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HEALING WITH HALLUCINOGENS

THE THERAPEUTIC BENEFITS OF PSYCHEDELIC DRUGS

DO MICROBES TRIGGER ALZHEIMER'S?

THE FUNCTIONAL CONSEQUENCES OF DNA BASE MODIFICATIONS

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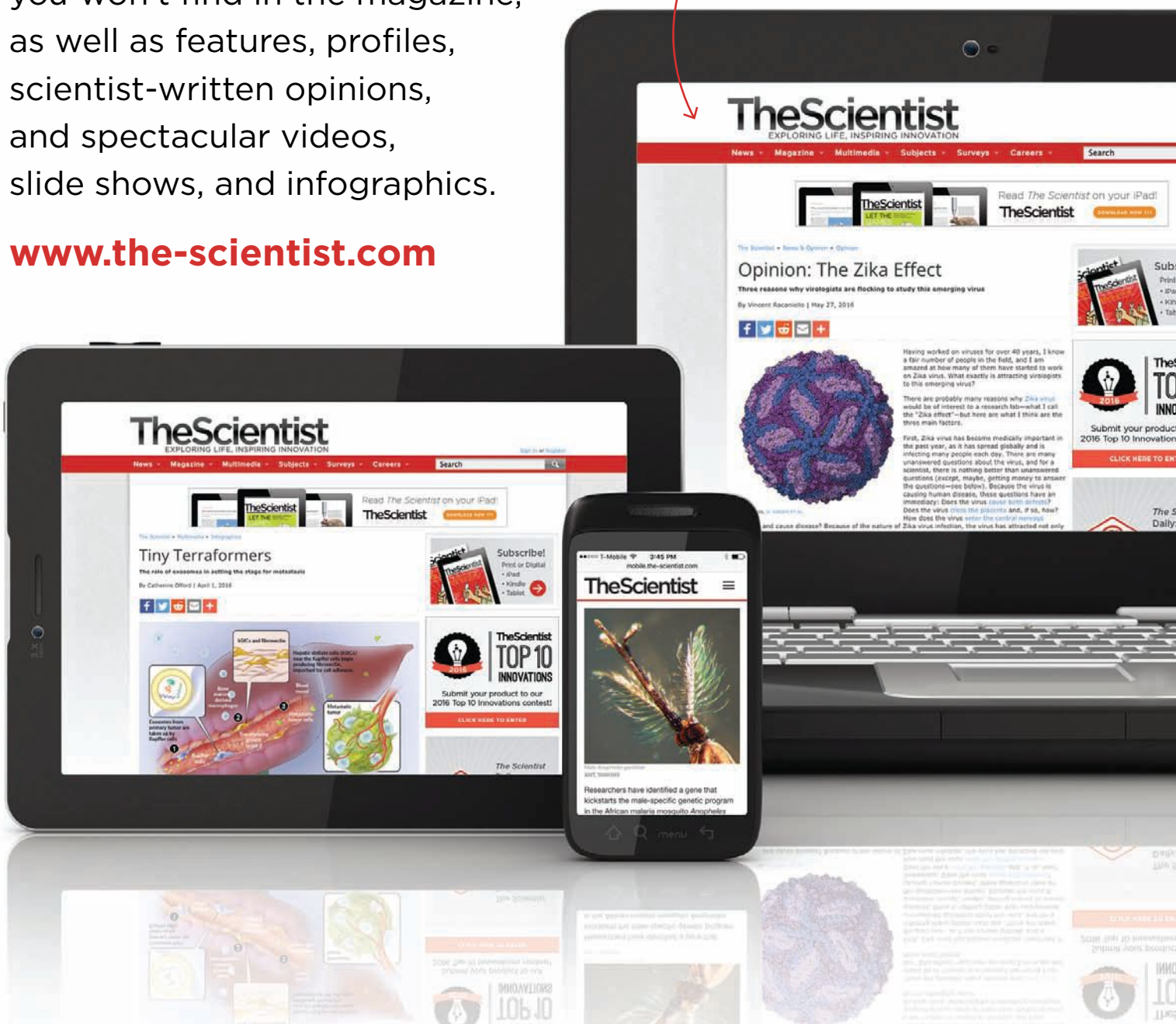

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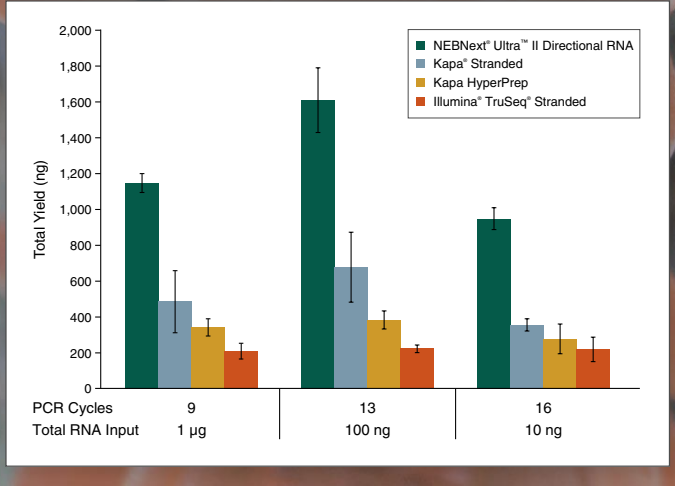

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Contents

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Features

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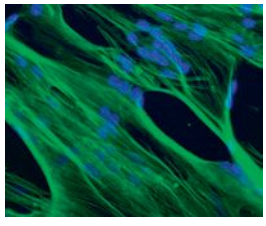

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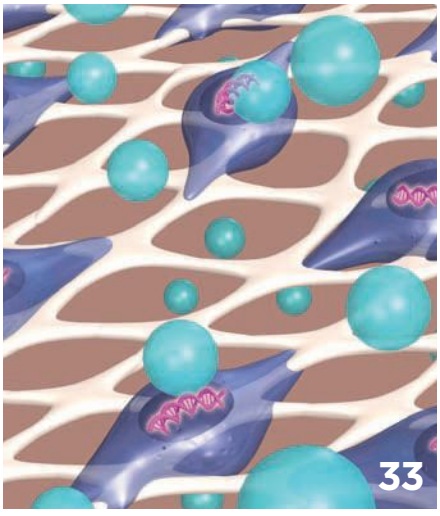
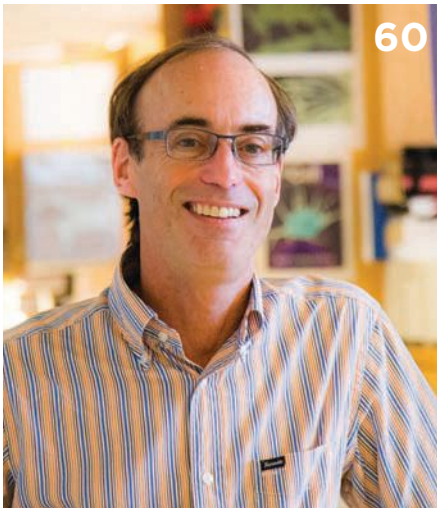


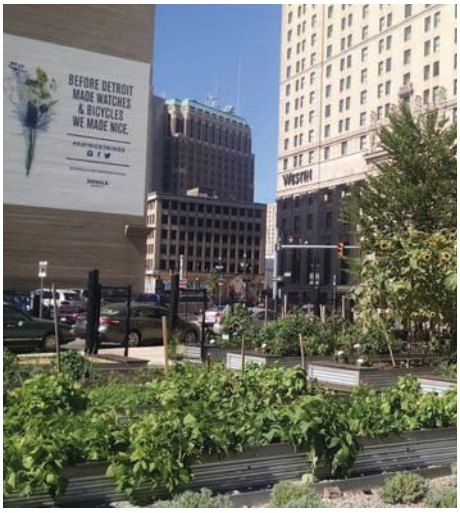


CORRECTIONS:

In the July/August issue of *The Scientist*, please note the following corrections: "Bacteriophages to the Rescue" stated that *Shigella* was a virus. It is a species of bacteria. "Identifying Predatory Publishers" failed to state that Virginia Barbour's term as chair of the nonprofit Committee on Publication Ethics (COPE) ended in May 2017. "Oceans' Ambassador" incorrectly stated that the National Science Board (NSB) is associated with the National Academy of Sciences. The NSB is associated with the National Science Foundation. "The Mechanobiology Garage" incorrectly stated that pore sizes in the microfluidic device designed by the Lammerding lab did not reflect actual capillary pore sizes. They do.

The Scientist regrets the errors.

Online Contents



THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Athlete Meets Machine
Alena Grabowski, a University of Colorado Boulder researcher, discusses her motivations for studying the interface between biology and mechanics in prosthetic devices.

VIDEO
Living Fabric
Have a look at the bacteria-powered, breathable clothing made by former MIT researcher Wen Wang and colleagues.

SLIDE SHOW
City Bees
See the urban landscapes in Detroit where researchers are studying the fates of pollinators that adopt a metropolitan lifestyle.

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Coming in October

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

- Making DNA data storage a reality
- Macrophages: more than just immune cells
- Could vaccinating farm animals drive the evolution of more virulent pathogens?
- Intrinsically disordered proteins as drug targets
- A primer on CRISPR patents

AND MUCH MORE

CITY BEES: PAUL GLAUM, UNIVERSITY OF MICHIGAN; PIG: © ISTOCK.COM/BAZILFOTO

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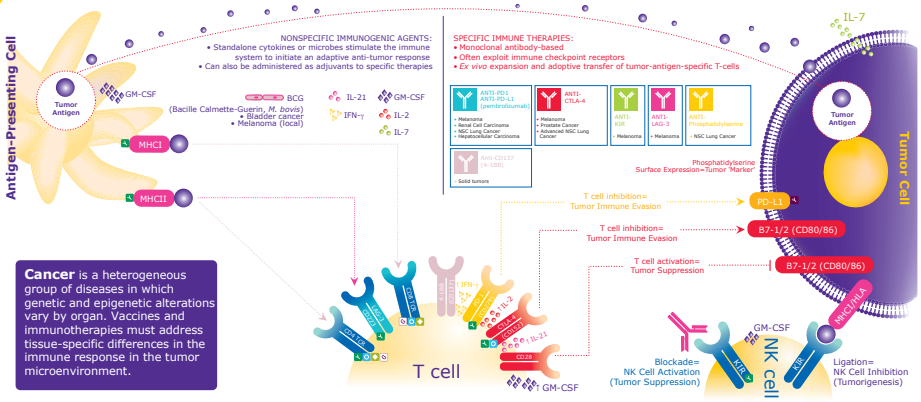
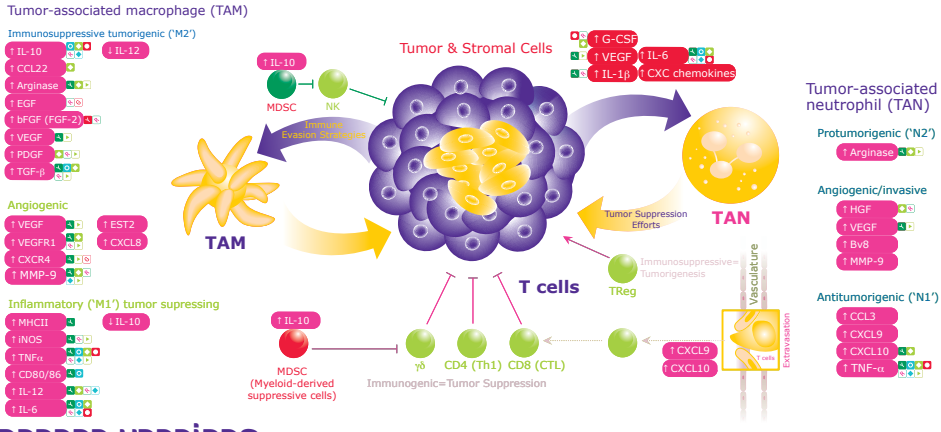
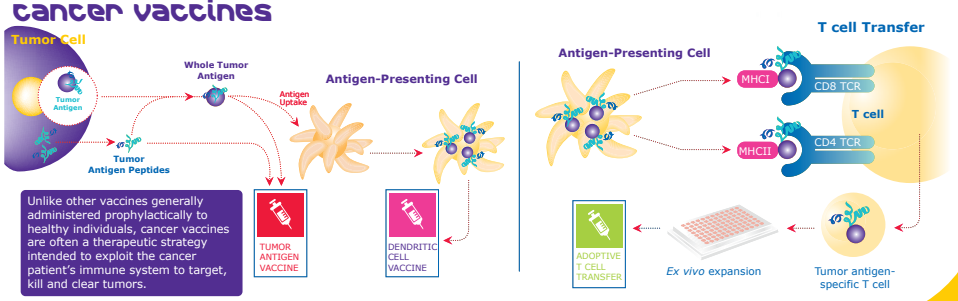


Cancer is a heterogeneous group of diseases in which genetic and epigenetic alterations vary by organ. Vaccines and immunotherapies must address tissue-specific differences in the immune response in the tumor microenvironment.

