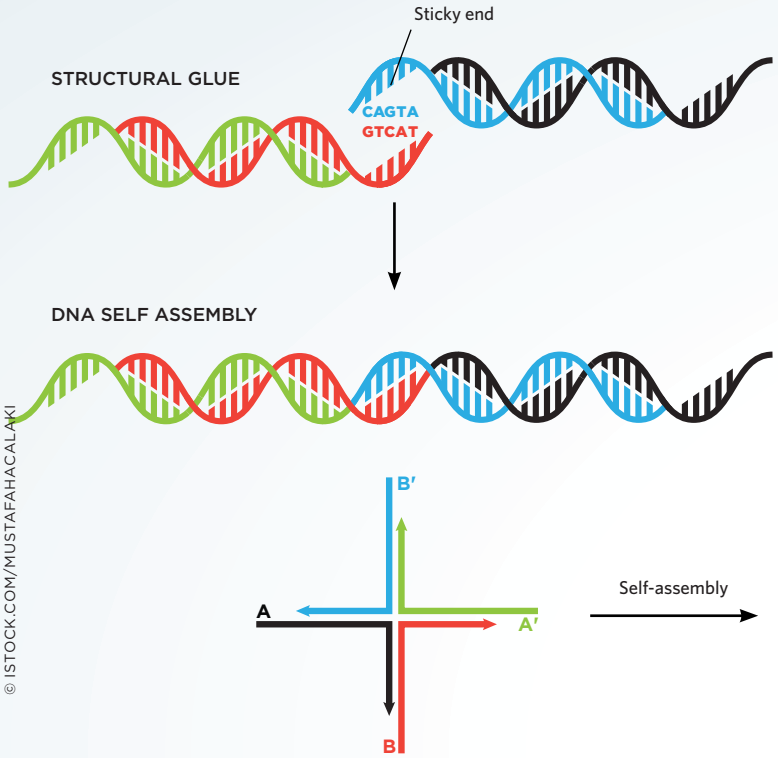
Welcome to the fold

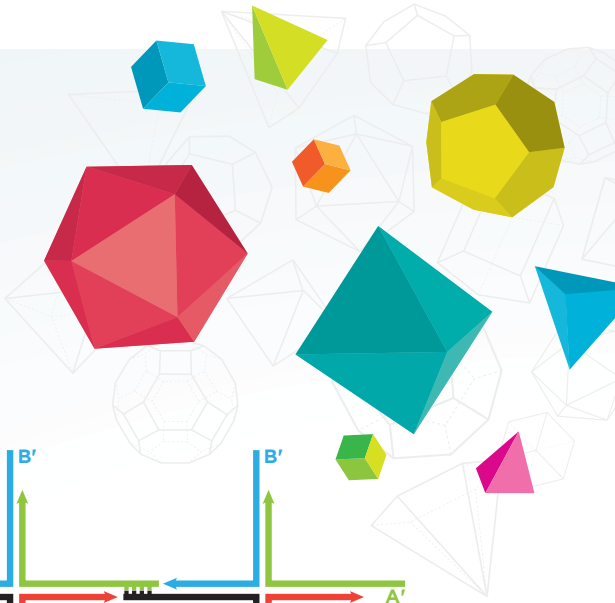
In 2006, a smiley face made out of DNA appeared on the cover of *Nature*. In a study published in that issue, Paul Rothemund of Caltech described the process, dubbed DNA origami, that he used to create the smiley face, which measured approximately 100 nm in diameter and was imaged using an atomic force microscope (AFM). He began with a long piece of single-stranded DNA, called the scaffold strand, from the viral genome of the bacteriophage M13 (~7 kilobases). Then, with the addition of hundreds of short complementary oligonucleotides called staple strands, the scaffold was folded into the desired shape.[5] As with hierarchical self-assembly, the structures were made using annealing procedures that involved heating and cooling the DNA mixture to facilitate binding of the short staple strands to different regions of the scaffold.

This strategy is largely dependent on the sequence and length of the single-stranded scaffold, thus limiting the size of the origami structure created. However, the ease of preparation and the flexibility of designing a variety of two- and three-dimensional structures has made DNA origami a go-to technique in the field. In addition, this method does not require purified DNA strands or exact stoichiometric mixtures of component strands.

Over the past decade, researchers have optimized the technique for higher yields, greater design complexity, and easier purification of desired structures, introducing isothermal annealing procedures, in which the temperature stays constant, for

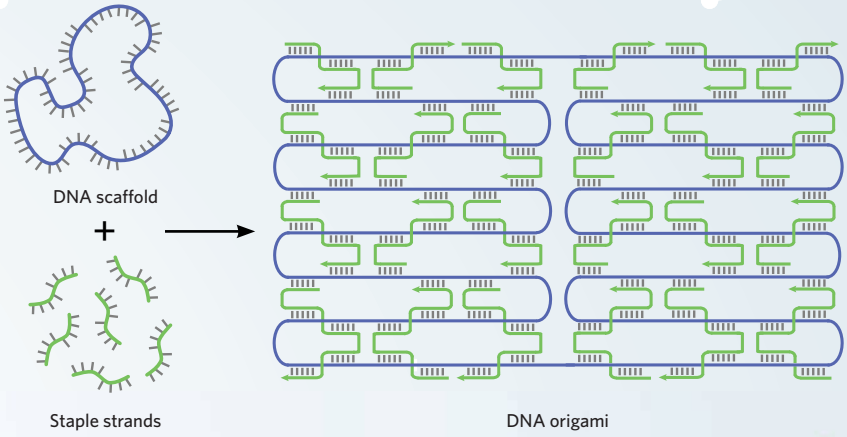
The ends of the molecules can be designed to contain single-stranded "sticky ends" that enable researchers to encode the predictable self-assembly of larger nanostructures.



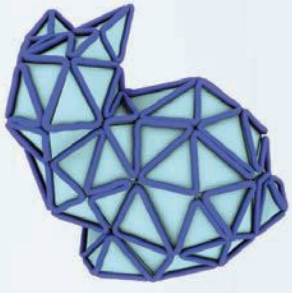


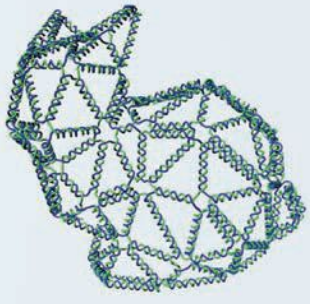
DNA ORIGAMI

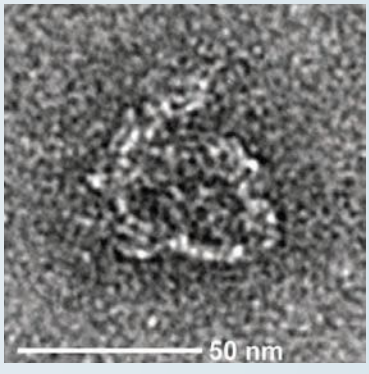
By folding a long, single-stranded DNA scaffold using short, single-stranded "staples," researchers can build any shape they like. For example, researchers recently designed a three-dimensional bunny, using computer algorithms to determine the folding pathways and DNA sequences that would be required to create the desired shape.




3-D design plan


Wrapping of scaffold DNA


3-D origami model



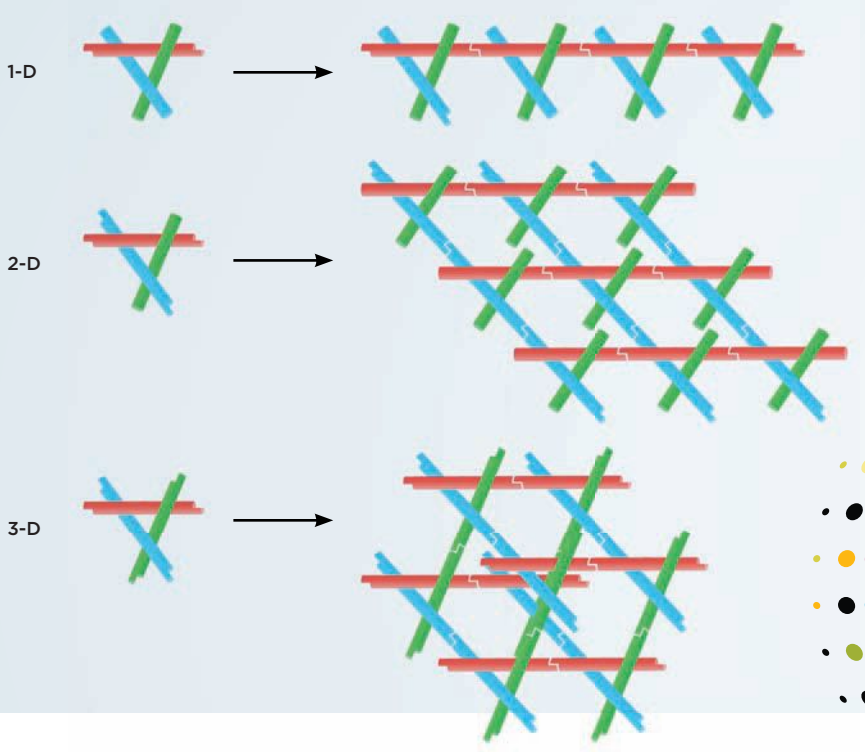
construction (instead of the traditional heating and cooling down of oligonucleotide mixtures). William Shih's group at the Wyss Institute extended the concept of DNA origami to create the first three-dimensional objects from pleated sheets or bundles of DNA helices. The architectures made using origami are much larger than the DNA objects assembled from DNA tiles. Hao Yan's group at Arizona State University produced DNA objects with curved surfaces such as hollow spheres and flasks, demonstrating the versatility of designing complex structures using DNA. Another notable example is the DNA origami box, developed by the groups of Kurt Gothelf and Jørgen Kjems of the Centre for DNA Nanotechnology at Aarhus University, Denmark. This box has a lid that could be unlocked by external DNA strands.

In 2015, Yan's group constructed complex wireframe architectures using DNA origami, creating shapes and contours similar to a freehand drawing. In contrast to conventional DNA origami structures that contain tightly packed helices, Yan's design allowed the creation of porous structures with a precision that is not attainable using traditional techniques, such as lithography. Such structures are potentially useful for the construction of delivery vehicles and fuel cells. They also used this strategy to create finite quasi-crystalline patterns, which remains a challenging task using other fabrication methods. That same year, Björn Högberg's group at the Karolinska Institute in Sweden reported an automated procedure to create similarly complex shapes using origami. They wrote a computer algorithm that can provide the folding pathway for the scaffold strand to create any desired shape in a mesh-like pattern. (See illustration above.)

The design of such complex structures using DNA origami is supported by computer programs such as cadnano and CanDo, which can help users create any pattern and analyze resulting shapes. Most DNA origami construction is based on the M13 genome as the scaffold strand. Researchers have also used other single-stranded scaffolds, ranging in length from just 700 bases to some 50,000 bases, to create origami structures from tens to hundreds of nanometers in size. Recently, Seeman's group designed a cross-shaped origami tile and created the first two-dimensional origami crystalline arrays, extending DNA origami's dimensions into the micrometer scale.[6] Single-stranded M13 DNA is now commercially available from companies such as New England Biolabs. Moreover, companies such as Tilibit now provide customers with a few options for scaffold strand lengths and also supply ready-made mixtures for prefabricated DNA origami designs.

MULTIDIMENSIONAL DNA ARRAYS

Researchers can also design small DNA motifs that self-assemble into lattices. Shown below is a "tensegrity triangle" motif with three double helical edges (represented as cylinders) that contain complementary sticky ends along one, two, or all three of its edges, leading to assembly of one-, two-, or three-dimensional arrays.



Putting DNA nanostructures to work

Seeman's original idea for using DNA as a building material was to spatially position "guest" molecules for the crystallization of hard-to-crystallize biomolecules. Over the years, researchers designed and assembled numerous DNA motifs into one- and two-dimensional arrays, and these have been used to host guests such as proteins, nanoparticles, antibodies, and quantum dots. But Seeman's vision of using a DNA scaffold as a framework for macromolecular crystallization wasn't fully realized until almost 30 years later, in 2009.

Seeman's group used a DNA motif that spanned three dimensions, called a tensegrity triangle. The motif had threefold symmetry with three double-helical edges designed in an over-and-under fashion and six sticky ends, giving the structure the ability to connect to other tensegrity triangles. The assembly continues in an infinite arrangement, thus forming a crystal.[7] (See illustration at right.) The cavities in this crystal provide space to accommodate guest molecules, thereby acting as a framework to obtain crystal structures. Seeman and his colleagues further showed that they could design such a DNA framework with bigger cavities, thus allowing researchers to encapsulate molecules of various sizes. In addition, the design could be asymmetric, allowing each edge of the DNA motif to have sequences for unique guests.

The fact that DNA structural scaffolds allow for the spatial positioning of proteins, specifically enzymes, allows researchers to use them for analyzing specific biomolecular interactions. For example, DNA motifs have been used to study the activities of a three-enzyme pathway—malate dehydrogenase/oxaloacetate decarboxylase/lactate dehydrogenase—with variable spatial distances and geometric arrangements. DNA scaffolds also allow researchers to increase the efficiency of enzyme cascades and to create synthetic enzyme pathways.

Beyond providing architectures that allow critical spatial positioning, DNA nanostructures can also be designed to respond to chemical cues, such as changes in temperature, pH, and ionic conditions. One of the most notable examples of such DNA nanodevices is a pH sensor called the I-switch, developed in 2009 by the University of Chicago's Yamuna Krishnan, then at the National Centre for Biological Sciences in India.[8] This device was based on the i-motif, a four-stranded structure containing stretches of cytosine repeat sequences that, under acidic conditions, pair with protonated cytosine (C^+) instead of guanine. This pairing resulted in a conformational change from an open linear structure under physiological conditions (pH 7.3) to a closed triangular structure when pH dropped. In the switch, Krishnan and her colleagues included two fluorescent tags that only emitted light when brought into close proximity with each other by the acid-triggered shape change. The researchers used the I-switch to map spatial and temporal pH changes associated with endosome maturation in *Drosophila* blood cells and in *C. elegans*, demonstrating that DNA nanomachines could be used to measure real-time pH changes in living systems. Recently, the researchers developed another DNA nanodevice, called Clensor, to measure the activity and location of subcellular chloride channels and transporters in *Drosophila* blood cells.[9]

In addition to environmental stimuli, DNA constructs can be tailored to respond to more-specific signals, such as external DNA strands that bind specifically to a region of the nanostructure and induce conformational changes. Such biosensors are used for detection of nucleic acid or protein biomarkers and might one day aid in disease detection and treatment. One example is a DNA origami platform, developed by Seeman, that can detect single nucleotide polymorphisms. Researchers designed surface probes to bind to a target oligonucleotide, and if there is a mis-

DIAGRAM: ARUN RICHARD CHANDRASEKARAN; © ISTOCK.COM/SHUOSHU

match between the probe and the target, the origami displays the alphabet letter—C, G, T or A—corresponding to the mismatched nucleotide when imaged using an AFM.[10] DNA nanostructures tagged with aptamers—oligonucleotide sequences that bind to specific proteins, peptides, carbohydrates, and small molecules—can detect disease-related biomarkers and even cancer cells. (See "Antibody Alternatives," *The Scientist*, February 2016.)

DNA-based machines have also been developed to perform specific tasks. In 2009, Seeman's group created a nanoscale assembly line: a DNA walker was able to pick up gold nanoparticle cargos from three different stations on a DNA origami platform. The assembly line can be designed so that the walker can pick up any of eight possible combinations of cargoes from the three stations. In 2014, another group of researchers created a different type of walker that was capable of transporting nanoparticle cargos along carbon nanotubes. And last year, Hendrik Dietz's group at Technische Universität München in Germany used DNA origami components to create a rotary motor.[11] Such innovations can be used to better understand the functioning of cellular machinery, such as the ATP synthase motor, as well as to create nanoscale devices that could potentially perform therapeutic functions in vivo.

Drug delivery and bioimaging

One of DNA's main attractions is its biocompatibility. DNA has been used to build nanocages to house therapeutic cargoes, for example, and nanoscale robots functionalized with specific biomarkers can direct these structures to specific locations in the body, where they release their cargo upon binding to specific molecules. These constructs can be used to encapsulate fluorescent dyes, antitumor drugs, and peptide- and nucleic acid–based therapeutics such as siRNA and CpG sequences.

One such example is a hexagonal barrel-shape DNA origami that encapsulated antibody fragments in its cargo binding sites.[12] The two halves of this clamshell-like structure were connected at the rear by scaffold strand hinges. The front of the barrel contained a DNA aptamer "lock" that can be opened in the presence of the correct aptamer antigen (the "key"), thus providing a mechanism to release the encapsulated cargo upon reaching its target. One potential cargo is the well-known cancer therapeutic agent doxorubicin. Doxorubicin-loaded origami structures have been shown to effectively fight cancer both in human cell cultures and in mouse models. DNA origami triangular structures containing gold nanorods have also been designed to reach cancer cells in mouse models. Once at the target location, a near infrared (NIR) laser heats up the gold nanorods, thus killing the tumor cells—a process called photothermal ablation.

One of DNA's main attractions is its biocompatibility.

DNA nanostructures have also been useful for bioimaging purposes. Krishnan's group, for example, has assembled a DNA icosahedron from five-arm junctions that can host cargoes within its cavity. The researchers encapsulated a fluorescent biopolymer within the icosahedron and used the complex to track cellular uptake pathways in *Drosophila* blood cells as well as in the whole organism *C. elegans*.[13] The group used a similar strategy, but with quantum dots, to follow cellular uptake in HeLa cells. And last year, Chunhai Fan's group at the Shanghai Institute of Applied Physics in China used tetrahedral DNA nanostructures with attached signaling peptides to track entry into cell nuclei.[14] Such deliberate control over the entry and fate of DNA nanostructures in cells can spur the creation of more-efficient drug delivery pathways. While there are no DNA nanostructure–based drug delivery carriers yet in clinical trials, research towards this goal is being pursued.

DNA nanotechnology has grown out of its infancy and into adulthood. With the endless chemical strategies available to functionalize DNA strands, DNA nanostructures can host a variety of molecules and position them in predefined spaces to allow proximity-directed chemical reactions. As researchers continue to develop new techniques for building structures out of DNA, which can now be synthesized for as low as $0.001 per base pair, they will no doubt discover even more applications, in fields ranging from biology to medicine to biophysics. ■

Arun Richard Chandrasekaran is a senior scientist at Confer Health, Inc., which develops clinical-grade diagnostics for home use.