The Tumor Microenvironment



Cancer Vaccines



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Contributors

Skirmantas Kriaucionis had been playing around with microscopes since his school days. But it was during a project with DNA methylation researcher Saulius Klimasauskas at Vilnius University in his native Lithuania that Kriaucionis got a real chance to dive into biological research. "The work was really exciting," he says. "My interest in it has continued throughout my life."

After relocating to the University of Edinburgh in 2000, Kriaucionis began a PhD with geneticist Adrian Bird on MeCP2, a protein that binds to methylated DNA. "It was a very exciting period to work on MeCP2," Kriaucionis recalls—Bird's lab had just developed a knockout mouse model, and mutations in the *MECP2* gene had recently been linked to Rett syndrome in humans. With Bird, Kriaucionis identified a previously overlooked isoform of the protein that accounted for more than 90 percent of MeCP2 in mouse brains.

Kriaucionis earned his PhD in 2004, and, after a one-year postdoc at Edinburgh, moved to neuroscientist Nathaniel Heintz's lab at Rockefeller University in 2006. There, he identified a new type of DNA methylation, 5-hydroxymethylcytosine (5hmC), occurring at high levels in neurons and absent from cancer cells.

Now, at the Oxford branch of the Ludwig Institute for Cancer Research, Kriaucionis's lab is investigating the role of DNA methyltransferase enzymes in cancer development and probing possible roles for modifications such as 5hmC in neurons. Kriaucionis describes his discovery of 5hmC and explores the possible functions of epigenetic modifications to DNA in his feature, "DNA Extras," on page 48.

As a biology undergraduate at Queen's University in Kingston, Ontario, in the late 2000s, **Britt Wray** was inspired by her lectures, but realized that a life in the lab wasn't for her. Instead, it was another pursuit that would provide direction after graduation: a student radio show about science. "I had a little recorder pack, and I'd go and find people in their labs and talk to them about their work," Wray says. "I loved it."

That experience was just the first taste of a successful career in radio journalism. Graduating from Queen's in 2008, and earning a graduate diploma in communication studies from Concordia University in 2010, Wray went on to produce and host several shows on CBC. This year, she appears as cohost on BBC's new science podcast, *Tomorrow's World*. Wray also holds a master's degree in art, media, and design from OCAD University in Toronto, for which she designed a six-month installation and workshop series to engage public interest in synthetic biology. The program allowed Wray to collaborate with artists and designers—people who "are asking questions from a sideways angle compared to how scientists might be approaching the topic," she says.

In 2014, Wray began a PhD in the University of Copenhagen's Department of Media, Cognition, and Communication, where she is exploring new methods to communicate advances in syn bio. She has also completed her first book, *Rise of the Necrofauna*—an exploration of efforts to recreate extinct organisms such as the woolly mammoth. She describes this project, and the science behind it, on page 70.

As a biochemistry major at Colorado College, **Shawna Williams** assumed she'd eventually go on to get a PhD and become a researcher—until she spent a summer genetically altering yeast cells. "It wasn't nearly as fun as just learning about the science," she says. So she switched gears and in 2002 enrolled in the University of California, Santa Cruz, graduate program in science writing. After graduation, she did internships at the European Organization for Nuclear Research (CERN) in Geneva and the Stanford University School of Medicine, before securing a permanent position as the communications officer at the Boyce Thompson Institute for Plant Research in Ithaca, New York, in 2004. Two years later, she accepted a position with Johns Hopkins University's Genetics & Public Policy Center in Washington, DC, writing about issues such as genetic privacy. In 2009, she again switched gears, moving to Chengdu, China, to teach English at Sichuan University. There, she met her husband, and remained in China working as a freelance writer and editor. In 2012, Williams spent six months in Japan as a science writer at the brand-new Okinawa Institute of Science and Technology, before returning to the U.S., where for the past five years she has been working as a communications manager at Johns Hopkins University in Baltimore. When she saw the opening at *The Scientist* for an associate editor position, Williams jumped on the opportunity, joining the staff in June. "[It] seemed like a job where I would get to do a whole lot of writing and editing and have a wider pick of a wider variety of stories. And it has been a great fit in that way."

MONTY RAKUSEN; ARDEN WRAY; KAT ONRUBIA

The Scientist wins more kudos for editorial excellence

CREATIVE CONTENT FOR CURIOUS MINDS

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

TheScientist

Far-Out Science

How psychedelic drugs and infectious microbes alter brain function

BY MARY BETH ABERLIN

ANDRZEJ KRAUZE

Having lived through it, I can free-associate for hours about the so-called Hippie Era. It's really (dare I say it) an invitation to fall down the rabbit hole of memory. Music comes to mind first: *White Rabbit* ("One pill makes you larger/And one pill makes you small/And the ones that mother gives you/Don't do anything at all"), of course, and *Lucy in the Sky with Diamonds* (tangerine trees, marmalade skies, kaleidoscope eyes), to name just two. Then there was the attire (patterns run wild, bell bottoms, beads); the pelage (long, wild, puffy), which got star billing in the 1967 musical *Hair;* and the books (Tom Wolfe's *The Electric Kool-Aid Acid Test* and Richard Brautigan's *Trout Fishing in America* come to mind).

But for a wordsmith like me, it's the associated vocabulary and the era's identifying dictums that I love: "Turn on, tune in, drop out," "Drop acid, not bombs," "Don't bring me down," or "Sock it to me," "That really blows my mind," and "Far out, man."

So when Diana Kwon turned in her cover story ("Trippy Treatments," page 34) on using psilocybin, mescaline, ayahuasca, and synthetic LSD as treatments for a wide variety of psychological ailments, not only did the article inspire a trip down memory lane, but it made me curious about the etymology of the word "psychedelic." Apparently, the term was coined in 1956 or 1957, just as the beatnik era was being supplanted by hippiedom. British psychiatrist Humphry Osmond and author Aldous Huxley (*Brave New World*) were searching for a word that was less of a downer to describe the effects of hallucinogens, then known as psychotomimetics—psychosis imitators. Huxley, who had written about his experiments with mescaline (*The Doors of Perception*), employed verse: "To make this mundane world sublime/Take half a gram of phanerothyme." To which Osmond countered: "To fathom Hell or soar angelic/Just take a pinch of psychedelic." (Both words mean "mind/spirit/soul revealing.") How groovy is that?

The drugs got a new moniker, but the counterculture's recreational use of psychedelics earned the compounds a bad name, and a 1970 Schedule 1 assignment of the drugs by the US Department of Justice basically stopped federal funding for research into how they worked in the brain and how they might serve medicine. Kwon neatly summarizes the state of research today, as scientists pick up on a handful of trials from the 1950s, '60s, and '70s and begin to work out the neural pathways that are altered during a psychedelic trip.

As an interesting coincidence, this issue also contains a feature about the opposite of mind expansion—the truly mind-altering condition of Alzheimer's disease (AD). Decades of research have been devoted to treatment strategies aimed at wiping out amyloid plaques or other pathological manifestations of AD dementia—to little or no avail. So, if attacking the plaques after they form is not effective, evidence about how AD initiates its destruction in the first place is even more warranted. Is inflammation the culprit? Or could the cause be some sort of infection? A recent article in *The New York Times* summarized research suggesting that a gene associated with Alzheimer's risk originally acted to help fight parasites, and, in their absence, may predispose the brain to an immune attack on itself. In "Brain Bugs," page 42, Jill Adams reports on a long-scoffed-at but decades-old theory that infection by certain microorganisms that cross the blood-brain barrier is involved in some cases of AD, triggering both inflammation and the telltale formation of plaques.

There are plenty of other mind-expanding articles in this issue of *The Scientist,* including one that links the gut microbiome to mental health (page 27); a report on whether limb prosthetics give athletes an unfair advantage (page 23); and an essay on de-extinction experiments aimed at bringing back some semblance of the woolly mammoth (page 70).

So chill out and tune in. We're socking it to you. ■

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

Speaking of Science

If every wildebeest was a penny, and you stacked the pennies, it would be two kilometers high. And those pennies are going to eat 4,000 to 5,000 tons of grass every day. That's an enormous amount of biomass being consumed, digested, and redeposited in some way.

—**Grant Hopcraft**, landscape ecologist at the University of Glasgow, who studies wildebeest migration in order to understand the animals' interaction with their ecosystems (*The Scientist,* August 15)

IMAGE COURTESY OF CHRISTOPHER DUTTON

Listen to experts better qualified than you are. Especially scientists. Be guided by evidence and reason, not gut feeling. By far the best way to assess evidence is the scientific method. Indeed, it is the only way if we interpret "scientific" broadly. In particular—since the matter is so urgent and it may already be too late—listen to scientists when they tell you about the looming catastrophe of climate change.

—Evolutionary biologist and author **Richard Dawkins**, when asked in a *Scientific American* interview what advice he would give to Donald Trump if he had the chance (August 10)

I'm simply stating that the distribution of preferences and abilities of men and women differ in part due to biological causes and that these differences may explain why we don't see equal representation of women in tech and leadership.

—A memo, penned by software engineer **James Damore**, decrying what he calls "arbitrary social engineering" aimed at increasing workplace diversity at Google, which fired him after the document circulated first inside then outside the company (August 5)

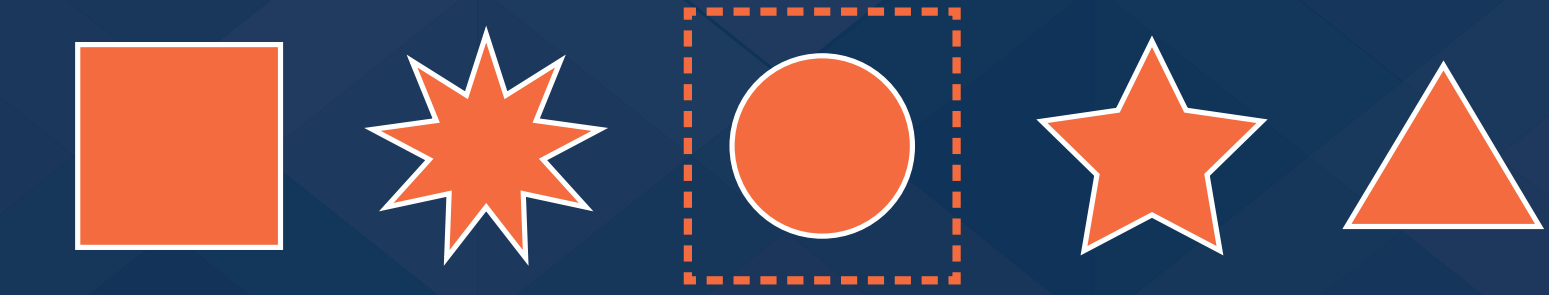
It is impossible to consider this field of science without grappling with the flaws of the institution—and of the deification—of science itself. For example: It was argued to me this week that the Google memo failed to constitute hostile behavior because it cited peer-reviewed articles that suggest women have different brains. The well-known scientist who made this comment to me is both a woman and someone who knows quite well that "peer-reviewed" and "correct" are not interchangeable terms.

—University of Washington particle physicist and philosopher of science **Chanda Prescod-Weinsten**, criticizing what she calls the "shoddy science" that propped up Damore's argument (August 9)

That's never made any sense to me. Why would resistance arise if you stop using your antibiotics? In fact, I think the adage should be that in order to ensure increased likelihood that you will successfully treat your infection, you should complete your full course of antibiotics, but bear in mind that the risk you run is, the longer you use antibiotics, you increase your risk of developing resistance.