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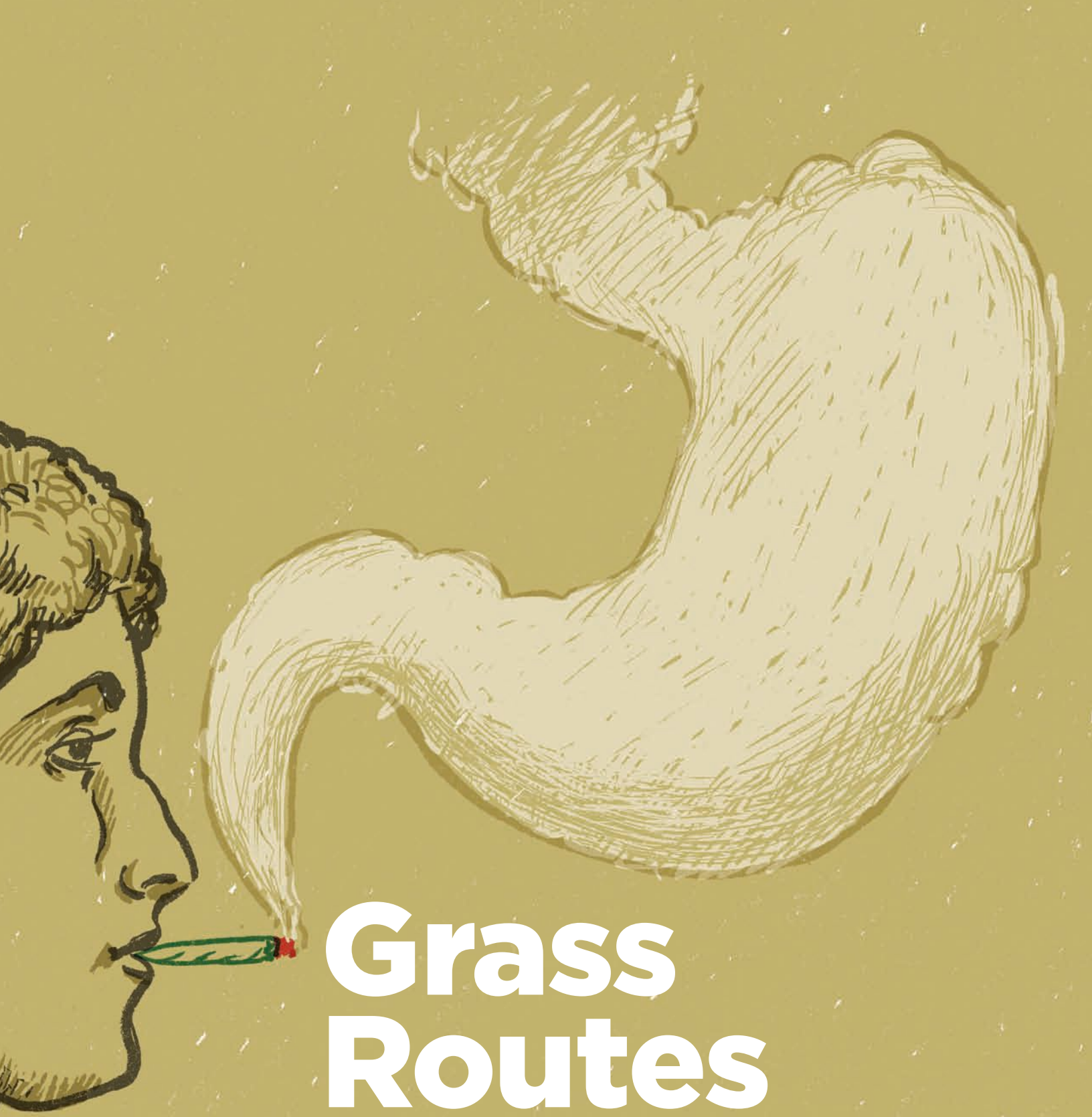


Grass Routes

Researchers are discovering a suite of new locations and functions of endocannabinoid receptors that play roles in sickness and in health.

BY MEGAN SCUDELLARI

europharmacology postdoc Nick DiPatrizio was stumped. His advisor, University of California, Irvine, researcher Daniele Piomelli, had discovered eight years earlier that hungry rats have high levels of endocannabinoids, endogenous molecules that bind to the same receptors as the active ingredient in marijuana.

Now, in 2009, DiPatrizio was trying to identify exactly where and how those molecules were controlling food intake in rats. But under specific feeding conditions, he couldn't locate any changes in endocannabinoid levels in the brain, which is flush with endocannabinoid receptors and the obvious place to look for behavioral signals.

Piomelli gently chastised his mentee. "He said, 'You're being neurocentric. Remember, there's a body attached to the head. Look in the other organs of the body,'" recalls DiPatrizio. So the young scientist persisted, and eventually discovered that hunger—and the taste of fat—leads to increased endocannabinoid levels in the jejunum, a part of the small intestine. Endocannabinoid signaling in the gut, not the brain, was controlling food intake in the rodents in response to tasting fats.[1]

The evolution of endocannabinoid research has mirrored DiPatrizio's early thinking: ever since the first endocannabinoid receptor was identified in the late 1980s, the field has been overwhelmingly focused on the central nervous system. The main endocannabinoid receptor, CB_1, was first discovered in a rat brain and is now known to be among the most abundant G protein–coupled receptors in neurons there. Plus, cannabis is well-known for its psychotropic effects. "That has led the research field to be very CNS-oriented," says Saoirse O'Sullivan, who studies endocannabinoids at the University of Nottingham in the U.K.

But recent work has provided evidence that the endocannabinoid system—a family of endogenous ligands, receptors, and enzymes—isn't exclusive to the brain. It is present everywhere in the body that scientists have looked: the heart, liver, pancreas, skin, reproductive tract, you name it. And disrupted endocannabinoid signaling has been associated with many disorders, including diabetes, hypertension, infertility, liver disease, and more. "There is so much that's still unknown about this system. It looks to be regulating every physiological system in the body," says DiPatrizio.

Now an assistant professor at University of California, Riverside, School of Medicine, DiPatrizio has trained his whole research program on the gut, where the endocannabinoid system appears to be a major player in human health and disease. In January, his lab suggested that endocannabinoid signaling in the gut drives the overeating characteristic of Western diets. In a rodent model, chronic consumption of a high-fat and high-sugar diet led to elevated levels of endocannabinoids in the gut and blood, promoting further consumption of fatty foods. Blocking endocannabinoids from their receptors decreased overeating in the animals, his team found.[2]

Because of that link to appetite, pharmaceutical companies have sought to target the endocannabinoid system to create the ultimate diet pill, a drug to reduce appetite or treat metabolic disorders. Those efforts have recently been subdued by two tragic and highly visible failures. (See "On Trial, Off Target" on page 38.) But some scientists still hope that by understanding the true nature of this system, they might identify new treatments, especially for conditions related to gut health and metabolism.

"We are now at a point where you have to understand how [endocannabinoids] can be so relevant in so many areas—literally everywhere in the body," says Mauro Maccarrone, head of biochemistry and molecular biology at Campus Bio-Medico University of Rome, Italy, who has studied the molecules since 1995. "There must be a reason why these endocannabinoids are always there."

It has been known for some time that the brain can modulate the gut. With endocannabinoids, it appears the gut can also modify the brain.

In the weeds

Researchers describe the endocannabinoid system as the most complicated and most ubiquitous signaling system in our bodies, yet no one knew it was part of human physiology until the 1980s. And that realization came from an unusual source—an oft-derided effort to understand how marijuana gets us high.[3]

In 1964, researchers seeking to understand the psychoactive component of the cannabis plant identified the compound Δ^9-*tetrahydrocannabinol,* or THC.[4]

More than two decades later, in 1988, investigators found the first direct evidence of an endogenous signaling system for THC—a receptor in the rat brain that bound a synthetic version of THC with high affinity.[5] Blocking the receptor with a chemical antagonist in humans effectively blocks the high typically experienced after smoking marijuana.

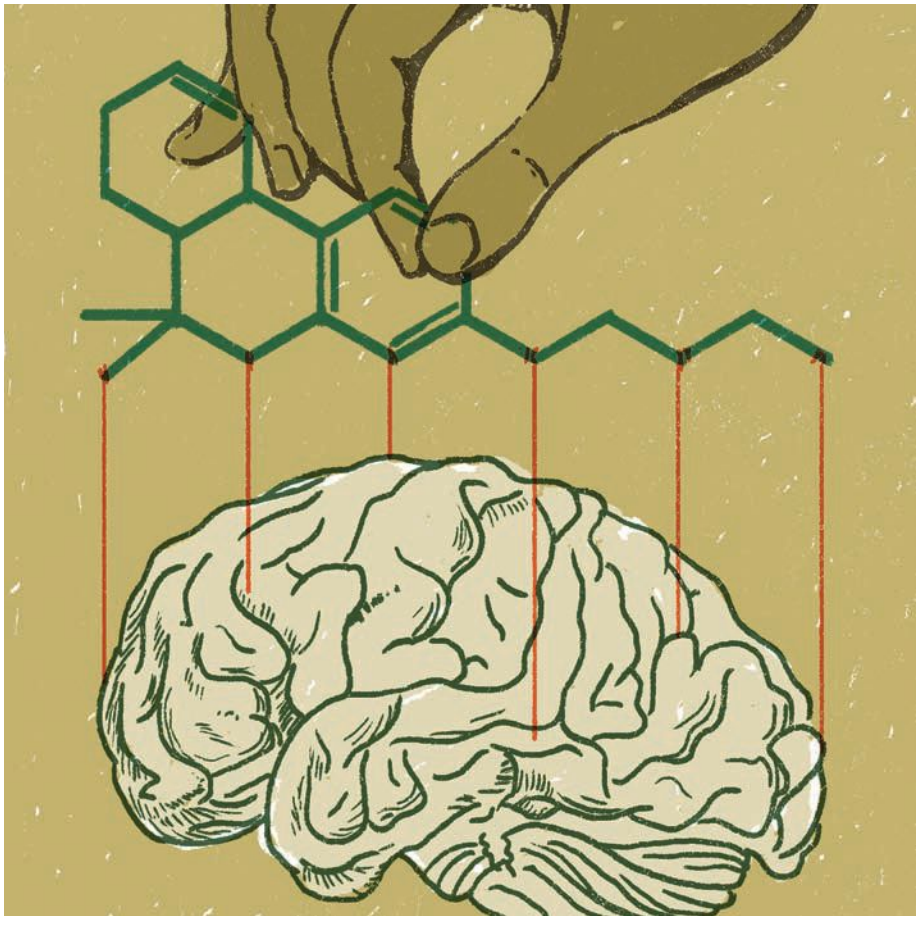
The receptor, called CB_1, was subsequently identified in other mammalian brains, including those of humans, and appeared to be present in similar density to receptors for other neurotransmitters, including glutamate, GABA, and dopamine.[6] A second cannabinoid receptor, CB_2, was discovered in 1993.[7] This receptor was first isolated in the rat spleen. That surprising finding was an omen of things to come; the endocannabinoid system functions far afield from the brain, practically everywhere in the body.

The presence of these receptors sparked a quest to find natural ligands that bind to them. The first endocannabinoid identified, a fatty acid-based agonist for both receptors, was named anandamide, based on the Sanskrit word *ananda* meaning "inner bliss." A second agonist, 2-arachidonoylglycerol (2-AG), did not get so groovy a name, but did appear to be present at high levels in normal mammalian brains.

By 1995, the so-called "grass route" was complete: over three decades, researchers had identified THC, its endogenous receptors, and endogenous ligands for those receptors. Maccarrone suspects that endocannabinoids are among the oldest signaling molecules to be used by eukaryotic cells. His team recently showed that anandamide and its related enzymes are present in truffles, delectable fungi that first arrived on the evolutionary scene about 156 million years ago, suggesting endocannabinoids evolved even earlier than cannabis plants.[8]

"They are kind of a master signaling system, and other signals have learned to talk to these lipids," says Maccarrone. In the brain, endocannabinoids interact with other neurotransmitters; in the reproductive tract, with steroid hormones; in the muscles, with myokines; and so on.

But even though researchers have documented the existence of the endocannabinoid system throughout the body, they still don't really know what role it plays outside the brain, where it is involved in synaptic signaling and plasticity. In healthy, non-obese animals, there is typically no consequence to knocking out endocannabinoid receptors in peripheral organs. "There is

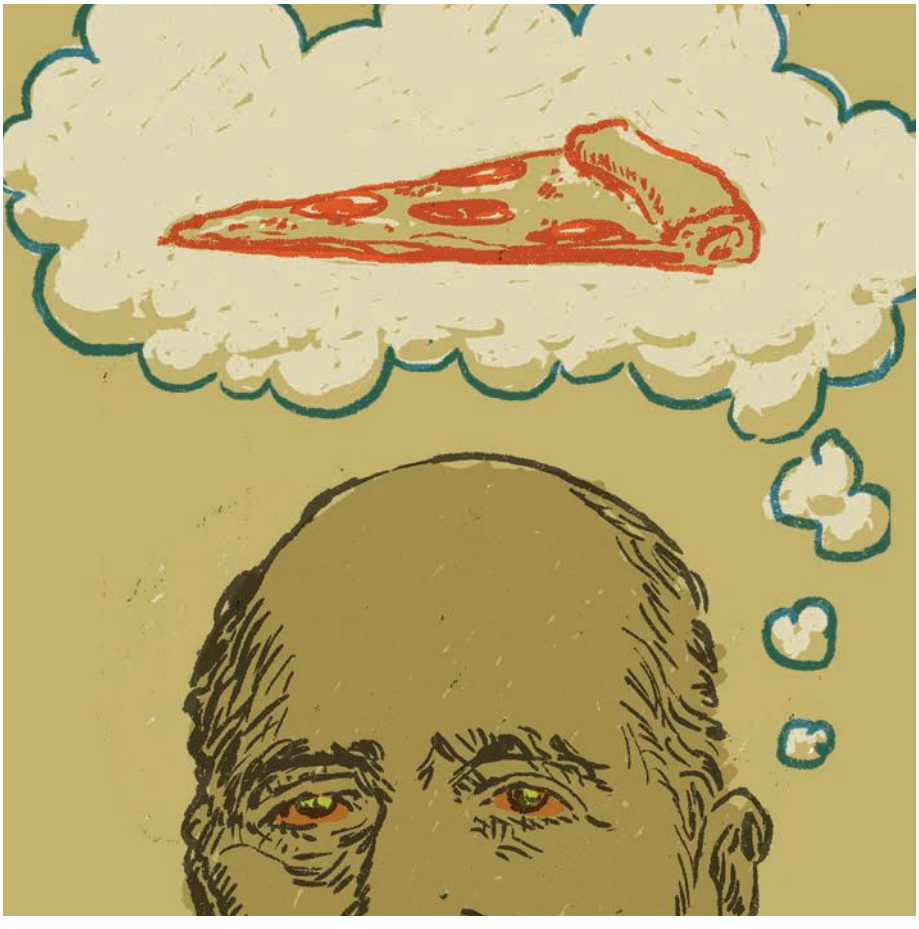


> We are now at a point where you have to understand how endocannabinoids can be so relevant in so many areas—literally everywhere in the body.
>
> —Mauro Maccarrone
> Campus Bio-Medico University of Rome

no detectable effect on any important biological function," says George Kunos, scientific director of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) at the National Institutes of Health.

What's the buzz?

The one exception to this functional black box is the gastrointestinal tract. The idea that cannabis—or, by extension, endogenous cannabinoids—affects the gut is not surprising. Preparations derived from marijuana plants have long been used to treat digestive conditions such as inflammatory bowel disease and vomiting. Even before CB_1 was discovered, scientists had suggested that cannabinoids regulate the motility of the gastrointestinal tract—the orchestrated movements of muscles that churn and move food through the intestines. For example, in 1973, Australian researchers showed that oral ingestion of THC slowed the passage of a meal through the intestines of mice.[9] Conversely, knocking out parts of the system is associated with increased movement of food through the colon, a common symptom of irritable bowel syndrome (IBS). These pathways are conserved among many species.[10]



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Both CB_1 and CB_2 receptors are present and active in the gut, though they appear to be involved in different gut functions. At the University of Calgary, Keith Sharkey and colleagues found that increased intestinal motility in the inflamed gut was reversed when CB_2 receptors, but not CB_1 receptors, were activated.[11]

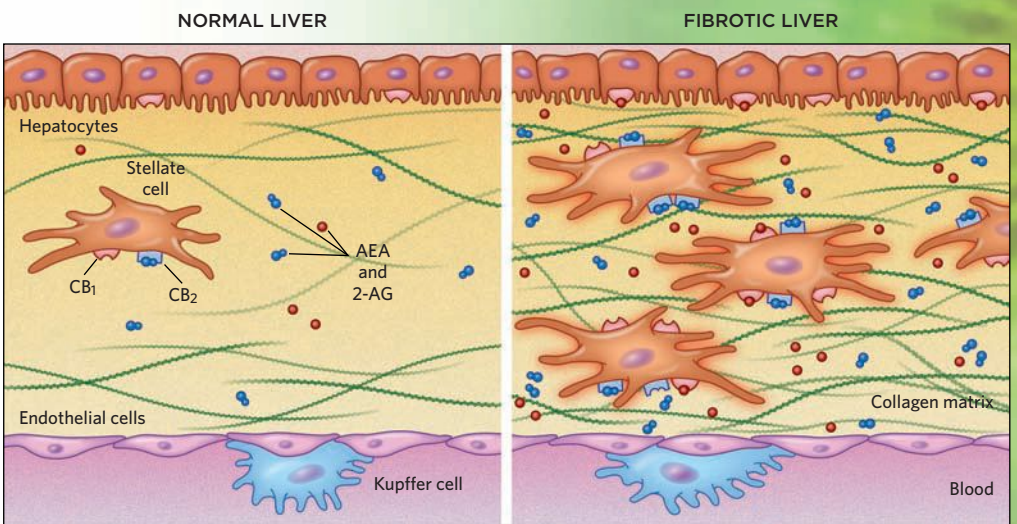
To make things even more complicated, there is a group of nonclassical receptors that interact with endocannabinoids in the gut, says Jakub Fichna, head of the department of biochemistry at the Medical University of Lodz in Poland. His lab studies the role of these receptors in inflammatory bowel disease (IBD) and IBS. Depending on the conditions in the gut, some of these nonclassical receptors don't even need an agonist or antagonist to become active, Fichna says. "It can even be the change in pressure or pH of the neighborhood. For example, if you have inflammation, most of the time you have decreasing pH, and this is already enough for some of the endocannabinoid receptors to be activated."