—MIT biologist **Jim Collins**, on the emerging understanding of the effects of finishing courses of antibiotics on the development of resistance in pathogens (*The Scientist*, August 11)

ONE WORKFLOW DOES NOT FIT ALL



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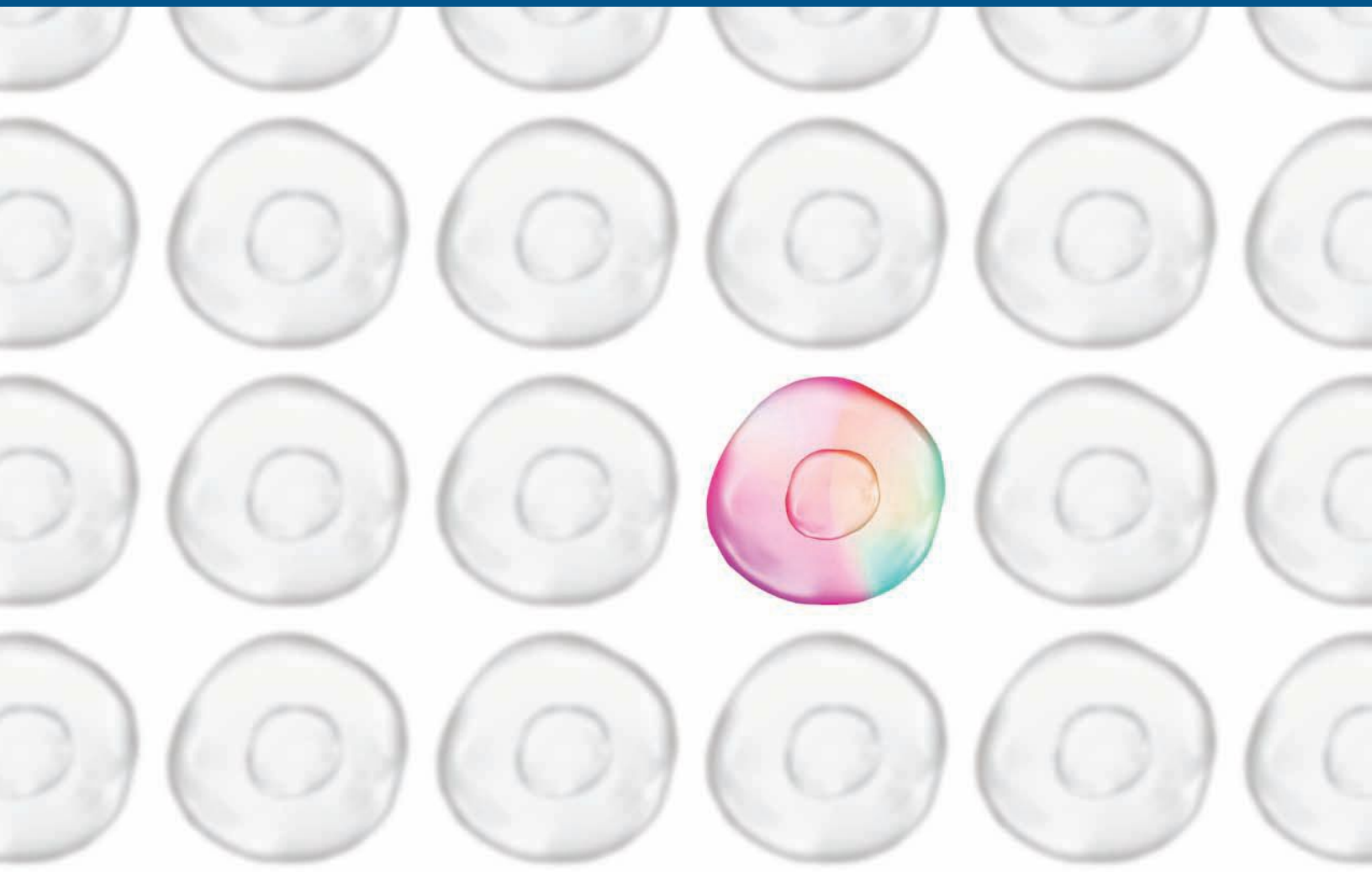

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Notebook

SEPTEMBER 2017

PAUL GLAUM, UNIVERSITY OF MICHIGAN

URBAN BUZZ: Community gardens, such as this one in Detroit, may serve as critical oases for city-dwelling bees.

Metropollen

A few years ago, Paul Glaum and fellow graduate students at the University of Michigan were talking about bees. The discussion centered on imperiled native bee populations in North America, and how to support the important pollinators in urban environments. Unlike intensive farming and pesticides such as neonicotinoids, which have been repeatedly linked with alarming declines in bee abundance, urbanization has a far less obvious impact.

Whether or not urban development harms bees "has been an open question for a number of years now," says Glaum, who is about to start his sixth year in Michigan's ecology and evolutionary biology grad program. "There's a wide variety of results in the literature." While some studies report that development is linked to decreases in bee abundance and species richness, others have identified higher species richness at intermediate levels of urban development; still more have found no relationship either way.

Discussing this uncertainty, Glaum wondered whether part of the problem came from lumping all pollinators, or even just all bees, into one group, thereby obscuring differences in the natural history of individual species or genera. So he and three other graduate students got together to design a study focusing on just one genus of well-studied wild pollinators: *Bombus*, or bumblebees. "There are certain everyday plants like tomatoes that honey bees just can't pollinate, and we need bees like bumblebees to do the work for us," Glaum says. Unlike honey bees, though, bumblebees build their nests directly on or just under the ground, and so could be particularly affected by impervious surfaces, such as the concrete and asphalt that blanket most cities.

The team picked five cities of varying size in southeastern Michigan, from Dexter, which gained city status in 2014, to the sprawling metropolis of Detroit, the state's largest urban center. Through collaborations with city farm and garden owners, the group obtained permission

A BOMBUS AMONG US: The common eastern bumblebee (*Bombus impatiens*) is a frequent visitor to Detroit and other urban centers.

PAUL GLAUM, UNIVERSITY OF MICHIGAN

to work at 30 sites and began recruiting undergraduates to help carry out numerous surveys. "It was an interesting exercise in learning to deal with controlled chaos," Glaum says, adding that because bumblebees don't forage in the rain, the students spent the summers of 2014 and 2015 at the mercy of Michigan's climate. "We checked the weather like nervous farmers," he says. "It was a great effort by everyone involved. Sometimes you look back and wonder how it all got done."

By the end of 2015, the researchers had amassed bee data from the sample sites, as well as estimates of the proportion of ground covered by impervious surfaces—a standard proxy for urbanization—in the surrounding areas. Surprisingly, when they compared bee abundance to the proportion of impervious surface in the 2 kilometers around each site—the approximate bumblebee flight range—the researchers found no relationship at all. It was only when sites from Detroit were excluded from the analysis that the team detected a strong negative correlation between the two variables; as impervious surface area went up, bumblebee abundance went down (*Roy Soc Open Sci*, doi:10.1098/ rsos.170156, 2017).

This correlation is fairly intuitive, says entomologist Dan Cariveau, head of the Bee Lab at the University of Minnesota. "Impervious surface could really affect nesting sites," he says. "Bees that nest above ground might be okay in urban areas, but when you have a lot of impervious surface, you lose a lot of ground-nesting habitat." Detroit, which showed relatively high bumblebee abundances, is an anomaly, he says, adding that it will be important to understand why this city bucks the trend. "If we can document why Detroit might be doing well for bumblebees, despite having such high impervious surface, I think that really could help to figure out how to manage the landscape for higher diversity in urban sites," he says.

Since this is such a human-controlled system, there might be an active role that humans can play to mitigate some of the negative effects on bees.

—Paul Glaum, University of Michigan

Although the current study does not address Detroit's curious bee abundance in detail, Glaum and his colleagues advance a theory in the paper they published earlier this year. "Detroit is a city with a unique physical setup," Glaum explains. "A long period of economic hardship has left much of the land vacant." Vacant lots in such "shrinking cities" might act as bee oases, he notes, adding that researchers need to take the heterogeneity of different cities into account when studying the ecological effects of urbanization.

Detroit wasn't the only surprise, though. Splitting the data on bee abundance by sex showed that the negative impact of urban development in cities other than Detroit was driven almost entirely by effects on female bumblebees—males seemed unperturbed. Again, Glaum suspects the result is tied to nesting habitat. "Male bumblebees have essentially one job, and that's to leave the nest and find a mate," says Glaum. "They live from flower to flower, so they don't have the limitation of having to nest underground. They just turn flowers into temporary bee motels overnight."

"It's an interesting result, that males are potentially less affected by the landscape," says community ecologist Katherine Baldock, a researcher at the University of Bristol who coordinated the UK's Urban Pollinators project a few years ago. "It's probably, as the authors point out, because they're using the landscape in a different way." However, she adds, there are factors other than nesting habitat that could influence bee abundance in city environments. "One thing I think would be really interesting is: What flowers are the bumblebees feeding on in these

sites?" she says. "I think there's more to this story."

Glaum says the team hopes to address these questions in further surveys, noting that growing the flowers that urban bumblebees frequent could perhaps help bees thrive in urban environments. "Working with farmers and gardeners in southeastern Michigan has exposed me to a very optimistic group of people," he says. "Since this is such a human-controlled system, there might be an active role that humans can play to mitigate some of the negative effects on bees." —Catherine Offord

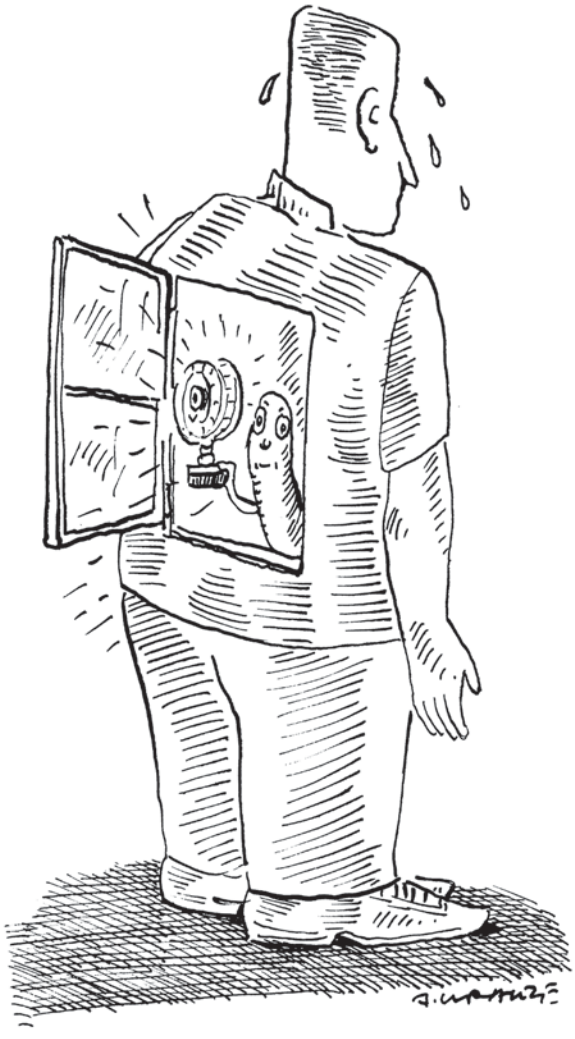
Sweat Shirt

In 2013, bioengineer Wen Wang, then a research scientist at MIT, attended a talk on how *Bacillus* spores shrink in response to falling relative humidity. The research, published the following year in *Nature Nanotechnology* (9:137-41), focused on using this property to extract energy, but it gave Wang another idea: What if she could use shape-shifting bacteria to develop a material that would ventilate upon sensing the sweat of its wearer? "Humans are a natural source of humid air," she says. "We thought maybe we can do something related to garments."

She teamed up with her friend and colleague Lining Yao, also a researcher at MIT's Media Lab, and began testing what caused the spores to change shape. Through a process of elimination, the team found that it was changes to the proteins inside the spores that contributed the most to the volume change, though DNA and polysaccharides also shifted configuration in response to changes in humidity. Sure enough, attaching pure bacterial protein to a fabric caused the material to become moisture sensitive. "Imagine you have a double-layer system: the top layer is the protein layer; the bottom layer is the fabric layer," Wang explains. "When the top layer starts to shrink [in response to dry conditions], the whole thing bends up."

Then came the challenge of designing a garment that would open in response to sweat, to allow the wearer to get additional ventilation as their body heat rose. Despite pinning the shape response on the bacterial proteins, Wang and her colleagues decided to use whole bacteria for this part of their project, in part because they are so easy to produce. "One bacterium, overnight, becomes millions," Wang explains. Whole bacteria are also more stable than naked proteins, she adds, and using whole bacteria could also enable the team to one day endow the garment with additional functions, such as consuming sweat, emitting light, or producing a pleasant-smelling odor.

Using a 3-D printer, the team laid down a layer of bacterial cells directly onto latex sheets. However, due to the high relative humidity of the printing conditions, the resulting material curled toward the



bacterial side of the fabric at normal room conditions. This is the opposite of what the researchers wanted: they were hoping to create a material that was flat at normal conditions and curled in response to high humidity. To solve this problem, the group decided to print bacteria on both sides of the fabric. This way, both sides would respond equally to room conditions, and the fabric would remain flat. But when one side was exposed to humidity, as would be the case for the interior of a shirt, that side would expand relative to the opposite side, and the material would bend outward.

With the help of added collaborators, the researchers used a version of the triple-layer fabric made with *Bacillus subtilis* to create responsive vents on the back of a shirt. The team then had volunteers wear the garment while running on a treadmill or cycling on a stationary bike, and monitored their skin temperature and humidity. In just five minutes, the vents started to open up, allowing the sweat to evaporate and lowering the wearers' temperature to a greater degree than experienced by people who wore a control shirt with nonfunctional flaps (*Science Advances*, 3:e160198, 2017).

That's the advantage of our garment—it's helping you remove the moisture immediately.

—Wen Wang

Wang herself tested out the prototype, along with the control shirt. "When I wore the control version, I felt really, really humid and hot," she recalls. "When I wore the functional one, once I started to sweat, it opened very naturally, and then I could feel air flow come to my back." This sort of ventilation system cools the body, while the shirt itself does not absorb the sweat, thus staying dry. "That's the advantage of our garment—it's helping you remove the moisture immediately [through evaporation]. Then body temperature will drop." Once that happens, the relative humidity equalizes on both sides of the garment, and the flaps close again.

"I think it's amazing," says Ozgur Sahin, a biophysicist at Columbia University who coauthored the 2014 *Nature Nanotechnology* paper but was not involved in the shirt development. "This is a very good example of the material directly responding to a stimulus—in this case, it's sweat—and it's responding in a way that helps the person lose heat."

Wang's team also made a prototype shoe using a nonpathogenic strain of *E. coli*, with responsive vents in the sole. As a proof of concept that additional functionalities could be incorporated into bacteria, the team equipped both species with the gene for GFP, which loses its ability to fluoresce under dry conditions. Sure enough, the shirt and shoe flaps started to glow as they opened up in response to increasing humidity. The researchers are now in the process of figuring out how to commercialize the products, including making the garment washable by having the bacteria or cellular materials bind covalently to the latex. New Balance was a sponsor of the research, and Wang says that the team had been approached by several companies interested in the technology.

Patrick Mather, a materials scientist at Bucknell University who was not involved in Wang's work, noted that this is not the first time researchers have devised materials that vent. "The idea is that, if you have a bilayer of two soft materials, and one reversibly contracts, then you can get these effects that can be used for venting," he explains. And some sportswear companies have already filed patents in this space. "There are different concepts out there for active sportswear; venting is kind of the lowest-hanging fruit."

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But this is the first time Mather has seen the use of cells to drive the response, and in other prototypes he's seen so far, venting is triggered by heat, not moisture. In Wang's work, "that the moisture sensitivity is coming from a biofilm—that's cool," Mather says. "The timescale is right, the humidity level is right, for the application in sportswear." Another benefit, he notes, is that the curling is gradual, with the angle changing slowly with relative humidity. "That's really nice. Instead of just being on and off, open and close, it's more gradual and continual."

Both Sahin and Mather say they're excited to see what might come next, especially if big-name companies start pouring money into the research. And even on the basic research side, "I think a lot of people will now run with this," Mather says. "When papers like this get published, it's good because it stitches communities together, and at the interface between two communities, usually that's where the big leaps happen. Because people are like, 'I'll try the simplest thing a microbiologist would ever try,' and that may be revolutionary for a materials scientist, or vice versa."

—Jef Akst

Athletic Prosthetics

Oscar Pistorius, a South African sprinter dubbed the "Blade Runner," made history in 2012 when he became the first double amputee to participate in the Olympics, running the 400-meter dash. Pistorius had been barred from the 2008 competition by the International Association of Athletics Federations (IAAF) after researchers in Germany reported that his prosthetic limbs provided an advantage over the legs of an able-bodied athlete (*Sport Technology*, 1:220-27, 2008). However, the IAAF reversed its decision after a team of researchers in the U.S. conducted a follow-up study that incorporated several additional parameters and concluded that Pistorius's artificial limbs, though mechanically different, were physiologically similar to biological ones (*J Appl Physiol*, 107:903-11, 2009).

Although the second study ultimately allowed Pistorius to compete in the Olympics, some of its authors later argued that the mechanical differences could help enhance running speeds. The others contended that there was insufficient evidence to make this claim. Years

BLADE RUNNER: A pair of the prosthetic devices that Alena Grabowski tested in her University of Colorado Boulder lab

PATRICK CAMPBELL, UNIVERSITY OF COLORADO

later—after the debate surrounding Pistorius's athletic performance was subsumed by a court trial that found him guilty of murdering his girlfriend—the question of whether these devices provide an advantage remains unanswered. However, researchers are now starting to shed light on how specific features of prostheses can affect performance.

"[After] doing some research on [Pistorius], I had a bunch of other questions about prostheses and what they're capable of and not capable of," says Alena Grabowski, a physiology and biomechanics professor at the University of Colorado Boulder and one of the authors of the second Pistorius study who said there was insufficient evidence to claim a prosthetic advantage. "That propelled me to this bigger research idea of trying to figure out how prostheses function."

Grabowski is currently part of a group of researchers investigating how adjusting various parameters of these devices affects athletic performance. In its latest study, the research team assessed five athletes with double transtibial (below-the-knee) amputations as they ran on a specialized treadmill. Each participant performed multiple trials with three different prosthetic models that were adjusted to various lengths and levels of stiffness.

"One of the major challenges of biomechanical studies related to the effects of prosthetic components is sufficiently powering the study," David Morgenroth, a professor of rehabilitation medicine at the University of Washington who did not take part in the work, writes in an email to *The Scientist*. "Although the small number of participants in this study may be seen as a weakness, relative to other published studies of running-specific prostheses in participants with bilateral amputations, this study has a larger number of participants."

By analyzing these five individuals, the team discovered that, contrary to what many believe, the vertical length of the prosthesis did not have a significant overall effect on key factors associated with running speed (*Interface*, 14:20170230, 2017). "There are two schools of thought," says study coauthor Paolo Taboga, a prosthetics researcher at California State University, Sacramento. One, he explains, posits that having taller legs means taking longer steps, which, in theory, could help you run faster. On the other hand, some believe that having a longer leg may make it harder to swing that leg, resulting in fewer steps.

It turns out that "both views are true," Taboga says. "You can take longer steps, but it takes a bit longer to take those steps—so in the end, the two effects counterbalance each other."

Even among athletes with amputations, prosthetic length has been a point of contention. In fact, Pistorius himself accused another double amputee Paralympian, the Brazilian runner Alan Oliveira, of artificially increasing his height with longer prostheses to improve performance. And although the International Paralympic Committee has a formula to determine maximum standing heights, "it's really hard and controversial to try to figure out what [the ideal] height would be," Grabowski says. She adds that this is due to the fact that many of these amputations are the result of congenital conditions (being born without certain bones, for example) and that these individuals may have different arm or femur lengths compared to an average nonamputee.

Other aspects of prostheses need to be taken into consideration as well. For example, in the same study, Grabowski and colleagues found that another measure, stiffness, did influence two factors associated with higher sprinting speeds. Increased stiffness improved the athletes' ability to generate large forces on the ground while decreasing the amount of time they spent on its surface. This effect, however, became less pronounced at higher speeds.

Now, the team is assessing whether length and stiffness can actually influence an athlete's maximum running speed. Although the results are not yet published, Grabowski says that their preliminary analyses suggest that neither measure has a significant effect.

"I think [this research] really is a tremendous contribution—they were able to address many of the hypotheses that were out there about how these devices should impact performance," says Craig McGowan, a biomechanics professor at the University of Idaho who wasn't involved in the work but has collaborated with the authors in the past. However, he adds, "I think the debates [about performance] won't go away anytime soon."

Is there a day that will come where we can create a prosthesis that's better than flesh and blood? We're not there yet, but I hope that day comes.

—Alena Grabowski
University of Colorado Boulder

Morgenroth adds that to thoroughly assess whether prostheses provide an advantage over natural limbs, "it is important to consider key disadvantages that sprinters and runners with amputations face, such as the potential for substantial discomfort, energy loss at the residual limb-socket interface, and limitations in ankle push-off power during acceleration."

Although more research is needed to settle disputes about the advantages or disadvantages athletes with amputations may possess, research has already resulted in changes on the track. For example, Grabowski says that the studies from her lab have helped inform the National Collegiate Athletic Association's (NCAA) decision to include athletes with amputations in track and field competitions with able-bodied individuals.

For now, one of the goals of Grabowski's research team is to use this work to help improve the process of providing prosthetic prescriptions that match athletes' abilities and their choice of sport. For example, while a sprinter might choose a stiffer prosthetic, distance runners tend to prefer softer ones, Taboga says. In another study (*J Appl Physiol*, 122:976-84, 2017), "we actually saw that decreasing stiffness allows you to run easier, [because] you consume less energy," he adds.

In addition to helping athletes, the researchers also hope to use these findings to build better prosthetics for every-

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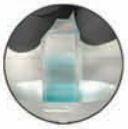

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day life. "[By] understanding some of the aspects that help with performance in a prosthesis, can we create something even better?" Grabowski asks. "Or is there a day that will come where we can create a prosthesis that's better than flesh and blood? We're not there yet, [but] I hope that day comes." —Diana Kwon

Marshalling Microbes

Stress, anxiety, and depression are emotions we all feel at some point in our lives, some people to a greater degree than others. Part of the human experience, right?

"It may seem odd that my research focuses on the gut if I'm interested in the brain," says John Cryan, a researcher at the APC Microbiome Institute at University College Cork in Ireland. "But when we think of how we express emotion in language, through sayings like 'butterflies in your tummy' and 'gut feeling,' it isn't surprising that they're connected."

In a recent study, Cryan and his colleagues reported a link between the microbiome and fear. By examining mice with and without gut bacteria, they discovered that the germ-free mice had blunted fear responses (*Mol Psychiatr*, doi:10.1038/mp.2017.100, 2017). Their findings may pave the way for the development of novel treatments for anxiety-related illnesses, including posttraumatic stress disorder.

Researchers at Kyushu University in Japan were the first to show, in 2004, that bacteria in the gut can influence stress responses, prompting many subsequent investigations. Yet despite mounting research, scientists remain uncertain about exactly how the gut microbiome affects the brain. While some bacteria influence the brain through the vagus nerve, other strains seem to use different pathways. It is known, however, that the population of the gut microbiome begins in early life, and recent research suggests that disruptions to its normal development may influence future physical and mental health (*Nat Commun*, 6:7735, 2015).