Endocannabinoids and their receptors also appear to be involved in gastric secretions, ion transport, and cell proliferation in the gut. And then there is appetite. Marijuana users often experience the "munchies"—a sharp and sudden increase in appetite after inhaling or ingesting the drug. Kunos wondered whether endocannabinoids cause a similar increase in appetite. In 2001, with the help of collaborators, he confirmed the suspicion: endocannabinoids acting on CB_1 receptors promoted appetite, and mice with CB_1 receptors knocked out ate less than their wild-type littermates.[12]

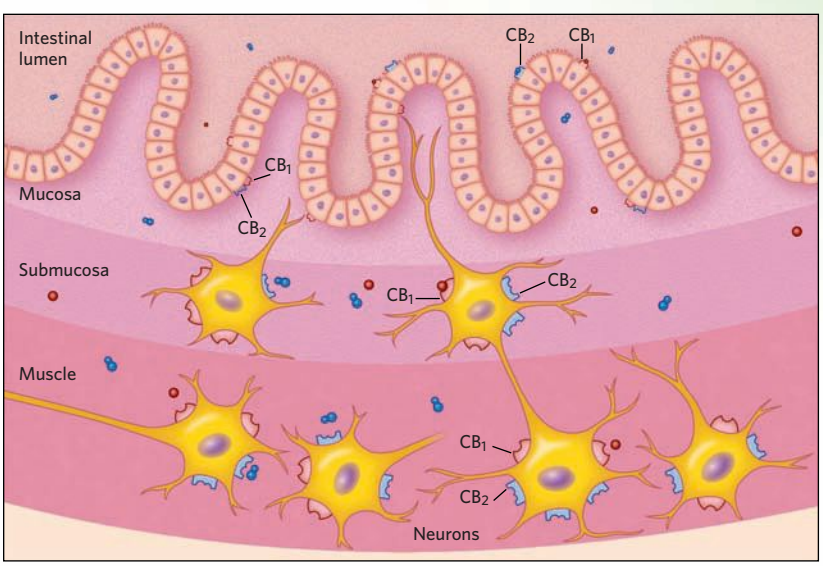
Additional research has supported that idea that endocannabinoids act as a general appetite-promoting signal. And as DiPatrizio's work showed, endocannabinoids control food intake not exclusively via the brain, but by way of signals gen-

ENDOCANNABINOID SIGNALING IN THE BODY

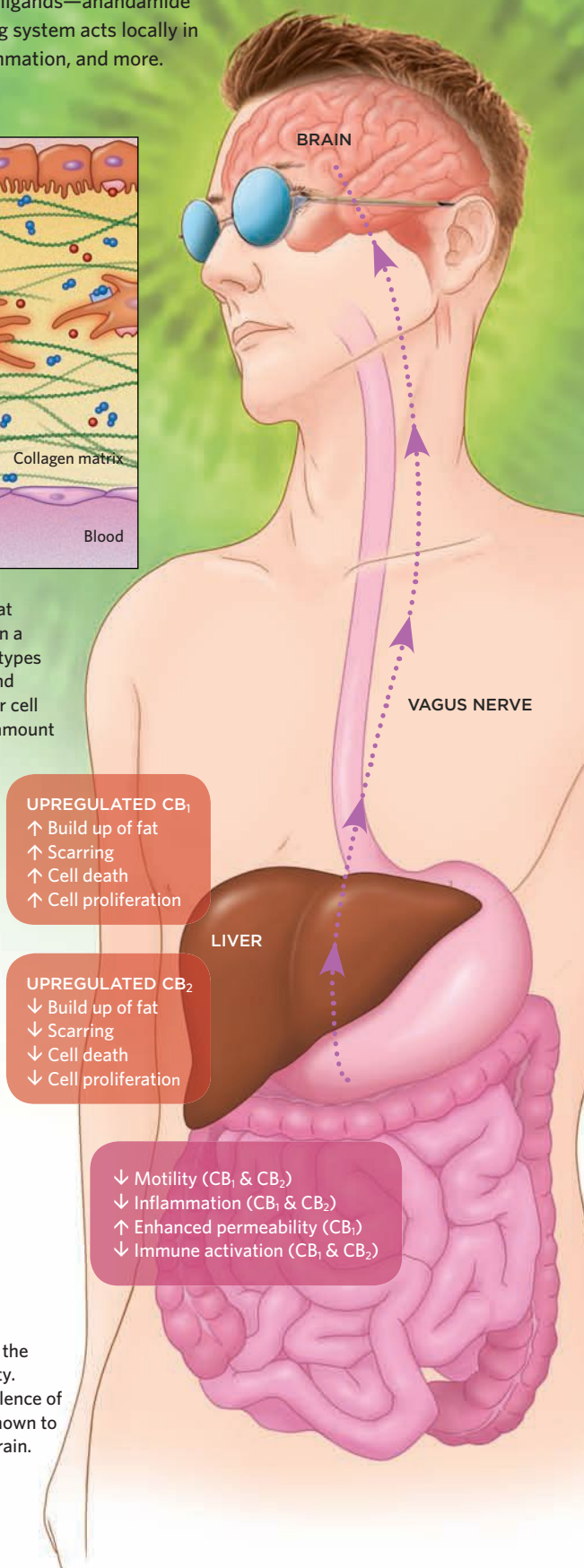
The two classical cannabinoid receptors, CB_1 and CB_2, are expressed by enteric neurons, immune cells, and other cell types within the gastrointestinal tract. The gut and the liver also synthesize two key ligands—anandamide (AEA) and 2-arachidonoylglycerol (2-AG)—for those receptors. Combined, this signaling system acts locally in the gut and liver, but also communicates with the brain to affect food intake, pain, inflammation, and more.



In the liver, endocannabinoids are thought to act almost like hormones, stimulating cell division at some times, cell death at others. In the healthy liver, expression of CB receptors is very low, but in a diseased liver expression increases, and endocannabinoid ligands are released from all four cell types shown here. Many ligands are produced and bind to CB_1 receptors, causing lipid accumulation and insulin resistance in hepatocytes, and increased proliferation of activated stellate cells, the major cell type involved in fibrosis (scarring) of the liver. Blocking CB_1 receptors with drugs decreased the amount of fibrosis in mouse models.



Both CB_1 and CB_2 regulate the rhythmic contractions of the intestinal tract, called gut motility. In the healthy gut, CB_1 predominates, but during intestinal inflammation, CB_2 also contributes to motility. Conditions such as inflammatory bowel disease and celiac disease often exhibit increased prevalence of these receptors, which results in decreased motility. Endocannabinoid signaling has also been shown to reduce inflammation, increase the permeability of gut epithelial cells, and signal hunger to the brain.



erated in the gut. It's a simple hypothesis with big implications for the management of obesity and other metabolic syndromes.

During his postdoc, DiPatrizio found that when rodents tasted dietary fats (just tasted, not swallowed), endocannabinoid levels increased in the rat small intestine—and nowhere else in the body. A CB_1 receptor antagonist blocked that signal, leading the rodents to decrease their ingestion of fatty foods. "This suggests to us that this is a very important and critical mechanism that drives food intake," says DiPatrizio.

From an evolutionary perspective, having a positive feedback mechanism for fat intake makes sense, he adds. When an animal in the wild detects high-energy foods, it is beneficial to stock up. However, that's not true for people in today's developed countries. "There's no period of famine. It's feast all the time, so now the system can drive us to overconsume," says DiPatrizio.

Sharkey sees the system as a regulator of homeostasis within the body, especially considering its roles in maintenance of food intake, body weight, and inflammation. "It seems to be very important in the conservation of energy," says Sharkey. "But in modern Western society in particular, those are the things that appear to have been dysregulated."

Times of trouble

Although the job of the endocannabinoid system remains mysterious in healthy tissues outside the brain and gut, diseases reveal clues. In obesity, both CB_1 and CB_2 receptors are upregulated throughout the body, including in the liver and in adipose tissue. And the activation of CB_1 receptors increases food intake and affects energy metabolism in peripheral tissues. In type 2 diabetes, endocannabinoids and their receptors are upregulated in circulating macrophages and contribute to the loss of pancreatic beta cells, which store and release insulin.

Interestingly, chronic marijuana users have no documented increased incidence of diabetes or obesity. Researchers speculate this is because chronic use results in downregulation of CB_1 receptors—a form of pharmacological tolerance. Another

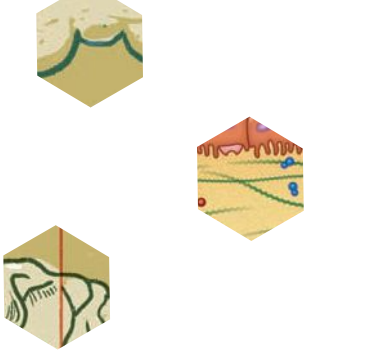
ON TRIAL, OFF TARGET

The endocannabinoid system has proven a tantalizing, if elusive, target for the pharmaceutical industry, especially for conditions related to appetite and gut health. Sanofi-Aventis was the first to market an antiobesity drug targeting endocannabinoid receptors. In 2006, the European Commission approved the CB_1 antagonist rimonabant (Acomplia) as a treatment to curb hunger. It did so effectively, but as a wider population of people began using it, dangerous side effects emerged. A small percentage of users suffered from serious psychiatric symptoms, including suicidal thoughts.[17] In 2008, the European Medicines Agency recommended suspension of the drug, and the company withdrew it from the market.

That withdrawal halted the development of the whole class of CB_1 antagonists, says George Kunos, scientific director of the National Institute on Alcohol Abuse and Alcoholism. Yet the side effects should have been predictable, he argues, as CB_1 receptors play an important role in brain reward pathways. Blocking them, he says, therefore is likely to cause an inability to feel pleasure.

Last January, the field was dealt a second blow. In France, six participants in a Phase 1 study of a compound known as BIA 10-2474 were hospitalized with neurological symptoms. Portuguese pharmaceutical company Bial was developing the drug as a candidate to treat a number of neurological disorders, including anxiety. But within days of receiving multiple daily doses of the drug, one participant was declared brain-dead, while others developed severe lesions on their brains.

BIA 10-2474 is an inhibitor of fatty acid amide hydrolase (FAAH), a key enzyme that breaks down endocannabinoids. Researchers had hoped that by targeting a downstream part of the endocannabinoid system, rather than the receptors themselves, they might avoid off-target effects in the brain and elsewhere. That was not the case. "That, again, scared regulators and the industry away from consideration of that system," says the University of Calgary's Keith Sharkey, who was not involved in the trial. There is still potential for drug development in the field, he emphasizes, but only under carefully controlled conditions with drugs that can be restricted to specific sites of action.



possibility, explored by Sharkey and colleagues in 2015, is that chronic THC exposure alters the gut microbiome, affecting food intake and preventing weight increase.[13] In liver disease, upregulation of CB_1 appears to contribute to cell death and the accumulation of scar tissue (fibrosis).[14] (See "Endocannabinoid Signaling in the Body" on page 37.)

Yet there remains debate as to whether endocannabinoid receptors are always the bad guys in disease. In some cases, endocannabinoid signaling even appears to be therapeutic. Animal studies suggest endocannabinoids are effective pain relievers, and the system has anti-inflammatory properties in certain contexts. In IBD, Sharkey's team found that activation of both CB_1 and CB_2 receptors resulted in reduced inflammation, suggesting the system may be activated as a protective force. Likewise, CB_2 activation appears to be anti-inflammatory in cases of atherosclerosis, says O'Sullivan, who focuses on endocannabinoids in the cardiovascular system. "It's a bit of a rescue receptor," she says. "In times of trouble, it gets upregulated." And several tantalizing studies suggest cannabinoids—from plants or from synthetic compounds that mimic botanical molecules and the body's own—might directly inhibit cancer growth by inducing cell death in tumor cells.

But the very thing that makes the endocannabinoid system so interesting—its ubiquity and varied roles in the body—is also what makes it a difficult drug target. Within the last 10 years, two drugs targeting the endocannabinoid system proved to have dire side effects in humans when the compounds crossed the blood-brain barrier. Off-target effects in other organ systems could also have long-term consequences, such as damage to a young woman's reproductive system. Indeed, in a recent review of the pharmacology of 18 different CB_2 ligands as potential drug candidates, Maccarrone and a large team of European researchers, in collaboration with Roche, concluded that just three of the compounds (none of which were developed by Roche) merited additional preclinical or clinical studies.[15] Many of the other compounds engendered too many off-target effects.

Researchers are now working toward second-generation drugs that more specifically target peripheral systems. "If the scientific community faces the challenge of really understanding how to direct certain drugs to the right target, then we could have wonderful drugs for the future," says Maccarrone. Most of those compounds are in preclinical trials, though Kunos hopes to have an Investigational New Drug approval from the US Food and Drug Administration soon for one agent his team has been working on as a possible treatment for nonalcoholic fatty liver disease. The compound does not penetrate the brain and is designed to accumulate in the liver, which may explain its efficacy in treating liver disease without causing psychiatric side effects in animal models, says Kunos.[16]

If researchers can figure out how to avoid the devastating off-target effects, there is one more reason why endocannabinoids may effectively help treat disease: they provide an indirect link to the brain. "We've known, for some time, that the brain can modulate the gut," says Sharkey. With endocannabinoids, it appears the gut can also modify the brain. It is now clear, for example, that there are very active communication pathways originating from peripheral nerves in the gut that are able to modulate brain function. Numerous studies suggest the vagus nerve is a major information highway between the gut and brain.

DiPatrizio is studying those communication pathways and hopes to identify ways to regulate feeding without ever getting near the brain with a drug. The research complements other evidence showing that the gut is able to modulate proinflammatory cytokines in the blood and even influence central nervous system disorders.

"We believe we can remotely control the brain from the gut, safely," says DiPatrizio. "That's why, once again, [endocannabinoid receptors] are very attractive targets." ■

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Round and Riveting

Recent research has revealed many surprises about circular RNAs, from findings that they are translated in vivo to links between their expression and disease.

BY CATHERINE OFFORD

RNA comes in many shapes and sizes. Over the past few decades, researchers have characterized at least two dozen different RNA varieties beyond the textbook classics. But a type of RNA that long flew under the radar due to its designation as a molecular mishap is now taking center stage.

Circular RNAs (circRNAs), or simply "circles" to many researchers, are just what they sound like: nucleotides of RNA arranged in a closed loop. Much about the function of these molecules remains a mystery, but for some time, at least one thing seemed clear: unlike linear messenger RNA (mRNA), circles were not translated into proteins in living organisms. "When you have any type of RNA, you wonder whether it's translated," says Sebastian Kadener, a molecular biologist who has spent the last few years researching circRNA at the Hebrew University of Jerusalem. Despite reporting the presence of one or more protein-coding exons in many circRNAs, multiple studies in the past few years failed to find evidence of the molecules associating with ribosomes in vivo. If circles were doing anything at all, many researchers agreed, they must be doing it as untranslated RNA.

A little over a year ago, however, Kadener and his colleagues detected something that would upend that assumption: an average-size (37 kilodaltons) protein encoded by a naturally occurring circRNA in *Drosophila*. Along with collaborators in Germany, Kadener's group used a method known as ribosomal footprinting to detect RNAs being actively translated in extracts from fly heads. Not only did the researchers discover more than 100 different circRNAs—ranging from around 300 to more than 2,000 nucleotides in length—apparently associating with ribosomes in the cells, they also identified a protein that, based on its sequence, could only have been translated from one of these circles, not from a standard linear transcript. "We could see the protein by Western blot," Kadener says. "It was being expressed in the synapses of flies."

Kadener's work was published earlier this year,[1] back-to-back in *Molecular Cell* with another group's study—on human and mouse cells—that had simultaneously come to the same conclusion: translation of circRNAs can and does occur in living cells.[2] For now, neither group has any hint of the function of these proteins, or of how common circRNA translation really is, but "you can imagine that it has some biological importance," Kadener notes. RNA researcher William Jeck, currently a fellow at Harvard Medical School, agrees. Many scientists had "written off translation," he says. "This is extremely exciting evidence that other circles may produce peptides that may be biologically relevant. . . . It's really changed the paradigm."

When it comes to circRNAs, though, such paradigm shifts are par for the course. First observed in electron micrographs of eukaryotic cells taken in the 1970s, circRNAs were for decades con-

sidered posttranscriptional mistakes expressed at low levels in the cell—perhaps the results of splicing gone wrong, generated when an exon's two ends are covalently joined together instead of to adjacent exons. But all that changed when Julia Salzman and colleagues at Stanford University set out to identify all types of RNA in human cells using an unbiased approach—one that diverged from standard methods by including RNAs that lacked polyA tails.[3] In 2012, the team discovered thousands of circles using this method. What's more, "we reported that there were hundreds of circular RNAs that were more abundant than their cognate linear transcripts," Salzman tells *The Scientist*. "I think people were in a bit of disbelief."

Around the same time, other labs were finding additional evidence to contradict the view of circular RNA as merely cellular "noise." Within the year, Jeck, then at the University of North Carolina School of Medicine, and colleagues reported that at least one out of every eight genes expressed in human fibroblasts gave rise to circRNAs.[4] "We were frankly shocked finding even one circular RNA," Jeck recalls. "We thought it was a fascinating novelty." The group also found that many circRNA sequences were highly conserved between humans and mice. And shortly afterward, two more groups published further evidence of circRNAs' abundance in humans and mice, and additionally in nematode worms.

Now, research on circRNAs is exploding, and the molecules' biogenesis is gradually becoming clearer. At least two proteins, Muscleblind and Quaking, have been linked to circle formation, which generally occurs when the cell's splicing machinery connects a downstream splice donor to an upstream splice acceptor, such as joining an exon's 5′ end to its own 3′ end or an upstream exon's 3′ end, in a process known as backsplicing. Recently, several additional mechanisms have been proposed (see "Making the Rounds" on page 44), and some circRNAs contain introns, either instead of or in addition to exons. Regardless of their genetic makeup, the lack of ends makes circles less vulnerable to exonuclease enzymes, allowing them to persist in cells for days, unlike their linear counterparts, whose life spans are measured in hours or minutes.

First observed in electron micrographs of eukaryotic cells taken in the 1970s, circRNAs were for decades considered posttranscriptional mistakes.

Despite a growing appreciation for the abundance—and now translation—of circRNAs in eukaryotes, there's still very little understanding of what exactly circRNAs do. "We don't even know how much of it is functional," says Jeremy Wilusz, an RNA researcher at the University of Pennsylvania Perelman School of Medicine. "What's the point of these circles? Why are they made?"

Salzman agrees that the topic is still wide open. "You can speculate to your heart's desire. There is currently no consensus about what they do."

In search of a function

Whether or not circRNAs are translated, it's possible that the vast majority of circles do nothing at all. "It's crazy to assume they're doing something just because they're there," says Nikolaus Rajewsky, an RNA researcher at the Max Delbrück Center for Molecular Medicine in Berlin who collaborated on both *Molecular Cell* papers. "The null hypothesis is that they're not doing anything." He adds that although thousands of circRNAs are expressed in various tissues, few are expressed at levels that are likely to be particularly biologically relevant. "It's not like there are thousands or millions of circles everywhere and they're all important," he says.