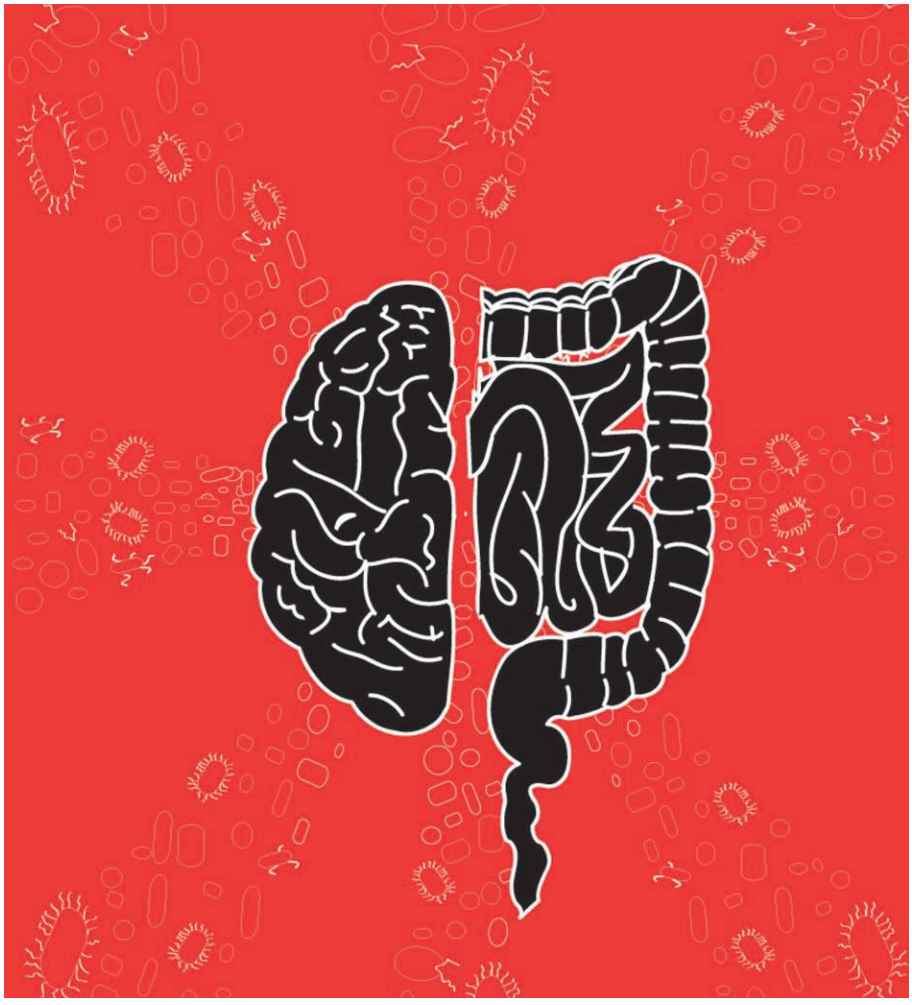
Researchers are finding that this gut-brain connection could have clinical implications, as influencing the gut microbiome through diet may serve to ameliorate some psychiatric disorders. Together with University College Cork colleague Ted Dinan, Cryan coined the term "psychobiotics" in 2013 to describe live organisms that, when ingested, produce health benefits in patients with psychiatric illness. These include foods containing probiotics, live strains of gut-friendly bacteria.

While there are many rodent studies linking probiotics and mental health, UCLA biologist Emeran Mayer and his colleagues were the first to test them in humans, using functional magnetic resonance imaging (fMRI) scans to assess the results. After administering probiotic yogurt to a group of healthy women twice a day for four weeks, the researchers found that the women had a reduced brain response to negative images (*Gastroenterology*, 144:1394-401, 2013).

"We reanalysed the data several times and convinced ourselves that it's real," Mayer says. "You can almost say it was a career-changer for me."

Having conducted this study on healthy participants, Mayer is reluctant to conclude that probiotics can cure mental illnesses such as anxiety. "It's a complex emotion, not just a reflex behavior like in the mouse," he says. However, Mayer says he's very supportive of the potential of prebiotics—fiber-rich foods that promote the growth of beneficial bacteria in the gut.

Researchers at Deakin University in Australia recently trialed a Mediterranean-style diet, which is predominately plant-based and fiber-rich, in a group of adults



KIRAN SANDHU, APC MICROBIOME INSTITUTE AT UNIVERSITY COLLEGE CORK

MELISSA DSOUZA, UNIVERSITY OF CHICAGO

THE GUT FEELINGS LAB: Graduate students Liz Ziegler (left) and Haitao Wang (right), along with postdoc Holly Lutz (center), work in the University of Chicago lab of Jack Gilbert to disentangle the connections between the microbiome and the brain.

with major depression. They found that one-third of the participants reported a significant improvement in symptoms after 12 weeks on the diet (*BMC Medicine*, 15:23, 2017). One of them was Sarah Keeble from Melbourne. "I've suffered from depression for 17 years. At the start of this study, I was right at the bottom of the barrel," she recalls. "After a few weeks, that sinking feeling slowly lifted, and my motivation and enthusiasm improved."

Just as activity in the gut seems to affect the brain, mental stress can lead to intestinal problems. Scientists have demonstrated this in research on irritable bowel syndrome. For example, a study by Mayer and colleagues linked early-life emotional trauma to an increased risk of developing the bowel disorder (*Clin Gastroenterol Hepatol*, 10:385-90, 2012).

As data on the brain-gut axis accumulates, many scientists are taking notice. Trinity College Dublin researcher Shane O'Mara says that there is "great potential" in this area, but cautions that it's too early to say whether targeting the microbiome will play a role in psychiatric treatment. University of Manitoba gastroenterologist Charles Bernstein also feels the research is promising but believes we are "far from manipulating the microbiome to treat mental health disorders."

Those spearheading this research are equally aware of the need for more studies, particularly in human subjects, but they are hopeful that change lies ahead. "I'm almost certain that in several years, diet will be considered one branch of therapy for many mental illnesses, alongside medication and psychiatric treatments," says Mayer.

"People with severe mental illness will still need something very strong, but this is a useful adjunctive," agrees Cryan. "I think when we go to our GP in future, we will not only have blood tests, we will have the microbiome tested."

"Within five years, I hope to see more clinical trials that demonstrate the efficacy of prebiotics and probiotics on mental health disorders," says University of Chicago microbial ecologist Jack Gilbert. "There needs to be a revolution in how we deal with mental illness in our society."

—Amy Lewis

Dealing with Rising Publication Costs

As article processing charges top $5,000 at some research journals, authors and institutions have means of negotiating better deals or finding less expensive options.

BY M. BISHR OMARY AND THEODORE S. LAWRENCE

A highlight in the career of any biomedical investigator, from a trainee to an established scientist, is when a research study is ready to be submitted for publication. Increasingly, this sense of gratification may be offset by sticker shock at the article processing charges (APCs) associated with publication. In the process of conducting research, writing and submitting a paper, and addressing reviewer and editor comments, APCs are often not at the front of an investigator's mind. However, APCs have increased significantly, and authors' historical indifference to publication fees may be changing as a result.

There are large variations in APCs—publication costs per manuscript can range from minimal or no fees to more than $5,000. These charges are influenced by a number of factors, including the journal and/or publisher, page count of the article, and the number and type of images. Open access (OA) journals include access fees in the APC; many other publishers offer optional open access for an additional fee.

To understand some of the issues that may be driving the rise in publication fees, one needs to consider multiple and competing factors, including costs borne by publishers to bring an article (and a journal) to its final formatted state, as well as the cost recovery and potential profits generated by not only APCs but also revenue streams including subscription fees, advertisements, and reprint or licensing/copyright fees for single articles. While publishers are clearly entitled to profit from the multifaceted value they add, high profit margins, such as the reported 37 percent profit on $2.1 billion USD in revenue for Elsevier in 2014, highlight the current financial model that is supported for the most part by APCs and institutional subscriptions. (By comparison, Apple's 2012 profit margin was 35 percent.)

It's important to consider the perspectives of three interrelated constituencies: publishers, authors, and institutions that purchase or license journal content. From a publisher's point of view, there are clear costs associated with publication, including the handling and review of submitted and revised manuscripts, copyediting, typesetting, and printing (for the print journals). The difficulty comes in assigning a true cost of publication, which varies from journal to journal. For example, some journals incur as expenses one or more of the following: salaries for professional editors, medical artists, biostatisticians, and support staff; costs for publishing non-reimbursed sections such as reviews, editorials, and commentaries; and honoraria for editors and associate editors. These are real and significant costs, but the fact remains that the profit margins of major publishers are substantial.

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More immediate and long-lasting change could be effected by institutions, funding agencies, and publishers coming together to define a fair solution, ideally with negotiations in harmony across continents.

From the authors' perspective, it is difficult to justify budgeting $10,000 in a grant to publish, for example, two papers in *Cell Reports* or *Nature Communications* (in this case, the open access charge is embedded in the APC) versus not having to pay any fees or paying the $2,000–$3,000 typically budgeted in a National Institutes of Health (NIH) grant.

It should be noted that authors often provide significant services to publishers for free, by providing peer review not only for published papers but for manuscripts that are reviewed and not published by a given journal. However, such peer review is essential, and journals typically select some of their top reviewers to join their editorial boards, which is accepted as a form of academic recognition. Although publication fees are subsidized in some institutions and countries, particularly in Europe, there is an ongoing—and occasionally heated—discussion that promotes "flipping" the current institutional subscription model to one based on APCs that authors would cover (presumably with assistance from their institutions). Although some experts support this model, others raise significant concern that this will shift (and potentially

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Nature	Not listed	Not listed
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**Society-published journals typically offer publication discounts to their society members. The listed fees are for nonmembers.
***Elsevier, which publishes *Cell*, has a wide range of open access charges for their open access and hybrid journals.
#Indicates open access journal. Some of the open access fees include a discount for authors from institutions with a site license.
+Author fees are identical for all American Physiological Society journals that publish original research.

COMPS: The table shows a sampling of biomedical journals and a range of fees per article charged by the publisher to the authors. Pricing was obtained from the listed websites (accessed July 25, 2017).

increase) the overall cost of publishing and access, leaving authors simply caught in the middle.

A final and important perspective is that of academic or other research institutions that collectively pay much of the cost to purchase journal subscriptions from the major publishers. Average subscription charges per title across all disciplines (arts and sciences) have steadily increased, with costs for biology and health science journals growing by 5 percent to 7 percent per year for 2014–2016. This likely unsustainable financial model for institutions has led to ongoing discussions regarding the rising costs and lack of transparency in institutional spending on journal subscriptions.

Although it is difficult to obtain costs that different institutions pay for journal subscriptions due to nondisclosure restrictions, a study conducted by the University of California Libraries (in collaboration with several other academic libraries in North America) provides some numbers. For example, the University of California system-wide package expenditure for 2013 for biomedical research disciplines and life sciences alone was $6.25 million. The issue of rising costs recently reached the boiling point in Germany. Beginning in January 2017, more than 60 organizations that are part of Project DEAL—which seeks nationwide licensing agreements with major publishers—either cancelled their subscriptions with Elsevier or allowed them to expire.

So what can be done to address the issue of rising APCs? We do not focus on

the issue of OA publishing because it has its pluses and minuses. Authors can do due diligence in reviewing their options, including OA, and choose not to submit manuscripts to journals that assess high APCs. While this may seem to be the simplest solution—and could exert pressure on the publishers if carried out by a sufficient number of authors—it is easier said than done, given the pressures on investigators and trainees to publish in some journals that may charge significantly more than other journals.

More immediate and long-lasting change could be effected by institutions, funding agencies, and publishers coming together to define a fair solution, ideally with negotiations in harmony across continents. The NIH and major international funding agencies, research-funding foundations such as the Howard Hughes Medical Institute, and research societies and organizations could join together and exert pressure on publishers similar to that being applied in Germany. For example, the Gates Foundation has recently dictated that the research it funds cannot be published in journals such as *Nature* and *Science* (and their affiliated journals) that do not comply with its open access policy, with a temporary arrangement with *Science* journals recently reported.

In addition, and similar to the efforts by Project DEAL, research institutions and their libraries that collectively pay large sums to the major publishers could unite within or across countries to develop a model whereby institutional subscriptions would also cover publication fees for researchers based in the subscribing institutions using a transparent and fair system.

Another plausible model is that used by the Sponsoring Consortium for Open Access Publishing in Particle Physics (or SCOAP3). SCOAP3 represents a partnership of several funding agencies and research centers, and several thousand libraries in 44 countries; it converts important journals in the field of high-energy physics to OA at no cost for authors or readers.

Retaining affordable options for investigators in developing countries is also critical, as exemplified by some journals that make their content available for free.

Recalibrating the market forces, hopefully with negotiation by the involved parties, is the prudent approach. Also, authors have the option to seek out journals that do not charge excessive fees, while still publishing their work in highly respected journals. ■

M. Bishr Omary is the executive vice dean for research at the University of Michigan Medical School, where Theodore Lawrence is the chair of the department of radiation oncology. Omary is currently an advisory board member for Cellular and Molecular Gastroenterology and Hepatology *and Lawrence is a senior editor for* Cancer Research.

Bubbles for Broken Bones

Ultrasound-stimulated microbubbles enable gene delivery to fix fractures.

BY RUTH WILLIAMS

Repairing limbs after serious injuries can be a challenge for orthopedic surgeons. If the loss of bone is too great, regrowth is impossible. Smaller fractures can also be problematic if bone growth is insufficient because of the advanced age or poor health of the patient.

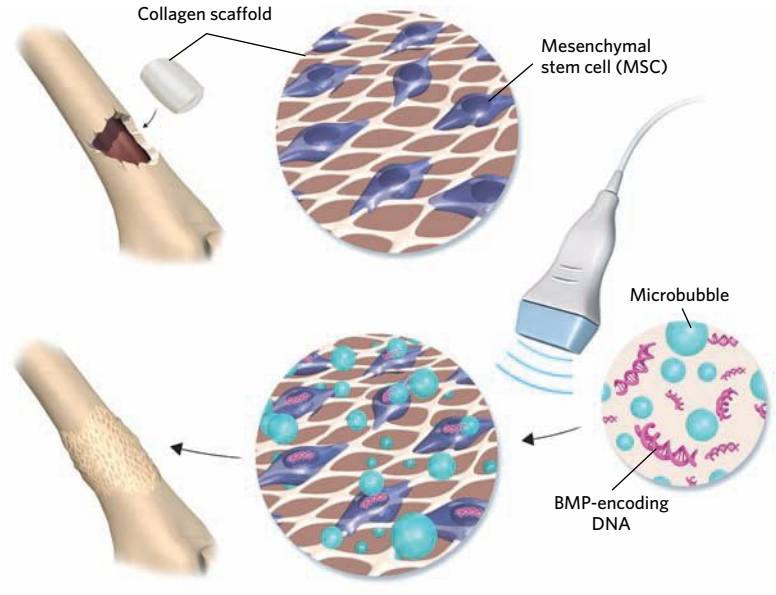
The gold standard for treating such nonhealing fractures is autologous bone grafts—where a segment of healthy bone (normally harvested from the patient's pelvis) is used to bridge the wound. But depending on the extent of the damage or the patient's health, this option is not always feasible. So in recent years, some doctors have begun to administer bone morphogenetic protein (BMP), which is incorporated into a bone implant to boost healing.

This strategy, too, has its pitfalls. "There are significant side effects," including bone resorption and bone formation in soft tissues, says skeletal regeneration researcher Dan Gazit of Cedars-Sinai Medical Center in Los Angeles, possibly because BMP is given in large doses. Rather than administer the protein itself, then, perhaps physicians could deliver the underlying gene to cells, providing more-physiological levels of BMP at the site of injury, and less elsewhere in the body.

The viral vectors used to deliver such gene therapies have raised their own safety concerns, however. To overcome this obstacle, Gazit and colleagues employed a delivery mechanism called sonoporation—in which ultrasound is used to induce the oscillation of lipid-shelled, gas-filled microbubbles, causing them to punch tiny, reparable holes in cells through which the DNA can enter.

The researchers also employed a strategy to ensure the DNA was targeted to endogenous mesenchymal stem cells (MSCs), which are proficient at producing BMP. In the fractured tibias of pigs, the team inserted collagen scaffolds, known to attract MSCs, then waited two weeks (for maximal MSC recruitment) before injecting a mix of BMP-encoding DNA and microbubbles at the site of the fracture and applying a pulse of ultrasound. Eight weeks after a single dose of the gene therapy, the pigs' fractures were mended, while those of control animals were not.

"[Gazit] has now got the proof of principle he needs," says Mayo Clinic orthopedics researcher Christopher Evans, who was not involved in the study. "That's really exciting." (*Sci Transl Med*, 9: eaal3128, 2017) ■



BONES AND BUBBLES: To repair a fractured pig tibia, researchers inserted a collagen scaffold that attracts mesenchymal stem cells (MSCs). Two weeks later, they injected a mix of microbubbles and DNA encoding bone morphogenetic protein (BMP) at the fracture site. Finally, they applied a pulse of ultrasound to encourage the MSCs to take up the DNA, and thus begin producing BMP. Within eight weeks, the bones were healed.

AT A GLANCE

TECHNIQUE	HOW IT WORKS	CLINICAL USE	COMPLICATIONS	BONE FORMING POTENTIAL
Autologous bone graft	Healthy bone is excised from the patient's pelvis and inserted into the fracture site to encourage bone growth.	Yes, well established	Patients often have pain at the site of the healthy bone excision.	Very good; limited only by graft-size feasibility
Sonoporation-driven BMP gene therapy	Plasmid DNA encoding BMP is injected at the fracture site together with lipid-encased microbubbles. Ultrasound causes the microbubbles to oscillate and create micropores in cell membranes. DNA enters the cells and BMP is produced.	No. It works in a large animal model, but safety testing is required for medical translation.	Unknown	The method works at least as well as an autologous bone graft in animals, and possibly better as it is not limited by graft size.

Trippy Treatments

Scientists are beginning to unravel the mechanisms behind the therapeutic effects of psychedelic drugs.

BY DIANA KWON

Lying in a room at Imperial College London, surrounded by low lighting and music, Kirk experienced a vivid recollection of visiting his sick mother before she passed away. "I used to go and see my mum in the hospital quite a lot," recalls Kirk, a middle-aged computer technician who lives in London (he requested we use only his first name). "And a lot of the time she'd be asleep . . . [but] she'd always sense I was there, and after about five minutes she'd wake up, and we'd interact. I kind of went through that again—but it was a kind of letting go."

Kirk choked up slightly while retelling his experience. "It's still a little bit emotional," he says. "The thing I realized [was that] I didn't want to let go. I wanted to hold on to the grief, because that was the only connection I had with my mum."

While this may sound like an ordinary therapy session, it was not what you would typically expect. Kirk was experiencing the effects of a 25-mg dose of psilocybin—the active ingredient in psychedelic "magic" mushrooms—which he had ingested as part of a 2015 clinical trial investigating the drug's therapeutic potential.

After his mother died, Kirk says, he fell into a "deep, dark pit of grief." Despite antidepressants and regular sessions with a therapist, his condition was not improving. "I was stuck in it for years," he recalls. So when he heard Imperial College London was recruiting participants for an upcoming trial studying the impact of psilocybin on depression, Kirk decided to sign up.

The study, led by psychologist and neuroscientist Robin Carhart-Harris, enrolled 12 patients with varying stages of treatment-resistant depression. Each participant took part in two guided treatment sessions, first with a low dose (10 mg) of psilocybin in pill form, then a high dose (25 mg) one week later. During each psychedelic session, subjects were closely monitored by at least one psychiatrist and an accompanying counselor or psychologist. "The guides [help] provide a safe space for the patient to have their experience," Carhart-Harris explains.

In addition to the deeply emotional encounter with his deceased mother, Kirk also recalls moments of "absolute joy and pleasure" during his sessions. He remembers having a vision of the Hindu deity Ganesh (the "remover of obstacles") and feeling an altered sense of self and his surroundings. "Your mind is always chattering and observing things," Kirk says. "And that was all shut down. For me, there was a feeling of new space."

Experiences like Kirk's are common among people who have participated in a psychedelic session (or "trip," as it was allegedly first called by US Army scientists in the 1950s). Reports consistently include feeling intense emotions, having mystical experiences, and entering a dreamlike state. Many also articulate a dissolving sense of a bounded self, coupled with a feeling of increased connectedness with others and the rest of the world.

When Carhart-Harris and his team assessed their study's participants three months after treatment, they found that most of the participants showed reduced depressive symptoms, with 5 of the 12 in complete remission[1]—including Kirk. It's now been two years since he received psilocybin therapy, and he says that he has not needed antidepressants or therapy since. "I got a new positivity that I didn't have for some time," he says.

These results are preliminary—the study tested a small sample size with no control group. But other recent trials, including some that were larger and included controls, have revealed additional therapeutic benefits. Last December, for example, two randomized placebo-controlled clinical trials of psilocybin in terminal cancer patients (51 and 29 patients, respectively) found that giving participants psilocybin in guided sessions could substantially decrease depression and anxiety—an improvement that persisted for at least six months after treatment.[2,3] In smaller pilot studies, psilocybin has also shown success in treating addiction. In two small trials, one involving smokers[4] and the other alcoholics,[5] most participants remained abstinent for months after treatment with the psychedelic.

A number of early studies have also reported evidence that other psychedelics, primarily lysergic acid diethylamide (LSD), have similar effects. Roland Griffiths, a psychiatry professor at Johns Hopkins University, describes the effects of psychedelics as a sort of "reverse PTSD" (posttraumatic stress disorder). With PTSD, there is "some discrete, traumatic event that produces some alteration in neurology and perception that produces [psychological] dysregulation going forward," he says. In a similar but opposite way, treatment with hallucinogenic substances is a "discrete event that occurs to which people attribute positive changes that endure into the future." While scientists are only beginning to understand the mechanisms behind these effects, what they've found so far already tells quite a compelling story.

Most psychedelics researchers believe that the session itself—the profound experiences individuals have during a trip—is key to the drugs' therapeutic effects. But whether this is a cause or consequence of underlying neurobiological effects is still unclear. Studies show that psychedelics disrupt established networks in the brain, potentially allowing new connections to form. Recent work has also begun to reveal that these drugs' effects—such as promoting neuroplasticity and reducing inflammation—are exerted through the serotonin 2A receptor.

"It's very exciting that we seem to be at a threshold of establishing the neurobiological basis for the range of effects that hallucinogens have, and specifically, the therapeutic range of action," says Charles Grob, a psychiatry professor at Harbor-UCLA Medical Center who conducted a pilot study of psilocybin for terminal cancer patients that was published in 2011.[6] "I think there is growing knowledge and appreciation that this work can be conducted responsibly and safely, and that it has the quite compelling potential to offer us very new and exciting treatment models."

The tripping brain

While on psychedelics, people commonly experience ego dissolution, a loss of the sense of a separate self, and an enhanced feeling of connectedness with the outside world. Recent neuroimaging studies have revealed that the intensity of this experience correlates with changes in brain activity, primarily in the default mode network (DMN)—a system of brain regions that is more active at rest than during tasks, and that is thought to be involved in, among other things, processing information related to the self.

What's been consistently found is that the brain or the mind during psychedelic states is in a different state of consciousness, and this is also reflected in how the brain is behaving.

—Rainer Krähenmann, University of Zurich

To understand what happens in the brain during a trip, Carhart-Harris and colleagues have been dosing healthy participants with psychedelics and scanning their brains using functional magnetic resonance imaging (fMRI) to measure cerebral blood flow, a proxy measure of neural activity. In 2012, for example, the researchers found that, following an intravenous injection of 2 mg of psilocybin, 15 subjects displayed an overall decrease in cerebral blood flow as well as decreased connectivity between the posterior cingulate cortex and the medial prefrontal cortex, two hubs of the default mode network.[7]

Follow-up studies using both fMRI and magnetoencephalography (MEG)—a technique to detect the tiny magnetic fields generated by electrical activity in the brain—on subjects dosed with LSD have revealed similar effects. This work also revealed a correlation between decreased connectivity in the default mode network and subjective ratings of ego dissolution.[8]

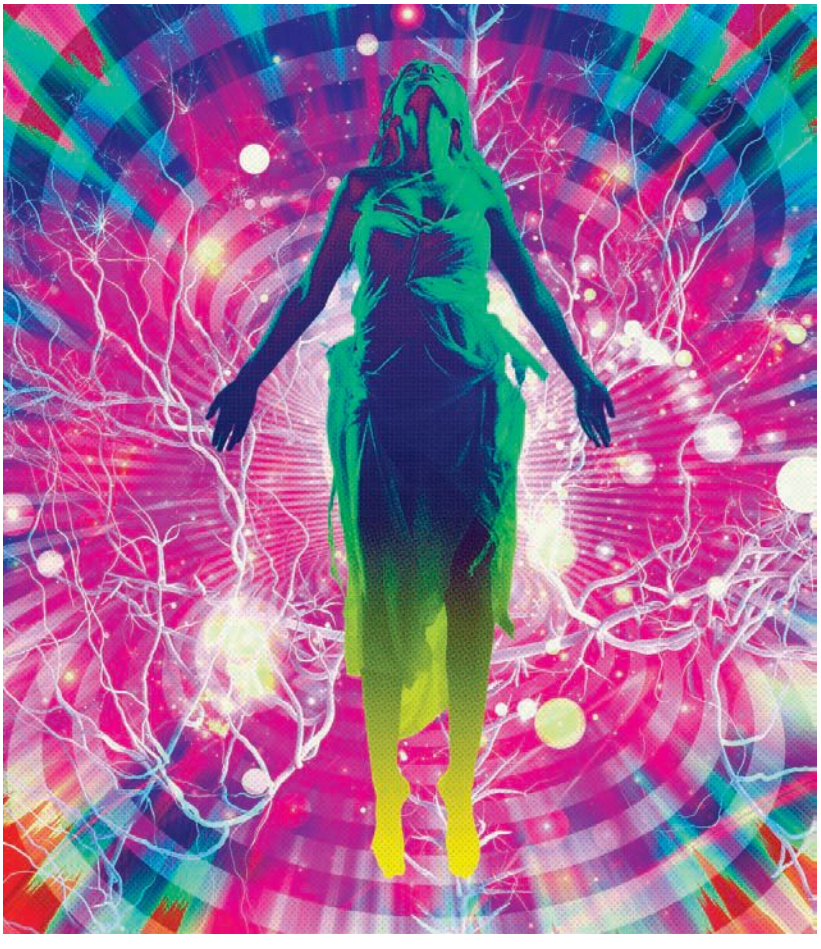
But while the two psychedelic drugs "share signature psychological effects," Carhart-Harris notes, "they differ in the potency [and] in their kinetics. The psilocybin trip is shorter, and for that reason is more manageable than an LSD trip."