But there are certainly reasons to believe that at least some circRNAs are more than just molecular accidents. In addition to the fact that many circRNAs are conserved across species, research suggests that circularization is regulated. If circles were merely by-products of normal splicing, their levels might correlate with levels of linear transcripts expressed from the same gene, Salzman says. But in 2013, her group found that different cells showed different ratios of circular to linear transcripts from the same gene—although how the relative stability of each RNA molecule contributes to the overall balance remains to be determined.[5]

A couple of years later, Rajewsky's team published hints that circRNAs play a role in the nervous system, showing that many circles in humans and mice are highly expressed in neural tissue, upregulated during neuronal differentiation, and enriched at synapses. "We looked at exactly what circles are expressed," he says. "Our data indicate that we're talking about a few hundred really interesting candidates in the brain." Many of these candidates are tissue-specific—with some circles enriched in the cerebellum, for example, and others in the cortex—and are expressed only at certain stages of neuronal development.[6]

Collectively, the studies hint at the functionality of circRNAs, but the exact nature of their roles has largely eluded researchers—though there have been a few tantalizing clues. In 2013, researchers discovered that some circRNAs act as molecular "sponges," soaking up large quantities of specific microRNAs—tiny, noncoding molecules about 20–25 nucleotides in length. That year, two studies—one by Thomas Hansen of Aarhus University in Denmark and colleagues and one by Rajewsky's group—simultaneously reported that a circRNA transcribed from the antisense strand of the

human *CDR1* gene and highly expressed in the brain, called CDR1as by Hansen and ciRS-7 by Rajewsky, has dozens of binding sites for a microRNA known as miR-7.[7,8] Hansen's group also showed that another circRNA, transcribed from the sex-determining region Y (*Sry*) gene and expressed in mouse testes, could bind microRNA miR-138.

Because microRNAs are involved in regulating translation—by binding to specific mRNAs, they trigger degradation of transcripts through a process known as RNA interference (RNAi)—Hansen and his colleagues speculated that the findings might indicate a general role for circles in regulating gene expression. "At that time, we were searching for other circular RNAs, but pipelines for detection weren't really established," Hansen tells *The Scientist*. When they found similar roles for two circRNAs, "we didn't know, but of course we hoped that it could be a general thing, that circular RNAs would emerge as [regulators] of these micro-RNAs—it made a lot of sense."

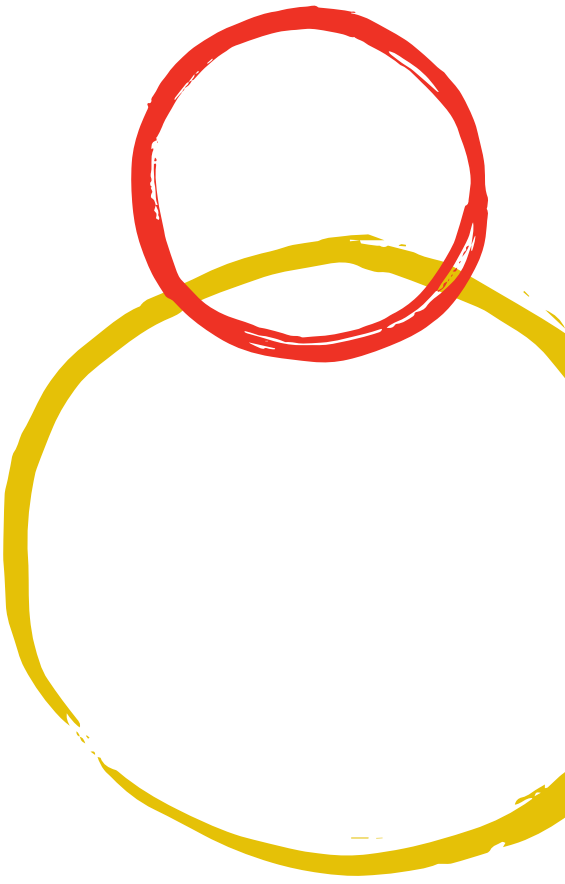
But researchers now believe that most circles are unlikely to act as microRNA sponges. As the number of known circRNAs has climbed into the thousands, only a handful of sponges have been identified. And a 2014 study using computational methods to predict sequences likely to make good sponges identified only a few other candidates.[9] The authors of that study "made a strong case that it wasn't a general function," says Salzman. Instead of sponging, circRNAs may be engaging in other types of microRNA interactions, Hansen notes. "I think [circles] could have more profound effects in terms of stabilizing [microRNA], or directing it to certain parts of the cell—although that's of course hypothetical at the moment."

CircRNAs also appear to associate with proteins, suggesting another suite of potential regulatory functions. For example, researchers recently showed that a circRNA produced by the *Foxo3* gene (called circ-Foxo3) interacts with proteins involved in cell proliferation, including a key cyclin-dependent kinase and one of its inhibitors, suggesting a role in the cell cycle. And while most exon-containing circles accumulate in the cytoplasm, those that retain introns are often found in the nucleus, where they encounter proteins involved in transcription. In 2015, scientists in China showed that a group of exon-intron circRNAs promoted transcription of their parent genes via interaction with RNA polymerase II.[10] Other studies have shown circles interacting with different RNA-binding proteins as well, including proteins now linked to circRNA biogenesis, such as Muscleblind and Quaking, and Argonaute proteins, well-known for their participation in RNAi-based gene regulation.

There's the possibility that the regulation of circRNA biogenesis itself constitutes a function, too. Because each RNA transcript can be either linear or circular, but not both, upregulating circularization could act as a mechanism to reduce the proportion of linear mRNA generated from a particular gene. A recent study by Rajewsky and Kadener showed that strong competition between circularization and linear splicing can occur, most likely due to overlapping dependence on the same splicing machinery—although the extent to which it constitutes a function per se is still unclear.[11]

With the recent description of in vivo translation of circRNAs comes an entirely new dimension of possible functions—one that researchers are only beginning to explore. "I'm sure that people are now going to be looking to see when these proteins are produced, where these proteins are produced, et cetera," says Kadener, adding that his team plans to further investigate the role of translated circRNAs in *Drosophila* brain function. "You can imagine so many hypotheses of what this translation might mean. . . . The protein made by the circle could modulate other proteins, for example. It opens a lot of possibilities."

Like all of the speculation about circRNA function, though, hypotheses about translation will have to be pursued with a healthy dose of skepticism, notes Wilusz. "It's certainly a very attractive idea," he says. "It would make sense in some way, that if you're making [a circRNA] from a protein-coding gene, you should make a protein. But there's a lot more work that needs to be done to prove that the proteins are being produced at high levels—or even do anything."



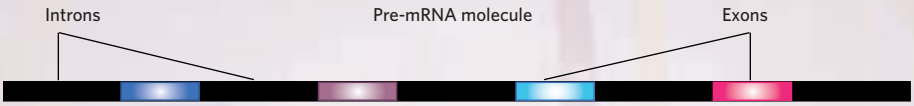
Putting circles to use

As RNA researchers continue to explore circles' possible functions, multiple labs have discovered that circRNA expression levels vary substantially with disease, leading to growing interest in how these molecules might be harnessed for diagnosis and treatment. Certain circRNAs are up- or down-regulated in cancers of the skin, liver, bladder, larynx, and stomach, to name a few. And it's not just cancer; abnormal expression of several circRNAs has also been linked to cardiovascular disease and to neurological disorders such as Alzheimer's and Parkinson's.

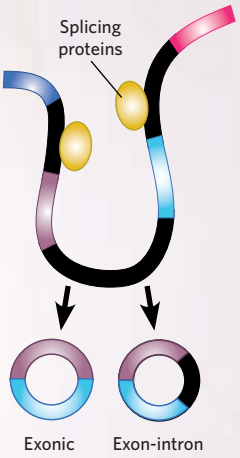
CDR1as, for example—one of the original microRNA sponges and the best-studied circle to date—is linked to a number of diseases, in several cases via its sequestration of miR-7. A well-characterized tumor suppressor, miR-7 inhibits cell growth, and its loss is associated with poor prognosis. "High expression of CDR1as is not very good in terms of cancer," Hansen explains, "because it inhibits the microRNA that would normally protect from cell prolifer-

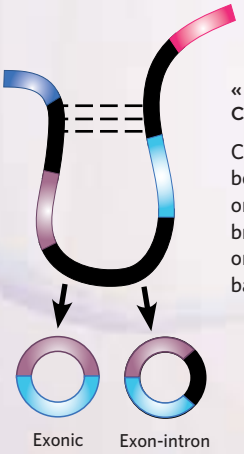
MAKING THE ROUNDS

Circular RNA biogenesis occurs when RNA fragments are bent into a closed loop of one or more exons and/or introns. This often occurs as the pre-mRNA molecule is processed into its final transcript via splicing, in which introns are removed and exons are linked together. Most circular RNAs are thought to be formed by a process called backsplicing, which joins one end of an exon to the other, or to an upstream exon, forming a circle. Researchers have recently published several models—not all of them necessarily mutually exclusive—to explain how different parts of the RNA molecule are brought into close proximity, encouraging backsplicing and turning a linear sequence into circular RNA.



In a general backsplicing model, proteins assemble to form the spliceosome that processes transcribed RNA. But instead of splicing exons together in a linear sequence, they join the end of one exon to the beginning of the same exon or to an upstream exon. Below are three mechanisms that can drive this backsplicing.



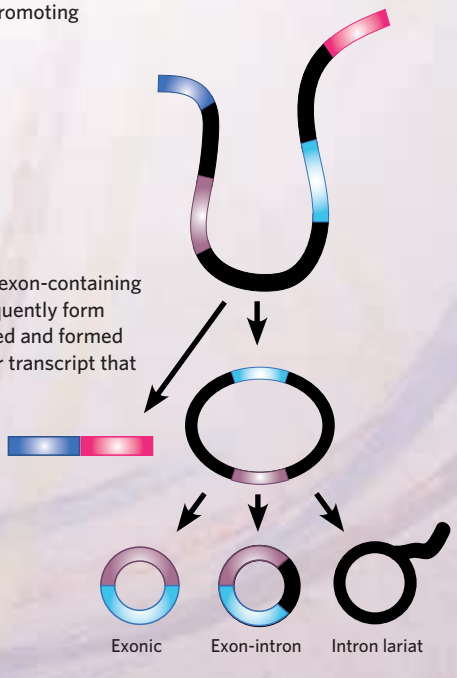


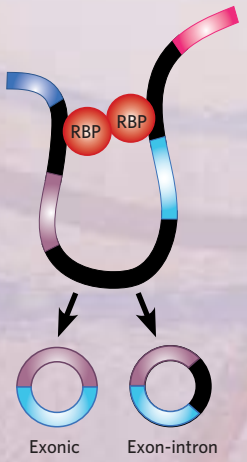
« INTRON-PAIRING-DRIVEN CIRCULARIZATION

Complementary base pairs formed between long intronic sequences on different parts of the transcript bring together different splice sites on an RNA molecule, promoting backsplicing.

LARIAT-DRIVEN CIRCULARIZATION »

Splicing proteins "skip" some exons, creating an exon-containing lariat—a lasso-shaped structure that introns frequently form during standard splicing. This lariat can be excised and formed into a circle while the leftover RNA forms a linear transcript that lacks the skipped exons.





« PROTEIN FACTOR–MEDIATED CIRCULARIZATION

RNA-binding proteins (RBPs) such as Quaking bind to sequences on either side of exons and pull these sequences into proximity with each other, forming circles even in transcripts that are normally spliced in a linear fashion.

ation." Looking beyond cancer, researchers in China reported in 2015 that overexpression of miR-7 in pancreatic islet cells led to impaired insulin production and diabetes in mice—an outcome the team suggested was normally kept in check by the sponging activity of CDR1as.[12] And reduced expression of CDR1as in the hippocampus has been associated with Alzheimer's disease.

Another disease-linked circular RNA, circTCF25, also appears to act as a microRNA sponge. Expressed at high levels, circTCF25 downregulates two microRNAs, leading to cancer cell proliferation in vitro and in vivo in humans—mechanisms that could explain the link between high circTCF25 levels and bladder cancer. And earlier this year, researchers described a complex pathway in which peptide-binding circ-Foxo3—downregulated in several cancers—regulates proteins involved in cancer cell death. The team showed that through interactions with several peptides, circ-Foxo3 increases levels of its parent gene's protein, Foxo3, which can trigger apoptosis in tumor cells.

These glimpses into circRNA's role in disease have sparked interest in exploiting the molecules as potential therapeutic targets. A study published earlier this year noted that silencing CDR1as using specially-designed short hairpin RNAs (shRNAs) inhibited proliferation and invasiveness of colorectal cancer cells in culture. And a team at Mount Sinai School of Medicine in New York used similar methods to target ciRS-E2, a circle consisting of a single exon that is highly expressed in cancers such as leukemia and melanoma. The group reported that shRNA treatment dampened ciRS-E2 expression by more than 80 percent in cultured cancer cells, and resulted in significantly reduced proliferation.

For now, though, while functions for the vast majority of circRNAs remain unclear, many labs are focused on exploring the more immediate goal of using circles to classify and monitor diseases with which they are associated. For example, "we're all very interested in trying to find ways to divvy up tumors into different categories of risk and potential response to

therapy," says Jeck. "CircRNAs do have one really nice feature, and that's that they are stable. That means if you can get a good sample of cells, you have a really good shot at identifying [them]."

Indeed, researchers recently found that circRNAs are present in circulating extracellular vesicles such as exosomes, and could in some cases provide more information about gene expression in healthy and unhealthy cells than their linear counterparts in easily accessible human fluids. In a 2015 study of blood-borne circRNAs, Rajewsky's lab discovered that detecting the circular transcripts served as a more faithful proxy for the expression of hundreds of genes than classical mRNA-specific assays.[13] "We would not 'see' these genes, so to speak, by normal RNA expression," he says. "So circRNAs could be molecules that tell you something about development or disease that normal molecules do not."

Specific, circRNA-based biomarkers for several diseases have already emerged from retrospective analyses of patients. In January, researchers described a combination of two circular RNAs, hsa_circ_0124644 and hsa_circ_0098964, that detected coronary artery disease with a specificity and sensitivity rivaling current methods, while presenting a cheaper and more convenient alternative. And other studies in the last two years have highlighted specific circular biomarkers for several cancers, including liver, stomach, and colorectal. Now, these candidates must be validated in studies that predict disease outcome, says Jeck. "There have been a lot of retrospective analyses, and that's all well and good," he says. "But I think the next step is to see if people can use circRNA expression in a prospective manner. That would be very exciting and potentially very useful."

Of course, how circRNAs come to be understood in the lab and possibly one day used in the clinic remains to be seen, as the study of these looped molecules represents an area that's still young. But if the past five years are any indication, the study of circRNAs is rapidly ramping up. "What's amazing to me is how fast this field has grown," says Wilusz, whose lab supplies plasmids expressing circRNAs to other research groups and has recorded a dramatic uptick in requests in the last couple of years. "It's really taking off."

Rajewsky, whose group is now focusing on circRNAs' interactions in the brain, agrees that the best is very much ahead. "We're really just at the beginning of an exciting journey," he says. "It doesn't happen often in molecular biology that you find such a fundamentally new phenomenon." ■

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SEEKING CIRCLES

For years, circular RNAs were overlooked, not least because traditional sequencing methods were not designed to identify them. One of the most commonly used transcriptome-sequencing approaches, RNA-Seq, often includes a selection step that picks out only RNA molecules such as linear mRNA that have polyA tails, a posttranscriptional addition that circRNAs lack. To retain circles in a sample, researchers have to skip this step, or deliberately select for RNAs lacking polyA tails instead.

Even when circles are retained, however, their identification is far from trivial. In 2012, Stanford University's Julia Salzman and colleagues described an approach to pick out exon sequences that had been scrambled in RNA relative to their sequences in the genome, as a mismatched order of exons could indicate a circular arrangement (*PLOS ONE*, doi:10.1371/journal.pone.0030733). But algorithms that identify these mismatches show little overlap in their predictions. One recent study comparing five current algorithms reported that up to 40 percent of predicted circRNAs were only flagged by one algorithm, and fewer than 20 percent of all the circles predicted in the study were identified by all five (*Nucleic Acids Res*, 44:e58, 2016).

As a further complication, scrambled exon sequences can result from things other than circularization of RNA, including certain unusual forms of splicing or, more commonly, artifacts from the sequencing technique itself, due to the use of enzymes such as reverse transcriptase. To combat this problem, some groups are working to develop statistical methods that estimate false detection rates, and distinguish real circles from by-products of the approach.

In the meantime, several researchers have pointed out that the challenges of finding circRNAs raise a deeper question about RNA research: If these abundant molecules were all but invisible to earlier RNA detection methods, what other structures could be out there that are currently being overlooked?

TS WEBINARS

COMING**SOON** | Is Immunotherapy Living Up to Its Promise?

Immunotherapy has been hailed as a breakthrough for treating the untreatable, but it has not yet lived up to its promise to eradicate cancer and infectious disease. While there have been a number of exciting advances, there are still several real-world hurdles for immunotherapy to surmount before it becomes a standard option for most patients. *The Scientist* is bringing together a panel of experts to weigh in on the progress that immunotherapy has made, particularly in the search for effective anticancer treatments, and to discuss the steps still needed before immunotherapy becomes standard treatment in the clinic. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.

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CEO and Director of Scientific Affairs
Cancer Research Institute

ALEX Y. HUANG, MD, PhD
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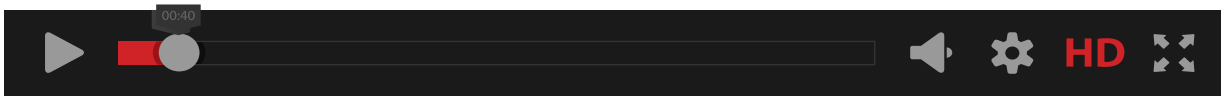

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ALEX DROHAT, PhD
Associate Professor of Biochemistry and Molecular Biology
University of Maryland School of Medicine

SAMUEL HONG, PhD
Postdoctoral Fellow, Department of Biochemistry
Emory University School of Medicine

TOPICS COVERED:

- The variety of epigenetic modifications and their influence on gene expression
- How editing or erasing epigenetic marks might be used in a therapeutic manner

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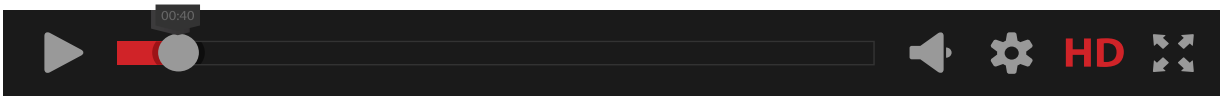

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KATHLEEN MAGUIRE-ZEISS, PhD
Associate Professor, Department of Neuroscience
Georgetown University Medical Center

COLIN K. COMBS, PhD
Professor, Department of Biomedical Sciences
University of North Dakota School of Medicine and Health Sciences

TOPICS COVERED:

- What factors induce microglial activation in neurodegenerative diseases
- Whether neuroinflammation is a protective state or agent of further damage

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ONDEMAND | Precision Medicine: Learning Lessons from the Microbiome

The influence of gut microbiota on human health has been well documented, particularly in the case of metabolic disorders, such as type 1 and type 2 diabetes. In light of the strong association between the composition of one's microbiome and human health, researchers have begun to target these microbial populations. For a detailed look at the state of the microbiome and its role in precision medicine, *The Scientist* brings together a panel of experts to share their research and discuss the next steps.

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ROB KNIGHT, PhD
Professor, Departments of Pediatrics and Computer Science & Engineering
University of California, San Diego

ROBERT A. BRITTON, PhD
Professor, Department of Molecular Virology and Microbiology
Member, Alkek Center for Metagenomics and Microbiome Research
Baylor College of Medicine

TOPICS COVERED:

- The human microbiome's influence on health
- Studying the microbiota of disease in order to better target treatments

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The Literature

EDITOR'S CHOICE IN GENETICS & GENOMICS

Glowing Down the Line

THE PAPER

A. Klosin et al., "Transgenerational transmission of environmental information in *C. elegans*," *Science*, 356:320-23, 2017.

When genomicist Ben Lehner and his colleagues at the Centre for Genomic Regulation in Barcelona engineered nematode worms to express a fluorescent reporter, they were hoping to learn about the control of gene expression. Fluorescence indicated activation of the promoter for the gene *daf-21*, which encodes an essential *C. elegans* heat-shock protein. Glowing worms meant high expression levels; dull worms, low expression. But during the project, the team stumbled across something else.

"Working with this strain, we noticed that if you had individuals that were brighter, their progeny tended to be brighter," says Lehner. With lab worms that are genetically identical, "this is something you don't normally see. There seemed to be inheritance."

Suspecting they had an epigenetic phenomenon on their hands, and knowing that *daf-21* is temperature-sensitive, the researchers decided to grow worms at different temperatures to see if it would affect gene-expression levels through the generations. Sure enough, worms grown at 25 degrees Celsius had offspring that were brighter at normal temperature (20 degrees) than the offspring of worms that had always been kept at 20 degrees.

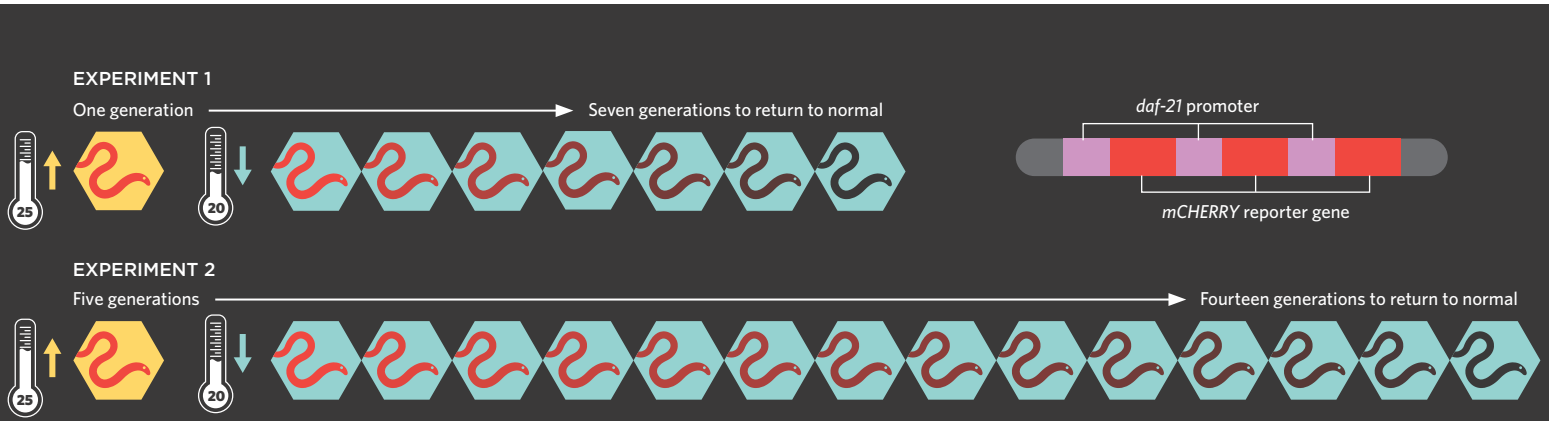
In worms engineered with multiple copies of the fluorescence transgene, this effect persisted for seven generations after the temperature spike, and even longer when the scientists raised multiple generations at 25 degrees. In one nematode line, the worms' glow persisted for 14 generations after the temperature had been dialed back to normal.

"The number of generations the worms were kept in high temperatures somehow counted," says Oded Rechavi, who studies inheritance in *C. elegans* at Tel Aviv University. "That's very interesting to see."

The basis of this inheritance remained unclear, however. DNA methylation is not extensive in *C. elegans*, but nematode studies by other labs, including Rechavi's, have shown that small RNAs could mediate epigenetic effects for multiple generations. So the Barcelona team performed a cross between bright and dull worms, expecting the trait to blend in later generations as the RNA became diluted.

But that's not what happened. In the second generation, some worms were very bright and others were very dull, just like their grandparents—a signature of Mendelian traits, not small RNAs. "Inheritance behaves like a gene," says Lehner. "Without doing any molecular work, we can see that this is inheritance with a locus."

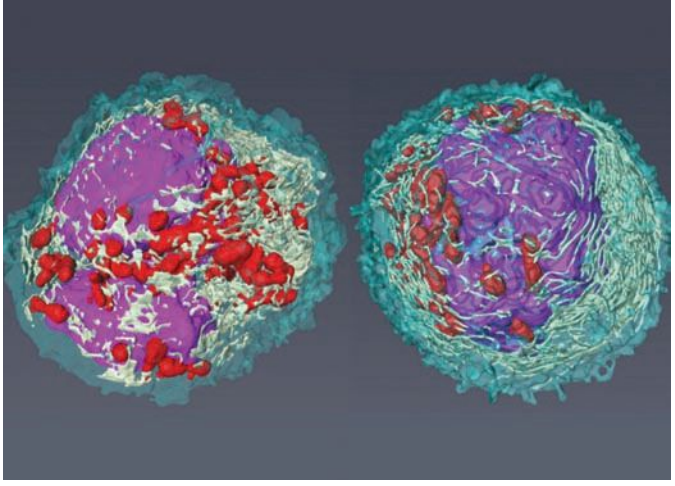
Looking more closely, the team found that offspring of worms grown in warmer temperatures showed reduced modification of histone proteins around the transgenes from an early stage of embryonic development. Over generations kept at normal temperature, this histone modification gradually returned to normal, suggesting epigenetic readjustment. These findings, says Rechavi, are "the most surprising and interesting part." —Catherine Offord



WARM MEMORIES: Researchers engineered *C. elegans* with multiple copies of a transgene called *mCHERRY* connected to a promoter for *daf-21*. When kept at 25 degrees, the worms began to fluoresce red and had progeny that showed similarly elevated expression of the transgenes, despite never having experienced the higher temperature—an effect that persisted for seven generations. When worms were kept at 25 degrees for five generations, the memory of the heatwave lasted longer, with expression levels taking as many as 14 generations to return to normal.

THE SCIENTIST STAFF

SEA STAR LUMINESCENCE: High sensitivity macrophotography captures a brittle star arm emitting light.



DISTINCTLY DIFFERENT: A newly discovered type of dendritic cell (left) exhibits notable differences from a standard plasmacytoid dendritic cell (right).

JÉRÔME MALLEFET, JÉRÔME DELROISSE; 3-D RECONSTRUCTION BY JAMES FLETCHER, IMAGE COURTESY OF KATHRYN WHITE, NEWCASTLE UNIVERSITY

EVOLUTIONARY BIOLOGY

Like Lights

THE PAPER

J. Delroisse et al., "A puzzling homology: A brittle star using a putative cnidarian-type luciferase for bioluminescence," *Open Biology*, 7:160300, 2017.

DEGREES OF SEPARATION

Although the long-tentacled brittle star (*Amphiura filiformis*) differs from the stout sea pansy (*Renilla*) in both appearance and phylogeny, researchers have now demonstrated that they share a similar luciferase—an enzyme that catalyzes the light-producing reaction that results in the invertebrates' bioluminescence.

UNEXPECTED HOMOLOGY

When an international group of researchers searched the brittle star's genome and transcriptome for known luciferase sequences, they detected sequences in the echinoderm that were homologous to those of the luciferase of the sea pansy—a cnidarian. The sequences were so similar, in fact, that antibodies specific to the sea pansy luciferase could also detect the brittle star luciferase.

DEFYING CONVENTION

Conventional dogma states that every taxonomic group has its own distinct luciferase, explains lead author Jérôme Delroisse of the University of Mons; but previous work has found similar homologies in distantly related species. How such different species acquired similar luciferases remains unclear, however.

AN EVOLVING HYPOTHESIS

The sea pansy's luciferase has known homology to a nonbioluminescent bacterial enzyme, and the authors uncovered similar proteins in other nonluminous organisms. The data suggest that both brittle star and sea pansy luciferases evolved "from a common ancestral protein originally not involved in light emission," says Delroisse, and that the gene for this protein horizontally transferred from bacteria to a common ancestor. It's becoming clearer that "not all independently evolved bioluminescent enzymes have to be structurally different," says Miriam Sharpe of the University of Otago who was not involved in the study. **—Aggie Mika**

IMMUNOLOGY

Hidden Cells

THE PAPER

A.-C. Villani et al., "Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors," *Science*, 356:eaah4573, 2017.

HIDING, NOT SEEKING

Dendritic cells and monocytes, essential pathogen-sensing immune watchdogs, fall into subtypes based on factors such as cell surface markers. But according to genomicist and immunologist Alexandra-Chloé Villani of the Broad Institute of MIT and Harvard, scientists only "use a handful of markers to define their favorite cell type," potentially overlooking subpopulations with similar features but different functions.

NEW CELL TYPES

To redefine traditional cell subtypes, Villani and her colleagues isolated human dendritic cells and monocytes, sequenced the transcriptomes of individual cells, then grouped cells based on similar expression patterns. They then identified cell-surface markers that were highly and specifically expressed in each group, uncovering two new monocyte and three new dendritic cell subtypes along with a novel dendritic cell progenitor. Using their new markers, researchers isolated and resequenced fresh dendritic cells to confirm their results.

JACKPOT

Among the newly characterized subtypes was a cell that had previously "hidden" among plasmacytoid dendritic cells (pDCs), known for producing interferons in response to viral invaders. Testing the functions of this new population, the team found that the cells potently activated T cells, while pDCs, on their own, did not.

A BETTER APPROACH?

"Single-cell transcriptomics methods are developing rapidly to become more scalable, robust, reliable, and affordable," the Sanger Institute's Sarah Teichmann, who was not involved in the work, told *The Scientist* in an email. This technology, Teichmann says, "is the method of choice" for studying cell type in immunology and beyond. **—Aggie Mika**

Oceans' Ambassador

Jane Lubchenco has embraced many roles: marine ecologist, science communicator, federal agency administrator, and sustainable fishing advocate.

BY ANNA AZVOLINSKY

Jane Lubchenco was a Harvard University assistant professor of ecology in 1976 when she and her husband, Bruce Menge, an ecologist at the University of Massachusetts, began to look for academic positions that would allow more time for each to spend with the family they hoped to have. The young couple realized that their biology fieldwork and teaching left little room for time with kids. Because neither wanted to put their career on hold, they proposed a novel idea to the departments where they interviewed. They asked to be treated as two independent, tenure-track professors, but to split one tenure-track position into two half-time positions. Most universities shied away, but at Oregon State University (OSU), both the faculty and the dean were willing to try the unusual arrangement.

The couple moved to OSU in 1977, and their first son, Alexei, was born the following summer. As a three-week-old, Alexei accompanied his parents on a research trip to Panama. Their second son, Duncan, was born three years later and spent his first summer on a field expedition, camping with his parents and brother on the Oregon coast. A decade later, both parents were tenured and ready to work three-quarters time. When the boys were 13 and 10 years old, the couple switched to full-time. "The positions allowed us the flexibility to organize our lives in the way that worked best for us," says Lubchenco.

> "Science can't tell society what to do. We must work together to identify problems and find solutions."

So many scientists inquired about the couple's positions that the two penned a personal account of their maverick job arrangement. While the setup is still uncommon, says Lubchenco, there are now thousands of similar split positions around the country, and universities in general have evolved in their understanding and flexibility of parents' schedules and demands.

Lubchenco's research on the causes of the patterns of distribution, abundance, and diversity exhibited by the plants and animals in marine communities evolved into an investigation of ecological trends on a global scale. As she began to engage more and more with the public, Lubchenco was appointed as a member of the National Science Board of the National Academy of Sciences and served as president of three professional scientific societies. Later, she served for four years as the head of a US government agency before returning to academia.

Here, Lubchenco talks about how her parents kept her and her five sisters busy, how a study she led became the springboard for her active role in public service, and how—with vinegar, calcium chalk, and water in hand—she demonstrated the deleterious effects of ocean acidification at a congressional hearing.

LUBCHENCO LAUNCHES

Water baby. Lubchenco grew up in Denver, Colorado, the oldest of six sisters. Her father was a surgeon and her mother, a pediatrician. "We were around a lot of science and medical talk, and that was second nature for us," she says. Growing up, the outdoors and swimming were constants for Lubchenco. "Our family had a membership to a man-made lake in what was then the outskirts of Denver, and all six of us would live in our swimsuits all summer long." Lubchenco's parents also encouraged the girls to participate in sports. After shuttling all six to their respective ballet classes, Lubchenco's mom figured out that all the girls could do swimming or diving at the same time. Motivated by a coach who was a former Olympic diving champion, Lubchenco continued to dive in high school and informally coached a men's diving team while she was in college. She was also an active Girl Scout and played basketball and volleyball at her all-girls high school. "In hindsight, the experiences of both individual and team sports were really helpful for learning to set my own goals and rely on myself, but also for learning to lead or to follow as part of a group," she says.

Laying out her path. Entering Colorado College in 1965, Lubchenco was one of twenty incoming students chosen to take part in a four-year program called the Ford Foundation Independent Study Program. The experimental program had no class requirements and only two exams during the students' four years. "Each of us had to figure out how we learned best and what we wanted to study. There was no feedback in the form of grades, and about half of the students dropped out because they wanted a more structured curriculum." Lubchenco loved it. As she had in high school, Lubchenco found an inspirational science teacher, Mary Alice Hamilton, who occasionally recommended a student to attend the invertebrate zoology course at the Marine Biological Laboratory in Woods Hole, Massachusetts. Lubchenco spent the summer before her senior year taking the course, mostly attended by graduate students, and was asked to stay on to do an independent six-week project with one of the course instructors, W.D. "Gus" Russell-Hunter, a malacologist. "It was a transformative summer for me. I was already comfortable with water and I absolutely fell in love with the ocean and its world of amazing biodiversity. I got tips about

JOY LEIGHTON

JANE LUBCHENCO

University Distinguished Professor and Advisor in Marine Studies, Oregon State University
Former Under Secretary of Commerce for Oceans and Atmosphere and Former Administrator of the National Oceanic and Atmospheric Administration (NOAA) (2009–2013)
Former US Science Envoy for the Ocean, US State Department (2014–2016)

Greatest Hits

- Identified the interaction patterns between plant and herbivore species in East and West Coast marine intertidal communities
- Spearheaded and led "The Sustainable Biosphere Initiative Project," which created a cohesive message on behalf of the broader ecology community that connects ecological research to society as a whole
- Cofounded nonprofit organizations to train scientists to be effective communicators of science to the general public, government officials, and the press
- As Under Secretary of Commerce for Oceans and Atmosphere and Administrator of the National Oceanic and Atmospheric Administration (NOAA) from 2009–2013, helped many fisheries become both sustainable and profitable; worked to restore coastal and ocean habitats, strengthened scientific integrity, and promoted climate science
- Served as the first Science Envoy for the Ocean within the US State Department, working with governments and citizens in China, Indonesia, South Africa, Mauritius, and Seychelles to promote awareness and action on oceans and climate change

graduate school from hanging out with graduate students. I did my first research project and realized I loved the hands-on work, posing hypotheses, designing experiments, and testing them," she says.

Ecological mosaic. To be close to the ocean, Lubchenco chose to enter the University of Washington's zoology PhD program, where she studied the foraging strategies of predators in rocky intertidal-zone communities. Lubchenco's advisor was Bob Paine, who is not included as an author on her papers because the convention in ecology is that direct data contribution is needed for authorship. That first year, she met a sixth-year marine ecology graduate student, Bruce Menge, who had just accepted a postdoctoral fellowship at the University of California, Santa Barbara. Lubchenco took a leave of absence from her program and moved with Menge, but continued to go back to conduct the independent experiments that she had begun at the Friday Harbor Laboratories in Washington on the coexistence strategies of competing species of sea stars in Washington State and the foraging habits of a sea snail, *Acanthina punctulata*. At the end of the year the couple married, and Lubchenco converted her research project into a master's thesis.

The newlyweds moved to the East Coast, where Menge was starting an assistant professorship at the University of Massachusetts. A year later, in 1972, Lubchenco entered Harvard's ecology PhD program. Her thesis focused on the herbivore-plant dynamics of intertidal marine communities, including the relationship between algae and an herbivorous marine snail that consumes them; Menge studied the predator-prey component of these communities. She and Menge often put their findings together to show the bigger ecological picture of these intertidal zones in New England. "The rocky intertidal seashores are relatively easy to manipulate using cages to remove consumers or competitors. This experimental marine ecology approach, partly initiated by Bob Paine, was a new way of getting to the causes of the patterns we see in nature. Most of ecology until that time was based only on correlations," says Lubchenco.

LUBCHENCO LEADS

Lessons in diversity. After the couple's move to OSU in 1977, Lubchenco initially worked on comparing the dynamics of the temperate seashores of New England with the tropical seashores in Panama. "There were a lot of studies on terrestrial systems, but not many on marine ones at the time," she says. As OSU faculty, she and Menge spent their first several winters and summers in Panama, with research positions at the Smithsonian Tropical Research Insti-

tute. They discovered that the tropical rocky seashores in Panama were home to an extremely diverse set of invertebrates and fishes, even though there was little seaweed diversity. "This was surprising because the dogma was that the tropics are more diverse in all species," she says. Another surprise, she says, was that many of the herbivore and predator species were generalists rather than specialists. "Ecologists had assumed that in such diverse, complex systems, the animals would be highly specialized."

A new role. Lubchenco and Menge continued to work on the factors that influence coastal communities, expanding their comparisons to communities in New Zealand, Chile, and South Africa, geographically distant locales that all have coastal ecosystems with similar types of oceanographic and atmospheric patterns. In 1988, Lubchenco became the vice president of the Ecological Society of America (ESA) and spearheaded the creation of a prioritized ecological research agenda "so that when the larger scientific community came together to discuss funding and budgets, ecologists as a group had their act together," she says. That work, published in 1991 as "The Sustainable Biosphere Initiative," laid out biodiversity, climate change, and sustainability as the core priorities of ecological researchers. "Essentially, we connected the dots between very basic, seemingly esoteric work of ecologists and its relevance to society. It sent the message that cutting-edge research that both advances knowledge and is immediately relevant to society is more important now than ever before. And it made the case for why ecology as a science is so important," says Lubchenco. The report brought ESA onto the radar screen of the Congressional Budget Office and various government funding agencies, and Lubchenco was suddenly being asked to make presentations on ecology to members of Congress and the Administration.