Researchers have found similar neurological effects during meditation—another altered state of mind associated with psychological well-being. Expert meditators also show an acute reduction in the activity of the default mode network.[9] Conversely, an increase in activity and connectivity in this network has been found in some individuals with depression. "In some ways, it kind of makes sense that psilocybin, which brings people very powerfully into the present moment, would be more similar to meditation than it would be to depression," says Griffiths. "In other words, people are riveted with interest in the present moment and what's happening here and now, rather than in the future or

in the past." Griffiths and his colleagues at Johns Hopkins are currently conducting a neuroimaging experiment probing the brains of expert meditators on psychedelic trips.

Using MEG, Carhart-Harris and colleagues have also discovered that psilocybin and LSD alter neural oscillations, rhythmic brain activity linked to various perceptual and cognitive functions, across the default mode network.[10] Individuals under the influence of these drugs experience a drop in so-called alpha rhythms, oscillations in the range of around 8 to 13 hertz, that correlate with their reports of ego dissolution. "When you plot out what rhythms contribute to the brain's overall oscillatory activity, you get this huge peak in the alpha band—this really prominent frequency that, in some ways, sort of dominates the rhythmicity of the brain," Carhart-Harris explains. "It's a really curious rhythm, because it's more prominent in humans than in any other species, and its prominence increases as we develop into adulthood. I see it as a kind of signature of high-level consciousness that adult humans have."

In contrast to the decrease in activity and connectivity within the DMN, imaging studies have revealed an increase in functional links between normally discrete brain networks during a trip, and such activity also correlates with reports of ego-dissolution.[11] Together with findings of changes in the default mode network and reduced alpha rhythms, these results are contributing to a hypothesis that the brain becomes "entropic"—more disordered, fluid, and unpredictable—during psychedelic use, disrupting certain pathways while allowing for new connections to be made. "What's been consistently found is that the brain or the mind during psychedelic states is in a different state of consciousness, and this is also reflected in how the brain is behaving," says Rainer Krähenmann, a psychiatrist and researcher at the University of Zurich. But, he adds, more research is needed to understand just what these changes mean. "I would not say that we can reduce it to certain areas or certain mechanisms," Krähenmann says. "The brain is still too complex to really understand what's going on."



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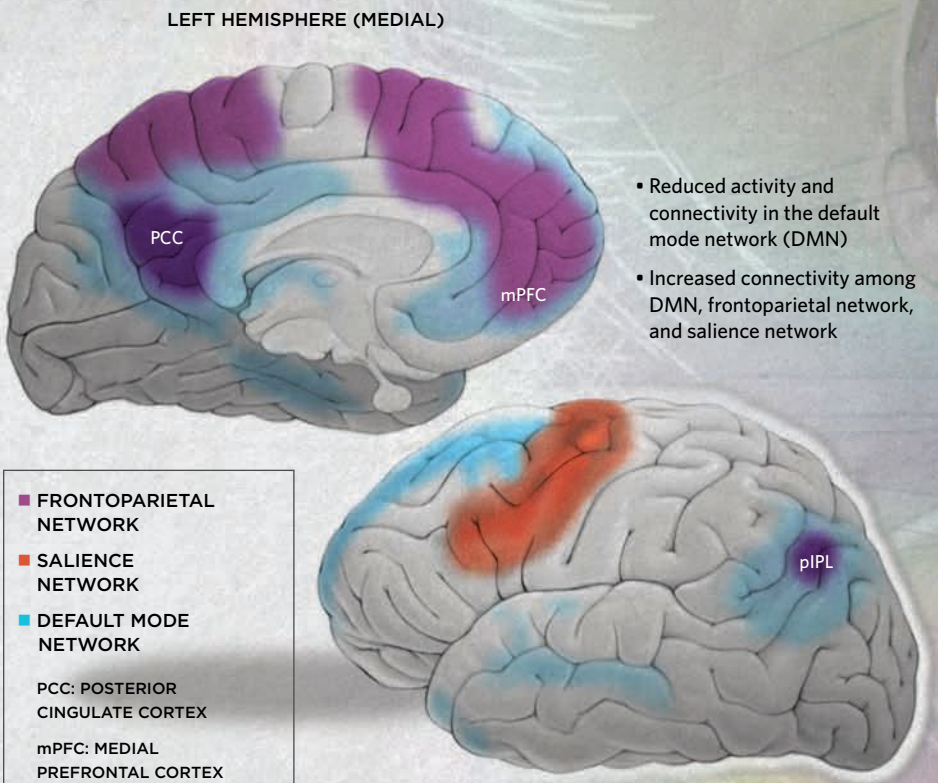
And of course, the biggest question that remains is how these neurological changes might be therapeutic. In a soon-to-be published study, Carhart-Harris and his colleagues found that changes in the connectivity of the default mode network predicted how well patients would do after psilocybin treatment, but the results are preliminary. "We know that there's fascinating things happening acutely in terms of these changes in the synchronization across brain areas," says Matthew Johnson, a behavioral pharmacologist at Johns Hopkins. "But the really tantalizing possibilities that a number of groups, including ours, are looking at is whether those types of changes persist and are related to long-standing clinical benefits."

Mind-bending molecules

All the classic psychedelic drugs—psilocybin, LSD, and *N,N*-dimethyltryptamine (DMT), the active component in ayahuasca—activate serotonin 2A (5-HT2A) receptors, which are distributed throughout the brain. In all likelihood, this receptor plays a key role in the drugs' effects. Krähenmann and his colleagues in Zurich have discovered that ketanserin, a 5-HT2A receptor antagonist, blocks LSD's hallucinogenic properties and prevents individuals from entering a dream-like state or attributing personal relevance to the experience.[12,13]

Other research groups have found that, in rodent brains, 2,5-dimethoxy-4-iodoamphetamine (DOI), a highly potent and selective 5-HT2A receptor agonist, can modify the expression of brain-derived neurotrophic factor (BDNF)—a protein that, among other things, regulates neuronal survival, differentiation, and synaptic plasticity. This has led some scientists to hypothesize that, through this pathway, psychedelics may enhance neuroplasticity, the ability to form new neuronal connections in the brain.[14] "We're still working on that and trying to figure out what is so special about the receptor

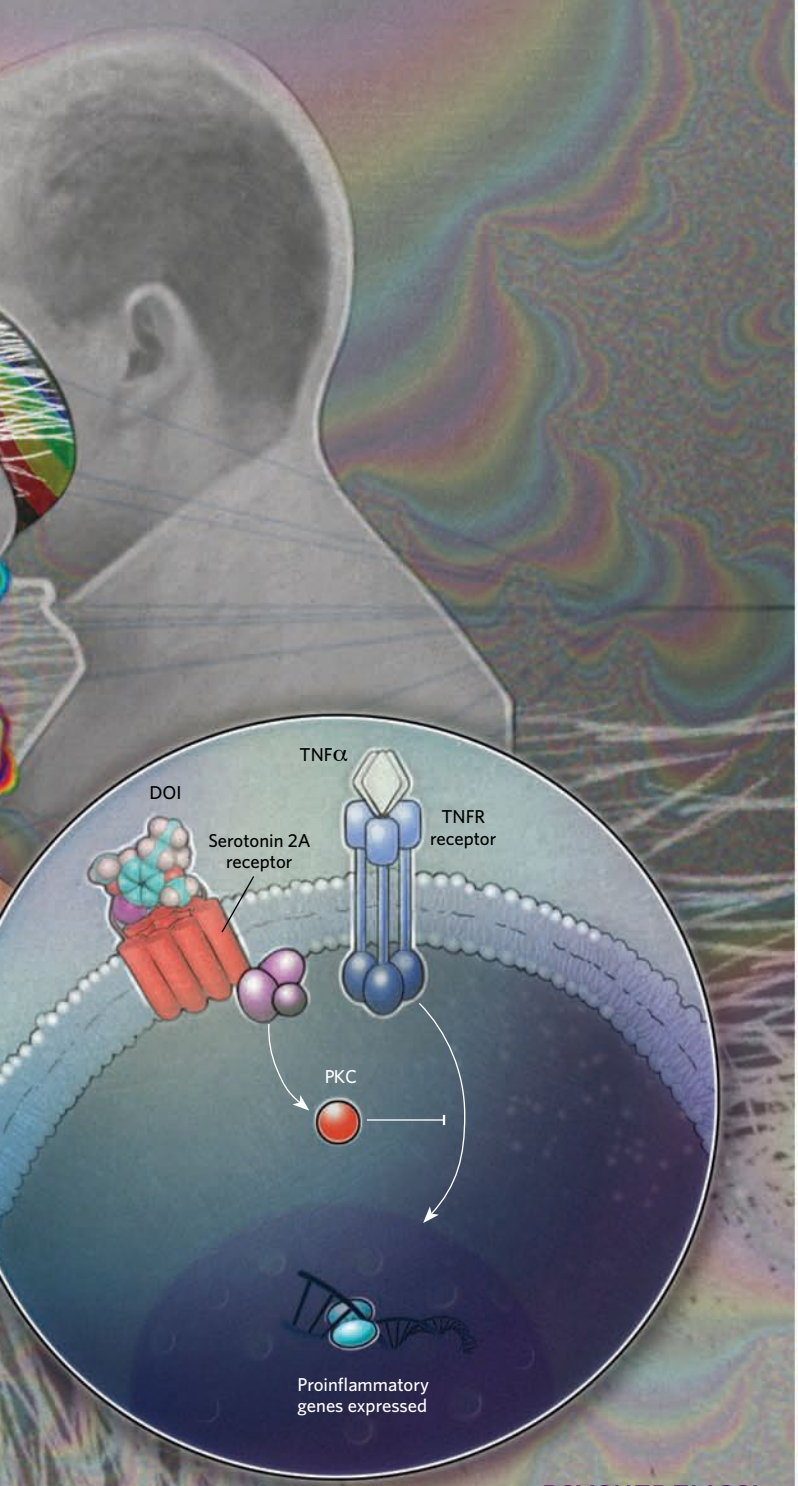


THE BRAIN ON PSYCHEDELICS

Key brain areas involved in the effects of psychedelic drugs are located in the default mode network (DMN), which is more active at rest than when attention is focused on the external environment. Neuroscientists first discovered this network while scanning participants' brains at rest: rather than a decrease in activity across the brain, they found that activity in some regions was actually higher when people were not engaged in a goal-directed task. Over the years, researchers have linked the DMN to a variety of functions, including autobiographical recollection, mind wandering, and processing self-related information.

Key hubs of the DMN include the posterior cingulate cortex (PCC), the medial prefrontal cortex (mPFC), and the posterior inferior parietal lobule (pIPL). Through neuroimaging, researchers have discovered that psychedelic drug use decreases activity in some of these brain areas, and also reduces connectivity within the DMN.

Neuroimaging studies have also shown that connectivity between brain networks is increased when psychedelics are administered. For example, the DMN; the salience network, which helps identify behaviorally relevant information; and the frontoparietal network, known to be involved in attentional control and conscious awareness, all show stronger connections to each other. Researchers believe that this increased crosstalk throughout the brain may play a key role in the drugs' effects.



PSYCHEDELICS' ANTI-INFLAMMATORY EFFECTS

Scientists have discovered that a number of psychedelics can reduce inflammation throughout the body. Animal studies with one of these drugs, DOI, which is an especially potent anti-inflammatory compound, are starting to reveal the mechanism behind these effects. According to one hypothesis, DOI binds to and activates the serotonin 2A (5-HT2A) receptor to recruit protein kinase C (PKC). This is thought to block the downstream effects of the binding of tumor necrosis factor-alpha (TNF-α) to its receptor (TNFR), which is known to initiate a signaling cascade that promotes the transcription of proinflammatory genes.

and where it is involved," says Katrin Preller, a postdoc studying psychedelics at the University of Zurich. "But it seems like this combination of serotonin 2A receptors and BDNF leads to a kind of different organizational state in the brain that leads to what people experience under the influence of psychedelics."