Engaging with society. For Lubchenco, the experience of conveying scientific information to government officials highlighted the importance of training scientists to communicate with nonscientists. To provide such training, she helped create the Leopold Leadership Program for midcareer environmental scientists as well as COMPASS, an organization that coaches and empowers environmental researchers to become better communicators and connects them with journalists, politicians, and business leaders.

LUBCHENCO LASTS

On to Washington. Lubchenco became more involved in bringing together the scientific community, engaging with individuals within the US government, and testifying before various congressional committees. Then, in 2008, she received a call from President-elect Barack Obama's transition team, asking if she would become the Administrator of the National Oceanic and Atmospheric Administration (NOAA). "After multiple conversations from Tasmania, where I was doing research, the head of the transition team called and asked me to fly to Chicago to meet with the president." She accepted the position and spent four years in Washington, DC, while Menge managed the OSU laboratory they shared. Despite the lagging economy at the time, the Deepwater Horizon oil spill disaster, and some of the most extreme weather on record, as well as a "partisan-heavy and legislation-light" four years, her tenure saw the accomplishment of many of the items on her and NOAA's to-do list. A major success entailed slashing overfishing and returning US fisheries to sustainability and profitability. NOAA also played a key role in creating the first-ever national ocean policy, established by executive order in 2010 with "A Healthy Ocean Matters" as the core message. Also under Lubchenco's administration, the role of science was strengthened, and NOAA created a robust Scientific Integrity Policy.

Congress as classroom. While asking Congress for money to fund a new NOAA weather and environmental satellite and conveying the importance of these satellites as a source of vital weather information, Lubchenco was told by a congressman, "I don't need your weather satellites, I have the Weather Channel," not understanding that the Weather Channel relies on NOAA satellites for its weather information. At a different hearing, Lubchenco, rather than simply giving Congress members an oral briefing on global warming and related ocean acidification, demonstrated the phenomenon by adding calcium carbonate, which makes up the bulk of coral, sea stars, and urchins, to water, a solution of water plus vinegar, or vinegar alone. "It's much more powerful to see the same substance that makes up the coral and other marine animals dissolve in an acidic solution."

Sustainable ocean fishing advocate. Although Lubchenco is now back running a joint ecology lab at OSU with her husband, she is still involved in projects aimed at understanding how to "use the ocean without using it up," she says. Such projects include supporting sustainable fisheries, improving aquaculture, and establishing protected marine areas. Lubchenco is currently advising on a project known as Seafood Business for Ocean Stewardship (SeaBOS), which aims to make fisheries and aquaculture more sustainable. "This is a collaboration between scientists and businesses. It is an opportunity for corporate leaders to understand the implications of climate change, ocean acidification, and overfishing for their businesses. It's an opportunity for them to be part of the solution rather than part of the problem. They have power to address destructive practices and be champions for policies that improve ocean health."

Unique education. "I was exposed to the different ecological world views at the three graduate schools I attended, each of which had distinct views and programs. As I look back, this was extremely valuable, because I ended up with a much broader view of what ecology is and how to do it."

An evolution. "I went from someone who just taught and did research to someone who began to engage more with society; then from leading other scientists to being a civil servant and policymaker; and then to being an international diplomat. . . . Science must be embedded in society and be its partner. Science can't tell society what to do. We must work together to identify problems and find solutions." ■

Emily Balskus: Microbe Miner

Associate Professor, Department of Chemistry and Chemical Biology
Harvard University. Age: 37

BY VIJAY SHANKAR

One day in high school, Emily Balskus was summoned by her chemistry teacher. To Balskus's surprise, the teacher praised her chemistry prowess before offering the teenager an after-school job in the chemistry lab. Balskus would help her teacher set up and break down experiments and test new lesson ideas for the lab.

"This was a turning point and made me realize that I should push myself to work hard, and that I could focus on science as a potential career," Balskus says.

As an undergrad at Williams College in Massachusetts, Balskus became fascinated with synthetic organic chemistry. She took the first steps towards synthesizing hennoxazole A, a complex antiviral compound produced by a marine sponge, and published her first research paper. Her work set the stage for completion of the total synthesis of the molecule a few years later, and Balskus published two more papers, coauthored with her chemistry professor Thomas Smith. "Her notebooks from that time are things of beauty," Smith says. "Emily set the bar extraordinarily high for future students in my lab."

Balskus went on to pursue a PhD in the Harvard University lab of organic chemist Eric Jacobsen in 2003. Jacobsen recalls Balskus's inventiveness and initiative, citing her novel idea of applying asymmetric catalysis—where a chiral catalyst aids in the formation of a favored stereoisomer—to control the formation of chemical bonds across large, cyclic molecules. This provided a way to study cyclic organic compounds, which are otherwise very challenging to assess, and resulted in a *Science* paper.[1] "From the very start, she worked on her own ideas and constantly led me and my group in new directions," Jacobsen says.

Balskus's independent streak followed her into the Harvard lab of Christopher Walsh, where she started a postdoc in 2008. There she helped discover the gene clusters in cyanobacteria that synthesize mycosporine and mycosporine-like amino acids, which act as sunscreens for the microbes.[2]

"Her work in my group was thought up and executed by her and had the wonderful feature" of teaching us many things about the phenomena we were studying, says Walsh.

Shortly after setting up her own Harvard lab, Balskus collaborated with Peter Turnbaugh of the University of California, San Francisco, to elucidate how the human gut bacterium *Eggerthella lenta* inactivates and detoxifies the heart-protecting drug digoxin.[3]

Recently Balskus has shown that *trans*-4-hydroxy-L-proline (Hyp) dehydratase, a newly discovered member of an abundant family of proteins, produced by gut bacteria, known as the glycyl radical enzymes, helps in metabolizing *trans*-Hyp, an amino acid that is rare in bacteria but is common in eukaryotes.[4]

"Emily had a major impact on our work and has really helped to dive deeper into the biochemistry and enzymology," Turnbaugh says.

"Given her fearlessness and willingness to learn new things, I would not place limits on what [Emily] might accomplish," Jacobsen adds. "I think she will make leading advances in elucidating the chemical aspects of the gut microbiome and its role in human health." ■

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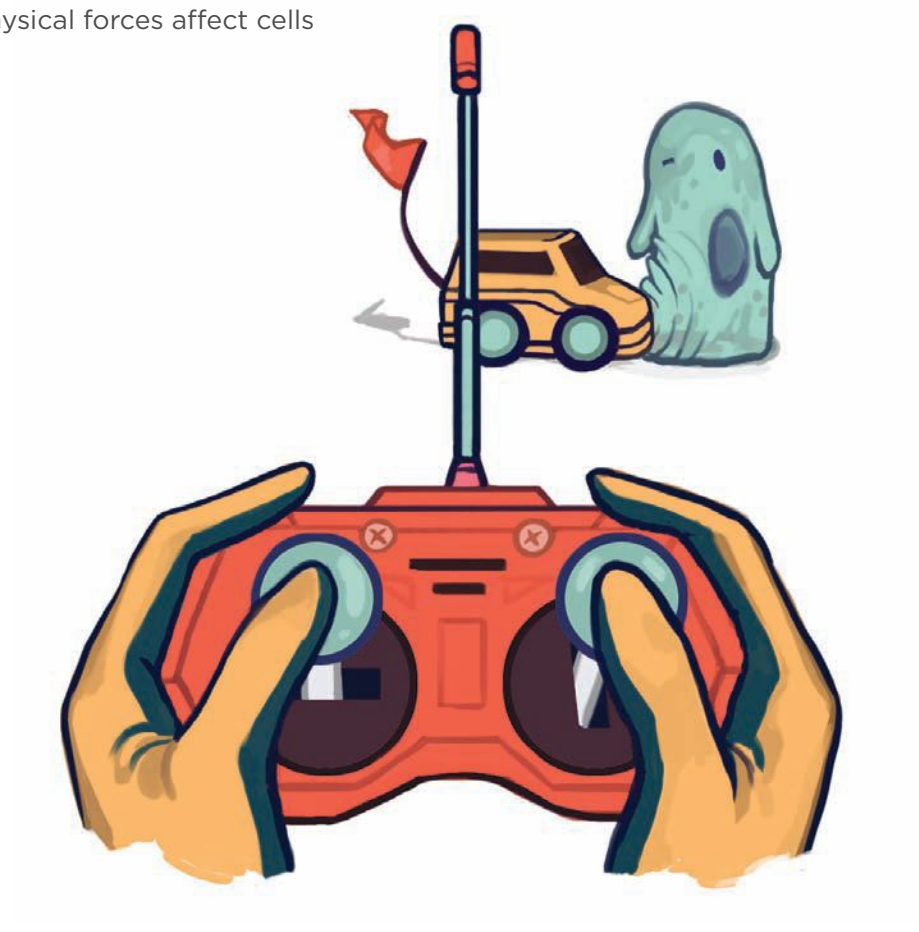
The Mechanobiology Garage

New tools for investigating how physical forces affect cells

BY ANDY TAY



Cells constantly interact with each other and with the surrounding extracellular matrix through physical forces such as tension, pressure, torque, and shear stress. Over the past 50 years, biologists have increasingly come to recognize the important role biomechanics plays in the function of cellular activities such as gene expression and signaling. (See "May the Force Be with You," *The Scientist,* February 2017.)

One key tool for studying how physical forces affect cells is the micropipette aspirator, a tiny glass pipette that applies pressure on a section of the cell membrane; testing gene expression, protein levels, or other factors can point to the effect of such forces on the cell. Researchers also use atomic force microscopy, which senses how cells respond to tiny mechanical pokes; apply fluid shear stresses to perturb membrane mechanosensors; and stick cells onto flexible polymers to investigate how their cytoskeletons are disrupted and repaired when the material is stretched and flexed.

However, these methods can only yield insights by looking at the whole cell at once, and not at smaller entities such as organelles and structural specializations. As the field delves into the mechanobiology of the nucleus, and investigates interactions between cells and between proteins and cell membranes, a new set of techniques has emerged. One widely used approach, three-dimensional microfluidics, segregates subcellular structures such as axons and dendrites into different microfluidic compartments to determine exactly where and how external forces affect cellular biology. Another emerging method deploys magnetic nanoparticles onto the cell to exert forces with better spatial and temporal control than conventional tools such as the micropipette aspirator.

These newer approaches have yielded surprising insights into intracellular processes, from how the cell deforms to how external force affects cell signaling and induce cell migration. For example, scientists have found that the stiffness of extracellular matrix can influence stem cell differentiation, that stretching chromatin can upregulate transcription, and that cytotoxic T cells use mechanical forces to recognize pathogens in order to eliminate them from the body.

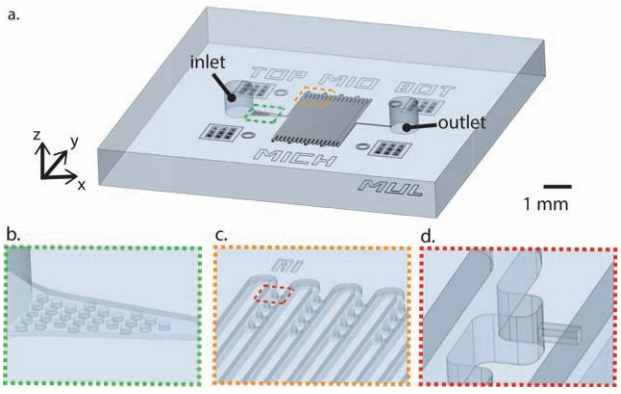
Here, *The Scientist* reports on recently developed methods—from upgraded versions of conventional tools to newer micro- and nanotechnologies—in the proliferating tool chest of cellular mechanobiology research.

MICROPIPETTE ARRAYS

RESEARCHER: Allen Liu, Assistant Professor of Mechanical Engineering, University of Michigan

PROBLEM: Micropipette aspiration of a section of an intact cell is a useful technique for measuring mechanical properties such as the cell's stiffness, but setting up the experiment is time-consuming, and throughput is extremely low (~10 minutes/cell).

SOLUTION: Liu and colleagues fabricated a microfluidic device consisting of a group of micropipettes that can perturb up to 128 cells simultaneously. They attached the micropipettes to a simple, cheap, calibrated pump that generates fluid pressure to exert forces on the cells through the



ALL TOGETHER NOW: The Liu lab's microfluidic apparatus (top) allows the measurement of mechanical forces on 128 cells simultaneously. The bottom panel shows a filtering unit (left) that removes clumps of cells and debris before single cells enter a continuous microchannel folded into 16 columns with 4 aspiration chambers per column (center). Each chamber connects to a micropipette through which mechanical forces can be applied to individual cells (right).

micropipettes. The team then used two computational models to describe how the deformations produced by the device reflect mechanical properties of the cells. The researchers showed that, consistent with previous findings, the stiffness of cultured metastatic breast cancer cells was lower than that of normal breast epithelial cells (*Lab on a Chip*, 15:264-73, 2015).

PROS:

- The device is much cheaper (~$3,000) than a conventional micropipette aspirator machine (>$10,000) as it does not require expensive electronics such as a piezo motor, an electrical motor that senses deflections and is used for force calibration.
- It offers higher throughput than a conventional micropipette aspirator.

CONS:

- Without the electronics of the traditional micropipette, the tool cannot exert small-magnitude forces of under a few hundred nano-Newtons and has limited sensitivity.
- The micropipettes are grouped together and cannot be controlled individually. Channels can get clogged by debris, which can affect the measurements and system throughput.
- The device cannot be applied to mechanoreceptors on cellular extensions such as neurites of neurons, because the cells, which are detached during mechanical phenotyping, lose their extensions.

FUTURE PLANS: Liu plans to develop a device to monitor mechanical cues in addition to pressure, to investigate how "multiple physical cues can orchestrate convergent mechanotransduction," he says.

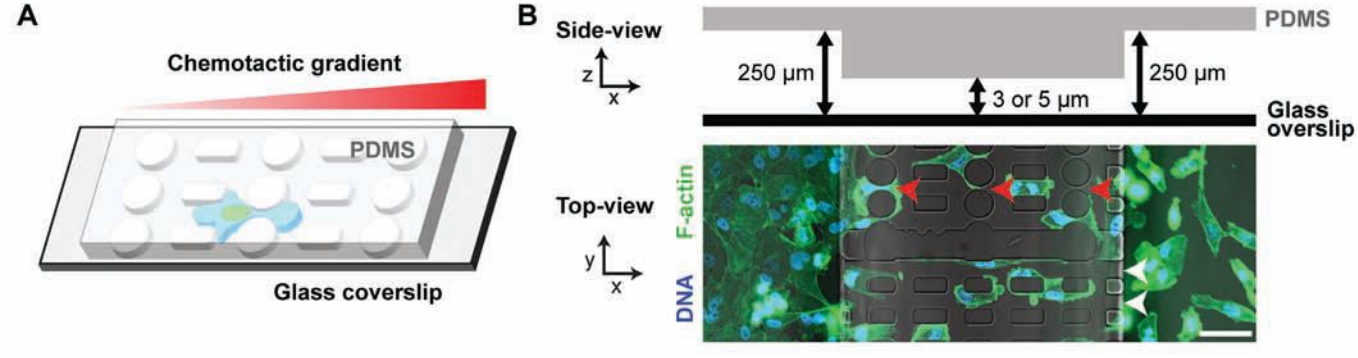
INDUCING NUCLEAR DEFORMATION

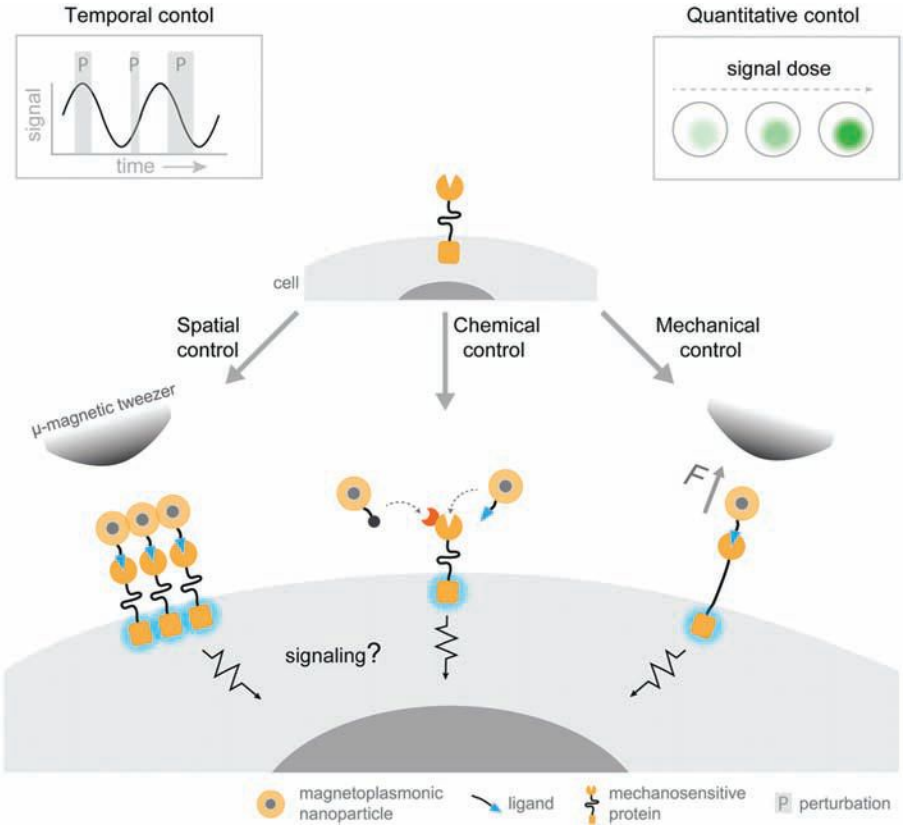
RESEARCHER: Jan Lammerding, Associate Professor of Biomedical Engineering, Cornell University

PROBLEM: Changes to the biophysical properties of the nucleus, such as loss of nuclear envelope integrity and nuclear pore selectivity, are linked to aging and diseases such as cancer. However, it is difficult to reproduce these changes in the lab in order to understand how they regulate DNA stability.

SOLUTION: To investigate nuclear deformation in cancer cells, Lammerding's group fabricated a microchannel device with a series of posts that narrow the cross-section of the channel as the cells travel along. By applying a chemotactic gradient, the researchers could induce cancer cells to migrate through pores with cross-sections that ranged from 5 μm² to more than 20 μm² in size. They tagged nuclear envelope proteins with green and red fluorescent markers and imaged the rupture of the nuclear envelope to determine how constriction affected the nucleus's contents. Cancer cells pass

MANEUVERING THROUGH TIGHT SPOTS: Cells passing through microchannel pores undergo nuclear deformations that destabilize their DNA. With this microfluidic apparatus (PDMS), scientists can gain insights into tumor metastasis, which involves cancer cells traveling through the bloodstream and squeezing between the endothelial cells that form capillary walls. Micrograph shows breast cancer cells labeled for DNA (blue) and actin (green). (Red arrowheads: 2 μm-wide constrictions; white arrowheads: 15 μm-wide channels. Scale bar: 50 μm)





MAGNETO-MECHANICS: Magnetic nanoparticles bind to mechanosensitive targets on the cell membrane and exert forces on them in the presence of a magnetic field. The effects of such forces on cell signaling can be monitored at different times and with different force magnitudes.

through tight spaces between endothelial cells lining capillaries during metastasis, so Lammerding's group used the tool to show that the DNA of cancer cells breaks as cells migrate through constrictions; the smaller the constrictions, the higher the probability of damage (*Science,* 352:353-58, 2016).

PROS:
- Lammerding's platform is higher-throughput than existing methods, such as the nuclear patch clamp technique, which can only deform the nuclear membrane of one nucleus at a time.
- Microchannels are easy to fabricate.

CONS:
- Pore sizes used for the microchannels (a few μm in diameter) do not correspond to actual capillary pore sizes (tens of nm), so the tool's physiological relevance is unclear.
- The platform probes nuclear deformation, but doesn't control for the effects of cell membrane deformation—a known biomarker of disease—which also occurs upon constriction.
- The technology does not account for the fact that different cells have different nucleus to cytoplasm volumetric ratios, which can affect nuclear envelope deformability.

FUTURE: Lammerding plans to further improve the tool's throughput to allow assessment of DNA damage in hundreds of cells per run. His lab is also developing software to automate the analysis of the video data it gathers.

MECHANOGENETIC TOOLKIT

RESEARCHERS: Zev Gartner, Associate Professor of Pharmaceutical Chemistry; Young-wook Jun, Associate Professor of Otolaryngology, University of California, San Francisco

PROBLEM: Cells respond to mechanical signals, but researchers lack effective tools to investigate how force magnitudes regulate the activities of specific mechanosensitive receptors on the cell membrane.

SOLUTION: The team synthesized magnetic nanoparticles coated with antibodies to target specific mechanosensitive membrane proteins. Using magnetic fields, they then applied forces on the mechanosensitive proteins via the nanoparticles bound to them. By varying the magnetic field strength, different forces could be applied to the mechanosensitive targets. With their setup, the team found that cells can sense different magnitudes of forces, influencing actin cytoskeleton assembly (*Cell*, 165:1507-18, 2016).

PROS:
- The nanoparticles are small enough (~50 nm) not to cluster on the cell surface or immobilize mechanosensitive receptors in the absence of external magnetic fields, avoiding these potential confounding factors.
- The shell of the ferrite nanoparticles used here allows clearer optical imaging than the microparticles used in most previous techniques.

CONS:
- The nanoparticles that Gartner and Jun designed cannot sustain as strong a force as can most previously used microparticles, so they don't engage mechanosensitive channels that are activated by forces beyond their limit of detection.
- This technology targets one cell at a time, limiting throughput and prohibiting its use in studying mechanotransduction in cellular networks, such as neuronal populations.

CELL, 165:1507-18, 2016

- Temporal resolution is limited by the use of alternating magnetic fields which operate on the millisecond time scale; probing faster (microsecond- to millisecond-scale) events such as synaptic transmission would require better time resolution.

FUTURE PLANS: Jun hopes to apply the technology to other mechanosensitive proteins such as ion channels. Duke University neurobiologist Jörg Grandl, who developed a similar tool but was not involved in this study, hopes to see improvements in methods for adding labeled antibodies to the nanoparticles to better target specific mechanoreceptors and to control force amplitude, space, and time. He envisions using this technology to "mechanically probe a protein, domain by domain," while analyzing the effects on protein properties and functions.

MAGNETIC MICRODROPLETS

RESEARCHER: Otger Campàs, Assistant Professor of Mechanical Engineering, University of California, Santa Barbara

PROBLEM: Mechanical forces in cellular microenvironments and their spatiotemporal variations are known to affect cellular behaviors such as migration, but there is no way to make direct in vivo and in situ measurements of such forces in tissues and organs.

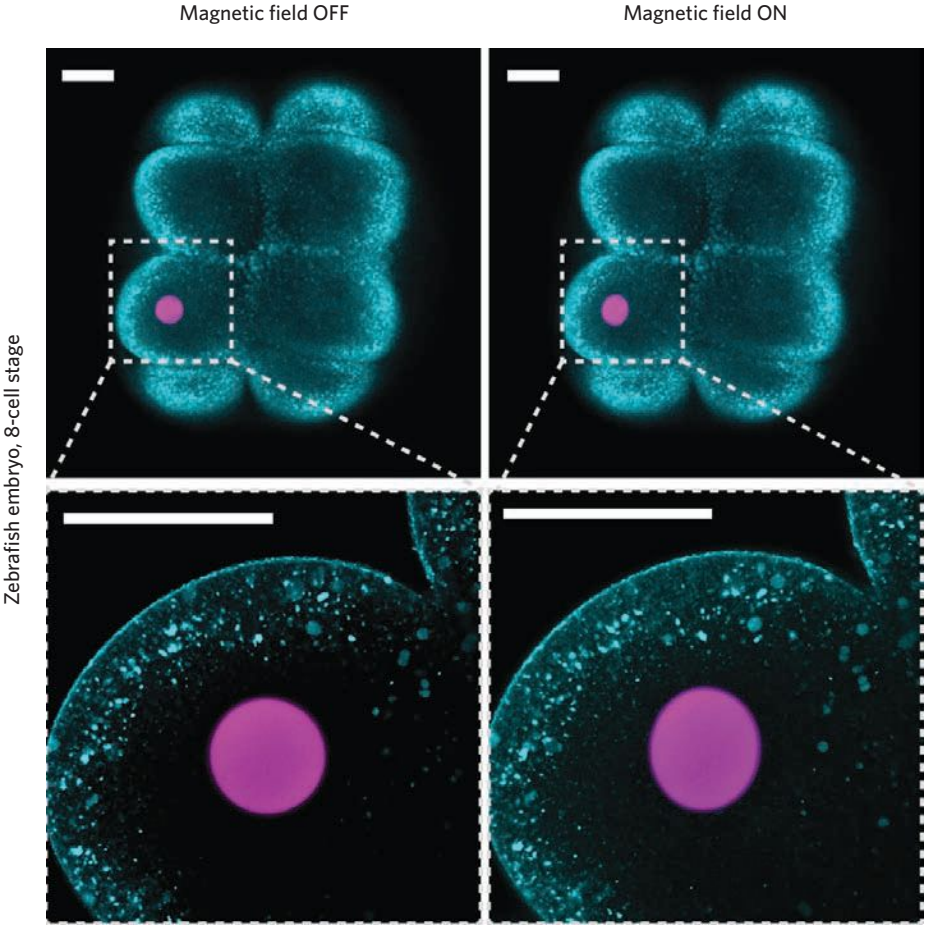
SOLUTION: The team created biocompatible microdroplets of ferrofluid oil—a suspension of magnetic iron nanoparticles—and injected single microdroplets into cells of early zebrafish embryos. To exert local and controlled forces within the cells, they then applied uniform magnetic fields to deform the microdroplets without exerting traction on them. Finally, they used high-resolution microscopy to observe tissue deformations in response to forces exerted by the shape change of the microdroplets, and estimated tissue mechanical properties by comparing the deformations of the tissues to a reference library generated from materials with known physical properties. Using the technique, they found that tissue stiffness in live, developing zebrafish embryos varies along the tailbud of the animals (*Nat Methods*, 14:181-86, 2017).

PROS:
- Microdroplets of ferrofluid are biocompatible and can be used in vivo, so this tool is widely applicable.

CONS:
- Injecting microdroplets into tissues is invasive, and immune responses could affect the readout.
- Magnetic fields decay rapidly across distance, so this method may not work in deep tissues or organs such as pancreas and brain.

FUTURE PLANS: Campàs is using his platform to study the mechanisms of tumor formation in multicellular spheroids and hopes to understand how abnormal biomechanics can cause or promote cancer and other diseases. ■

Andy Tay is a bioengineering graduate student in the lab of Dino Di Carlo at UCLA, where he uses and develops tools to probe the role of mechanics in cancer metastasis and neural stimulation.



IN LIVING VOLUME: Using magnetic fields, scientists can exert forces on magnetic microdroplets injected into cells to study and experimentally perturb the mechanics of cellular/tissue development. These micrographs demonstrate the effect on a droplet (magenta) injected into a cell of an early-stage zebrafish embryo. (Scale bar: 50 μm)

NAT METHODS, 14:181-86, 2017

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TheScientist

Identifying Predators

How to tell reputable journals from shady ones

BY TRACY VENCE

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When Björn Bauer began his research career, he was eager to establish himself as an expert in his field. So he was delighted when, in 2011, the publisher of a journal called *Pharmacologia* emailed him an invitation to join the publication's editorial advisory board. As requested, Bauer sent a copy of his biographical sketch. Soon after, his name appeared on the *Pharmacologia* website. Aside from a few administrative emails, he did not hear from the publisher, which, he says, never contacted him to review any manuscripts or consult on other editorial decisions.

"I looked at their website, and it looked like a new start-up journal," recalls Bauer, now an associate professor of pharmacy at the University of Kentucky. But after months—then years—of silence, he began to suspect something was awry.

Following a 2013 conversation with a journalist at *The Scientist* who identified *Pharmacologia* as a publisher of plagiarized material, Bauer requested that his name be removed from the journal's board. (See "Rampant Plagiarism in Two Journals," *The Scientist*, May 22, 2013.) Again, he did not hear from the publisher. Today, nearly all traces of *Pharmacologia* and its publisher, Science Reuters, have been scrubbed from the Internet.

The reason I call certain publishers predatory is because they prey on people. They try to trick honest researchers, and sometimes they are successful.

—Jeffrey Beall, University of Colorado Denver

While there are several reasons reputable scientists might get involved with fake journals, by and large it's because they don't realize what they're dealing with. "They're tricked. They think it's a legitimate journal, they get the invitation in the mail, and they accept it," says the University of Colorado Denver librarian Jeffrey Beall, who coined the term "predatory publishing" nearly a decade ago. (See "Predatory Publishing," *The Scientist*, August 2012.) "The reason I call [certain publishers]

predatory is because they prey on people. They try to trick honest researchers, and sometimes they are successful."

Tricky business

In a 2015 analysis, information-systems scientists Cenyu Shen and Bo-Christer Björk of the Hanken School of Economics in Helsinki, Finland, reported that, between 2010 and 2014, predatory journals had increased their publication volume from 53,000 to 420,000 articles per year. Considering the number of papers and average article-processing charge (APC) fees for around 8,000 titles, Shen and Björk estimated that the predatory publishing market was worth around $74 million in 2014 (*BMC Medicine*, 13:230).

We encourage people to look beyond a single identifier; there is no single indicator of quality research out there.

—Andy Pleffer, Macquarie University

Predatory publishing "has really turned into a plague," says Chad Cook, a musculoskeletal clinical researcher at Duke University. Some days, he receives more than a dozen invitations to publish in and join the editorial boards of scientific journals. "I get asked to join the editorial boards of diabetes journals, sexual dysfunction [journals], infectious disease [journals]—a number of areas that are not my expertise." Cook—who is an editor at the *British Journal of Sports Medicine* and the *Journal of Orthopedic and Sports Physical Therapy*, both reputable publications—has set up various spam filters to deal with the deluge of suspicious email invitations.

In 2009, Beall began helping scientists like Cook distinguish legitimate invites from spurious ones by launching a list of journals published by organizations he considered predatory. The list went dark this January, however—a choice that Beall says was not his own. In a recent *Biochemia Medica* opinion article, Beall alleged that he faced "intense pressure" from his institution to shut the list down, though the university denied the allegations. Beall says he has says he has no plans to resume publication at this time.

"It was him against the world for a while," says Cook, referring to Beall's public finger-pointing. "He elevated the level of communication [on predatory publishing]. To me, that's the most powerful thing that has been done at this point."

With the help of Beall's list, more scientists have become aware of the problem of predatory publishing, and some scholars have even turned the tables on tricksters, submitting obviously sham papers to suspect journals. (See "Opinion: Why I Published in a Predatory Journal," *The Scientist*, April 6, 2017.) Researchers reported publishing fully made-up papers in some "peer-reviewed" journals with ease, provided they paid an APC fee. In March, academics at the University of Wrocław in Poland described in *Nature* how they had created a phony persona, then succeeded in placing the faux scholar on the editorial boards of 48 of 360 journals they had contacted (543:481-83, 2017).

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In 2013, the predatory publishing discussion transcended scholarly circles when the US Department of Health and Human Services (HHS) wrote in a letter to OMICS Group Inc., which puts out a variety of suspect titles, stating that the publisher should "cease and desist from employing [HHS's] name or the name of any of our agencies, institutes, or employees on your website for other than true factual statements." (See "OMICS in Hot Water," *The Scientist*, May 14, 2013.) And last year, the US Federal Trade Commission (FTC) filed a complaint against OMICS Group alleging violations of the FTC Act, a broad law passed in 1914 meant to, among other things, protect consumers against deception. "In numerous instances, individuals who have agreed to serve as peer reviewers for [OMICS Group journals] either never receive any manuscripts to review or discover that, when they access the online manuscript review system to review their assigned articles, the articles have already been approved for publication," the agency noted. (See "US Gov't Takes On Predatory Publishers," *The Scientist*, August 29, 2016.)

Beall says he believes the predatory publishing problem is waning, but adds that it still poses a risk to legitimate scholarly pursuits. "Scholarly publishing and science are threatened because of all the junk that's being published."

The way forward

At Macquarie University in Sydney, Australia, administrator Andy Pleffer helps faculty decide when and where to publish their work. In his experience, even senior scholars are sometimes tricked by publishing scams. While junior scientists are more likely to be targeted by predatory publishers, "it can be across the whole [career] spectrum," says Pleffer. "On some level, over time you get experienced researchers who can sniff a rat, but in other cases, because of limited experience or limited information, they're just as easily able to fall into these traps."

Virginia Barbour, chair of the nonprofit Committee on Publication Ethics (COPE), says her organization has not extensively examined predatory publishing practices, but has partnered with the "Think. Check. Submit." initiative, which aims to help researchers identify legit journals. "Our approach has been much more towards building a positive culture rather than attempting to catalog the behavior of the suspect journals," Barbour told *The Scientist* in an email.

To that end, several groups have replaced what many considered a so-called blacklist—Beall's—with "whitelists" highlighting reputable publishers. In addition, COPE, the Directory of Open Access Journals, the Open Access Scholarly Publishers Association, and the World Association of Medical Editors have outlined "principles of transparency and best practice in scholarly publishing." Among these standards are that the journal clearly state its peer-review policies, APC fees, and editorial board membership.

These guidelines can help researchers as they decide where to publish their work or whether to join a journal's editorial board, Pleffer says, but it's ultimately up to the scientist to judge for herself whether associating with a particular publication is likely to be beneficial. Any individual journal-evaluation system, he says, may be subject to potential inaccuracies, and could quickly become outdated.

"We encourage people to look beyond a single identifier; there is no single indicator of quality research out there," Pleffer says. "More than anything," he adds, "it's important for individual researchers to be actively engaged in this space so that they have a good level of understanding and control about where they are publishing their research."

Still, predatory publications have found myriad ways to mimic legitimate journals. The best defense, says Bauer, is open communication. "Whenever I make mistakes," he says, "I always tell people around me, [so they] don't make the same mistakes."

He and his wife Anika Hartz, also an associate professor at the University of Kentucky and co–principal investigator of their blood-brain barrier research lab, have used Bauer's personal experience of being associated with a predatory publisher as a teaching tool for students and trainees. "I would assume you find this with more-junior people, who are eager to demonstrate acceptance into the field [and] have something to put on their CV in the early stages of their academic career," says Bauer. "That's exactly the situation I was in." ■

DECIDING WHERE TO PUBLISH

Get started early. While it's often an afterthought, consider where to submit your manuscript early on, says Andy Pleffer of Macquarie University in Sydney, Australia. "Think about it up front so you've got a longer lead-in time and you can create a longer list of where you might publish. Especially if you've got a particular journal on your radar, they might have a special issue coming up that ties in quite neatly with your particular expertise."

Scan the TOC. Are there any familiar names in the journal's table of contents? Do you recognize any members of the journal's editorial advisory board? If the answers to both are no, it's probably worth looking into alternative titles, says Chad Cook of Duke University.

Read the journal's policies. Familiarize yourself with the publication's peer-review process, author fees, and policies pertaining to copyright, access, and conflicts of interest. All should be clearly outlined on the journal's website.

Beware of "Contact us." While not always a sign of a suspect publication, journals that do not list editorial staff phone or email contact information—instead, offering only a "contact us" form—is "usually a red flag," says Pleffer.

Check DOAJ. Look to see if the publication is listed in the Directory of Open Access Journals and other scholarly databases, and is indexed on PubMed or by the Institute for Scientific Information. If it's not, proceed with caution.

SHOULD YOU JOIN THE BOARD?

Have you published in the journal? If yes, how was the overall experience? If no, have any of your colleagues or your collaborators' colleagues?

Email overload. "If you get an invitation through email, be extremely suspicious," says Jeffrey Beall, a librarian at the University of Colorado Denver. "Most high-quality journals don't go looking for editorial boards through email. It's usually the other way around: people want to serve on a particular journal's editorial board, and they will send an email to the journal."

Standing members. Examine the journal's existing board. Do you recognize any names? Are any of the board members senior scientists? "What I noticed from the beginning was that there were really no well-known people [on the board]. A lot of the people were junior people, like myself," the University of Kentucky's Björn Bauer says of his experience with *Pharmacologia*. Additionally, do the board members list their participation with the journal on their CVs or biosketches? "If they back that up on their profile, that's generally a good sign," says Pleffer.

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Send In the Phages

Employing biological armies, such as bacteriophages, to fight disease and crop pests could propel medicine and agriculture into the 21st century and reduce humanity's reliance on toxic chemicals.

BY EMILY MONOSSON

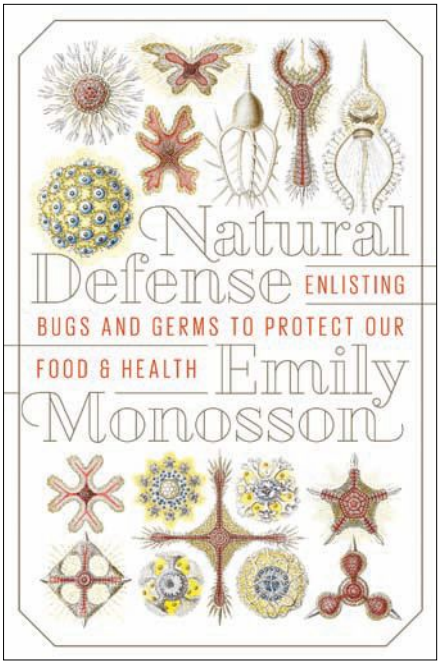
This year I gave a presentation to public-health students at a university about options for controlling pests and pathogens that didn't depend on industrial-age chemicals such as antibiotics and pesticides. When I asked if they'd ever heard of phage therapy—the use of bacteria-attacking viruses to fight infection—I was met with blank stares. When I finished sharing stories of desperate patients miraculously cured of antibiotic-resistant infections within days, I sensed a bit of skepticism, as if the crowd's politeness was keeping them from asking: "If it's so effective, how come we've never heard of this?" In this age of alternate truths and quack cures, it's an appropriate question.

But phage therapy is nothing new, nor is it some fringe remedy. It was first used to cure *Shigella* virus infections early in the 20th century, to miraculous effect (although at the time, scientists were unaware of the nature of viruses). Once treated with phages isolated from fecal samples of spontaneously recovering dysentery sufferers, patients' *Shigella*-induced fevers and bloody stools subsided within 24 hours. (See "Viral Soldiers," *The Scientist*, January 2016.) Within a decade, pharmaceutical companies on both sides of the Atlantic began developing various phage therapies. But then came antibiotics. And poor production practices by some pharmaceutical companies (some commercial products in the U.S. were found to be lacking in potency, for example) led to a couple of damning reviews of phage therapy in the *Journal of the American Medical Association*. All of this helped to close the door on phage therapy in Western medicine. The Cold War kept that door closed for decades to come.

Meanwhile, Russia, France, and Poland continued refining the therapy. Noticing the bacterial propensity to evolve resistance under pressure from killer viruses, researchers understood that they could capitalize on the even greater capacity of viruses for rapid evolution, updating phage cocktails as newly resistant bacterial strains emerged. The Phage Therapy Center in Tbilisi, Georgia, currently offers phage treatments.

Despite encountering skepticism about the effectiveness of phage therapy, I have also been asked by students desperate to find a cure for themselves, or a loved one, to recommend phage-friendly doctors here in the U.S. Other than suggesting that they ask their physician to look into phage therapies or do a Google search, I have had little to offer. But there are glimmers of hope. Now, Western scientists and physicians are trying to introduce the therapy into the American pharmacopeia. In July 2015, the National Institutes of Health organized a meeting of international bacteriophage scientists, entrepreneurs, and regulators hailing from the United States, France, Georgia, China, and elsewhere. Another workshop will be held this July in Rockville, Maryland. There are now clinical trials of phage-therapy products underway in the U.S. and in Europe. If successful, developers will soon be knocking on the FDA's door.

Phage therapy is just one example of a disease-control approach that is more in tune with nature, whether we are concerned about protecting our kids or a field of strawberries. In my new book *Natural Defense: Enlisting Bugs and Germs to Protect Our Food and Health*, I explore a range of strategies that can help us reduce our dependence on chemicals, from antibiotics to pesticides. The control of microbial infections in humans, in particular, shares many characteristics with agricultural strategies. Rapid, more-accurate diagnostics will help both in the hospital and on the farm: new technological solutions promise to enable rapid disease detection and identification. Prevention can help protect us and the crops we grow. And sometimes the solutions in field and body are the same—phages are useful allies against bacteria in the food industry and in human medicine. As I write in the preface, these are just a few strategies. Some may work, others may not, but such combined efforts can help to reduce our dependence on 20th-century chemical cures. For too long, we have considered ourselves separate from the environment. But the sooner we begin working with, rather than against, nature for our food and health, the better off we will be. ■



Island Press, June 2017

Emily Monosson is an environmental toxicologist. She is an independent scholar at the Ronin Institute and an adjunct professor at the University of Massachusetts, Amherst. Read an excerpt of Natural Defenses *at www.the-scientist.com.*

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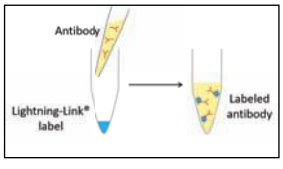

- ALEXA Fluor® 488, one of the most popular fluorescent labels used by life science researchers, has now been added to Lightning-Link® kits
- The addition of ALEXA Fluor®488 now enables easy, direct conjugation of the label to primary antibodies
- Allow for conjugation of antibodies and proteins in less than 20 minutes, using a simple protocol which is free of separation steps

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Lonza's RAFT™ 3D Cell Culture System for Tissue Modeling

Lonza's RAFT™ 3D Cell Culture System for Tissue Modeling

Lonza's RAFT™ System is designed with usability in mind, taking advantage of simple, easy-to-follow protocols that allow researchers to set up 3D cultures in less than an hour. In the RAFT™ System, cells are embedded within a collagen solution and biocompatible absorbers are utilized to remove the medium and create robust, high-density collagen scaffolds. The resulting 3D cultures are easy to handle for downstream assays and are more representative of the *in vivo* environment, enabling cells to grow and interact more effectively.

The versatile RAFT™ Kit is available in 96- or 24-well formats and is suitable for analysis using a wide variety of imaging, biochemical, and histological techniques. The 96-well format is well-suited for scale-up and has been successfully combined with automation systems from a number of leading manufacturers.

An important advantage of the RAFT™ System over other 3D platforms and 2D culture methods is its ability to create complex *in vitro* models, especially those where a multi-layer co-culture setup has proven difficult to achieve via alternative methods. Additionally, the use of permeable membrane inserts expands the applications of the system, such as facilitating the generation of airlift models.

Lonza's RAFT™ System is compatible with a variety of cell types and is currently being utilized in a number of research areas including oncology, barrier modeling, and pulmonary research. In combination with Lonza's human primary cells and media solutions, the RAFT™ System empowers researchers to create biologically relevant cell culture models for research use.

To see how the RAFT™ System works, watch the video at www.lonza.com/raft-3d-culture.

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scientific.support@lonza.com
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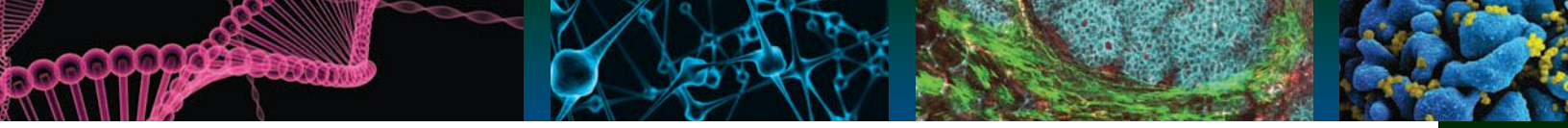

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Scientific Organizers: **Maureen Coetzee**, University of Witwatersrand, South Africa
Josiane Etang, Institut de Recherche de Yaoundé, Cameroon
Stephen Torr, Liverpool School of Tropical Medicine, UK
Scott L. O'Neill, Monash University, Australia

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Louis J. Muglia, Cincinnati Children's Hospital Medical Center, USA
Yoel Sadovsky, Magee-Womens Research Institute, USA

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October 15–19, 2017 | Pok Fu Lam, Hong Kong | China

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Scientific Organizers: **Paul K.H. Tam**, University of Hong Kong, China
Urban Lendahl, Karolinska Institutet, Sweden
Freda D. Miller, SickKids, Canada

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Scientific Organizers: **Gautam Dantas**, Washington University School of Medicine, USA
Jennifer A. Leeds, Novartis Institutes for BioMedical Research, USA

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Frontiers of Serotonin Beyond the Brain

November 12–15, 2017 | Park City, Utah | USA

Scientific Organizers: **Fusun Kilic**, University of Arkansas Medical School, USA
Michael D. Gershon, Columbia University, USA
Luc Maroteaux, UMR-S 839 INSERM / UPMC, France

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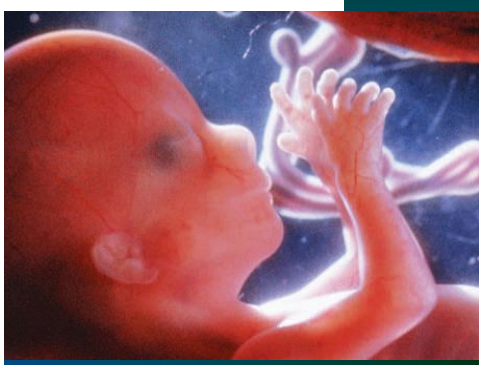
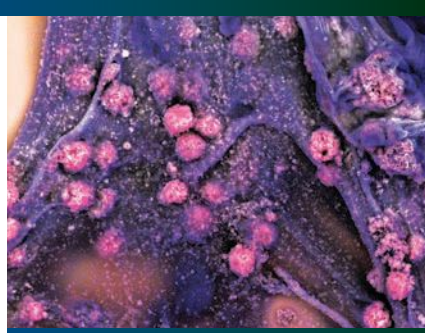
Submit an abstract three months before the meeting to be considered for a short talk! Check website for specific deadlines.



CUTTING-EDGE PRESENTATIONS



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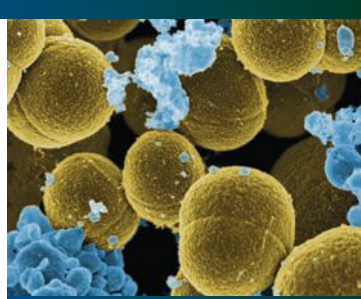

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Demonstrating Discontent, May 21, 1990

BY ANDREA ANDERSON

National Institutes of Allergy and Infectious Disease (NIAID) director Anthony Fauci raced down the stairs of Building 31. Roughly 1,000 AIDS activists filled the National Institutes of Health (NIH) campus in Bethesda, Maryland, armed with signs ranging from relatively benign expressions of discontent with the state of AIDS research ("We're Fired Up") to attacks directed at Fauci himself ("Fuck You, Fauci").

Groups split off to perform varied protest actions. Some staged die-ins, lying down across NIH's lawns as if they were dead. Peter Staley, a member of "AIDS Coalition to Unleash Power" (ACT UP), went for high ground. Flanked by two other activists, he approached building 31 and used his friends' hands to springboard onto the concrete awning.

Fauci, who had met with Staley and other activists previously, most recently at a dinner held at NIAID deputy director Jim Hill's house not long before the current demonstration, spotted police trying to pull Staley down and became "very concerned because I didn't want him to get hurt." By the time Fauci reached the ground floor, Staley's hands were zip-tied behind him, and he was being removed by an NIH police officer. "As he walked by," Fauci recalls, "he looked up at me with a big grin on his face and said, 'Tony, I did it! I was the first one to get arrested.'"

The "Storm the NIH" demonstration marked the climax in a series of public events aimed at drawing attention to the AIDS crisis, community concerns, and activist-led clinical-trial critiques. "After you make a phone call, after you write a letter, after you request a meeting, and those requests or either ignored or denied, then you need to create more pressure," says ACT UP alumnus David Barr, now a senior consultant at the Fremont Center, an HIV/AIDS advocacy organization in New York State. "The demonstrations were a way of both creating pressure and bringing the issues to the public."

By 1990, activists had made headway pushing for access to promising experimental drugs before clinical trials were complete. But many felt the NIAID director had fallen short on promises to afford them research access and input. The approved antiretroviral azidothymidine had shaky effectiveness and taxing toxicity. There was a dearth of treatments for opportunistic infections, not to mention concerns over funding, opaque clinical trial protocols, and trial requirements that deterred participation and neglected women, minorities, and injection drug users.

"There was a feeling by ACT UP and others that [trials] needed to be more open to the communities that were dealing with AIDS at the time," explains Mark Harrington, who was a member of ACT UP's Treatment and Data Committee and is now executive director of the Treatment Action Group, an advocacy organization that has its roots in the ACT UP movement. "Some of us wanted to participate in the scientific discussions and decisions that got made."

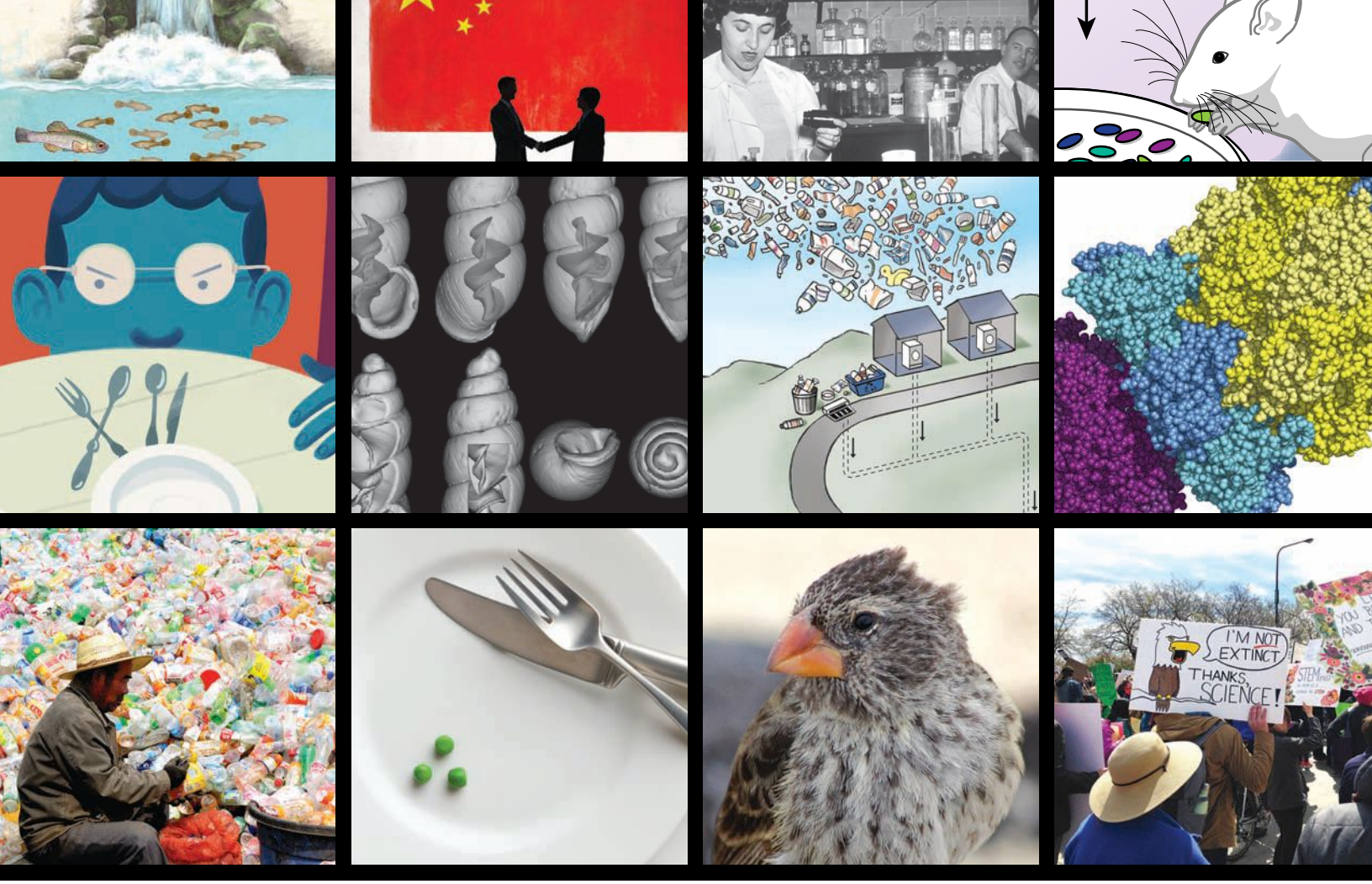
SPEAKING OUT, ACTING UP: AIDS activists from around the country came together to "Storm the NIH" on May 21, 1990, setting off colored smoke bombs en route to buildings where NIH and NIAID directors had their offices. The demonstration "made a huge statement" about activists' demands for increased patient access to clinical trial decisions, says activist Peter Staley. "The people whose minds were ultimately changed: this action made very clear to them how important this goal was to us."

NIH HISTORY OFFICE

Despite these frustrations, the demonstration in Bethesda that day was "a blast," Harrington says. "Everybody knew what we wanted. It was exhilarating. It was great to be with everyone, and it was great to be making our points."

The very next month, things started to change. At the International Conference on AIDS in San Francisco, Fauci and Staley gave speeches highlighting researchers' and activists' shared goals. Shortly after, NIAID's AIDS Clinical Trial Group (ACTG) opened its doors to HIV advocates and community members, who would go on to play active roles on ACTG committees and to serve as advisors at ACTG clinical trial sites across the country.

"That had never happened before in the history of the NIH," says Barr, who believes the NIH demonstration was one of several factors prompting increased inclusiveness and openness by investigators, "and it was a model that was replicated in other disease areas." ■



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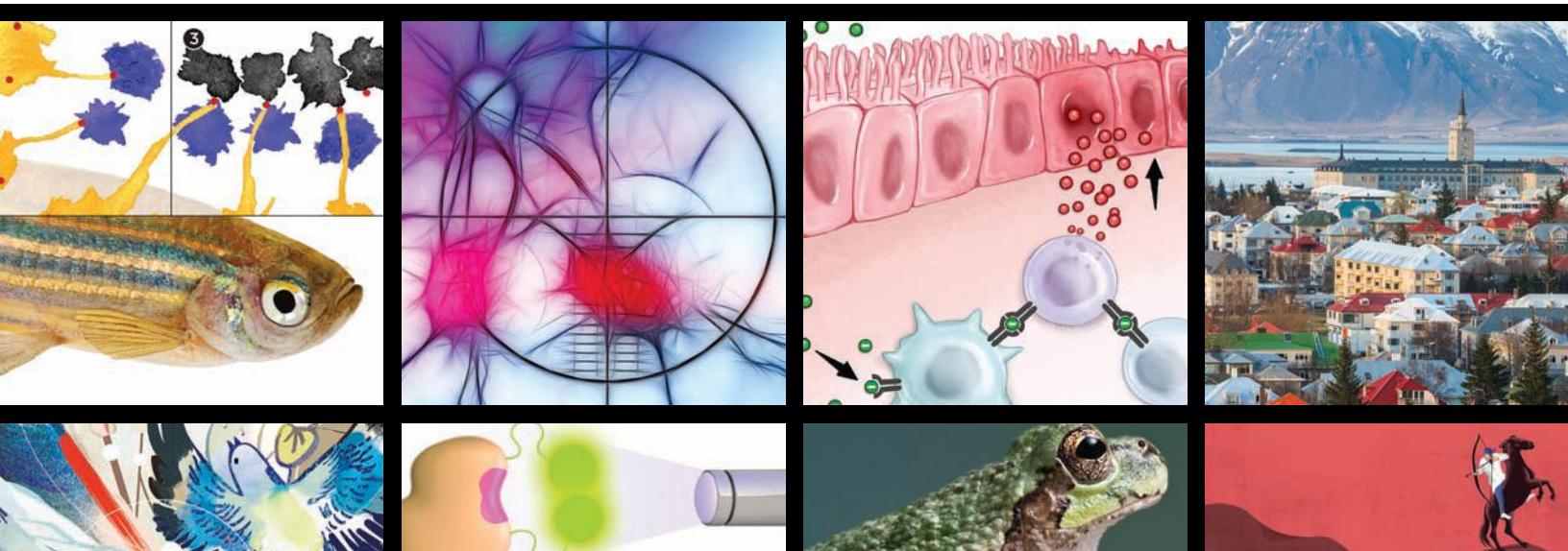

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