This serotonin receptor isn't limited to the central nervous system. Work by Charles Nichols, a pharmacology professor at Louisiana State University, has revealed that 5-HT2A receptor agonists can reduce inflammation throughout the body. Nichols and his former postdoc Bangning Yu stumbled upon this discovery by accident, while testing the effects of DOI on smooth muscle cells from rat aortas. When they added this drug to the rodent cells in culture, it blocked the effects of tumor necrosis factor-alpha (TNF-α), a key inflammatory cytokine.

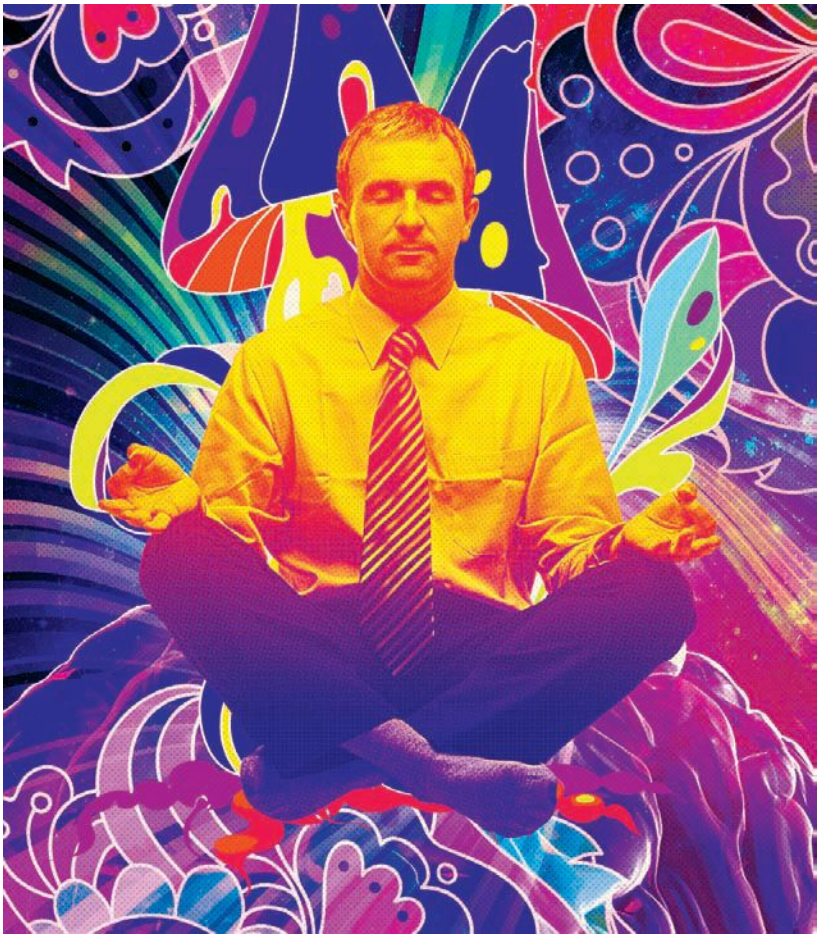
"It was completely unexpected," Nichols recalls. The effects were so bewildering, he says, that they repeated the experiment twice to convince themselves that the results were correct. Before publishing the findings in 2008,[15] they tested a few other 5-HT2A receptor agonists, including LSD, and found consistent anti-inflammatory effects, though none of the drugs' effects were as strong as DOI's. "Most of the psychedelics I have tested are about as potent as a corticosteroid at their target, but there's something very unique about DOI that makes it much more potent," Nichols says. "That's one of the mysteries I'm trying to solve."

After seeing the effect these drugs could have in cells, Nichols and his team moved on to whole animals. When they treated mouse models of system-wide inflammation with DOI, they found potent anti-inflammatory effects throughout the rodents' bodies, with the strongest effects in the small intestine and a section of the main cardiac artery known as the aortic arch.[16] "I think that's really when it felt that we were onto something big, when we saw it in the whole animal," Nichols says.

The group is now focused on testing DOI as a potential therapeutic for inflammatory diseases. In a 2015 study, they reported that DOI could block the development of asthma in a mouse model of the condition,[17] and last December, the team received a patent to use DOI for four indications: asthma, Crohn's disease, rheumatoid arthritis, and irritable bowel syndrome. They are now working to move the treatment into clinical trials. The benefit of using DOI for these conditions, Nichols says, is that because of its potency, only small amounts will be required—far below the amounts required to produce hallucinogenic effects.

In addition to opening the door to a new class of diseases that could benefit from psychedelics-inspired therapy, Nichols's work suggests "that there may be some enduring changes that are mediated through anti-inflammatory effects," Griffiths says. Recent studies suggest that inflammation may play a role in a number of psychological disorders, including depression[18] and addiction.[19]

"If somebody has neuroinflammation and that's causing depression, and something like psilocybin makes it better through the subjective experience but the brain is still inflamed, it's going to fall back into the depressed rut," Nichols says. But if psilocybin is also treating the inflammation, he adds, "it won't have that rut to fall back into."



If it turns out that psychedelics do have anti-inflammatory effects in the brain, the drugs' therapeutic uses could be even broader than scientists now envision. "In terms of neurodegenerative disease, every one of these disorders is mediated by inflammatory cytokines," says Juan Sanchez-Ramos, a neuroscientist at the University of South Florida who in 2013 reported that small doses of psilocybin could promote neurogenesis in the mouse hippocampus.[20] "That's why I think, with Alzheimer's, for example, if you attenuate the inflammation, it could help slow the progression of the disease." (See "What Causes Alzheimer's?" *The Scientist*, September 2011.)

Research revival

Although researchers have only recently started to test psychedelics' effects in controlled clinical trials, evidence that these drugs could help treat conditions such as depression and terminal cancer–related anxiety has existed since the middle of the 20th century. (See table on opposite page.) Despite promising results, the counterculture that emerged around LSD use led to the criminalization of it and other psychedelics in 1966. Since 1970, almost all of these compounds have been Schedule I controlled substances, which imposes strict prohibitions on their use, even in research.

"If the drug war hadn't started, and we didn't have this demonization [of psychedelics], we'd know a lot more about what makes people happy, sad, depressed," says David Nichols, a professor emeritus of pharmacology at Purdue University and a pioneering psychedelics researcher (also the father of Charles Nichols). "That's the tragedy—that none of that has happened because [the research] basically died in 1970."

Now, psychedelics research is slowly starting to regain ground, though it's still not easy to win federal funding for these studies. But with support from private organizations such the Heffter Research Institute and the Multidisciplinary Association for Psychedelic Studies (MAPS), scientists have begun to probe the mechanisms underlying the drugs' psychological effects and the enduring changes they can bring about. The answers to these mysteries may help scientists gain insight into what happens to the brain in disease, and perhaps learn more about the nature of consciousness itself.

"There are many different questions to ask, and in some ways, the therapeutic ones are among the most mundane," says Griffiths. "Our understanding is so primitive that I think it's important that we not be so naive as to think that our current technologies are going to be able to unravel the many, many subtleties that account for some of these kinds of sustained effects. That's why [the study of psychedelics is] such an interesting, important, and rich field of investigation for neuroscience." ■

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CLINICAL STUDIES WITH PSYCHEDELICS

Anxiety in terminal cancer patients

1950s to 1970s	Unblinded trials suggested that psychedelics such as LSD could reduce anxiety and depression in terminal cancer patients.
2011	A small placebo-controlled trial of 12 subjects with advanced-stage cancer reported that treatment with psilocybin reduced anxiety for up to six months after treatment.
2016	Two larger randomized, placebo-controlled clinical trials, at Johns Hopkins University and New York University (NYU), found that psilocybin can substantially reduce death-related anxiety and depression in terminal cancer patients.

Depression

2016	A pilot study at Imperial College London found that psilocybin had antidepressant effects that persisted for more than three months in a subset of participants.
2017	Researchers at the Federal University of Rio Grande do Norte in Brazil published a preprint for their randomized, placebo-controlled trial of ayahuasca for 35 patients with treatment-resistant depression, reporting improved symptoms one week after treatment.
2017	At the University of Zurich, researchers are in the process of developing a double-blind, randomized, placebo-controlled trial of psilocybin as a treatment for major depression that is scheduled to start later this year. Similar plans are currently underway at Imperial College London.

Addiction

1950s to 1970s	Researchers conducted early studies of therapeutic use of LSD for treating alcoholism and heroin addiction, showing that the psychedelic could reduce substance abuse.
2016	A small study of 15 cigarette smokers at Johns Hopkins University found that psilocybin treatment led to an 80 percent abstinence rate at six months.
2016	At New York University, researchers found positive effects in a small study of 10 participants who underwent psilocybin-facilitated treatment for alcohol dependence.
2014 to 2017	Survey studies show that people who have taken psychedelics subsequently choose to abstain from cigarettes, alcohol, and other drug dependencies.
2017	Researchers at both Johns Hopkins and NYU are currently conducting larger, randomized trials with control groups for both smoking and alcohol dependence. A group at the University of Alabama at Birmingham is currently conducting a pilot trial of psilocybin-assisted treatment for cocaine addiction.

Schizophrenia

1950s to 1970s	Psychiatrists examined LSD treatments for schizophrenia patients. Preliminary studies, many with small sample sizes and no control groups, reported beneficial effects in some children who received this treatment. Around the same time, state-approved tests of psychedelic drugs were also conducted on inmates in the U.S. diagnosed with schizophrenia by doctors who believed in the drugs' therapeutic potential. Some psychiatrists also examined the effects of various psychedelic drugs on healthy individuals as a way to elucidate the experiences of patients with schizophrenia and to improve treatment.
1990s to 2000s	Recent studies have focused on using these drugs to model psychotic states rather than to treat them.

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Brain Bugs

Long relegated to the scientific fringe, the idea that infection may trigger some cases of Alzheimer's disease is gaining traction.

BY JILL U. ADAMS

In late 2011, Drexel University dermatology professor Herbert Allen was astounded to read a new research paper documenting the presence of long, corkscrew-shape bacteria called spirochetes in postmortem brains of patients with Alzheimer's disease.[1] Combing data from published reports, the International Alzheimer Research Center's Judith Miklossy and colleagues had found evidence of spirochetes in 451 of 495 Alzheimer's brains. In 25 percent of cases, researchers had identified the spirochete as *Borrelia burgdorferi,* a causative agent of Lyme disease. Control brains did not contain the spirochetes.

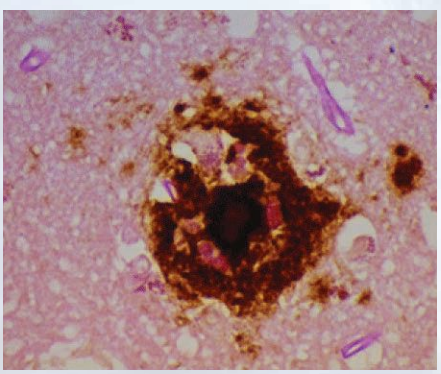
The study made Allen think back to 40 years earlier, when he was an intern at Johns Hopkins University and had treated a patient diagnosed with neurosyphilis, a neurological syndrome that included dementia and resulted from the invasion of the syphilis spirochete into the brain. "The parallel between Lyme disease and syphilis had me intrigued," he says.

Allen had recently proposed a novel role for biofilms—colonies of bacteria that adhere to surfaces and are largely resistant to immune attack or antibiotics—in eczema. He suggested that because biofilms block skin ducts and trigger innate immune responses, they may cause the stubborn skin condition. Allen knew of recent work showing that Lyme spirochetes form biofilms,[2] which led him to wonder if biofilms might also play a role in Alzheimer's disease. When Allen stained for biofilms in brains from deceased Alzheimer's patients, he found them in the same hippocampal locations as amyloid plaques.[3] Toll-like receptor 2 (TLR2), a key player in innate immunity, was also present in the same region of the Alzheimer's brains but not in the controls. He hypothesizes that TLR2 is activated by the presence of bacteria, but is locked out by the biofilm and damages the surrounding tissue instead.

Spirochetes, common members of the oral microbiome, belong to a small set of microbes that cross the blood-brain barrier when they're circulating in the blood, as they are during active Lyme infections or after oral surgery. However, the bacteria are so slow to divide that it can take decades to grow a biofilm. This time line is consistent with Alzheimer's being a disease of old age, Allen reasons, and is corroborated by syphilis cases in which the neuroinvasive effects of spirochetes might appear as long as 50 years after primary infection.

Allen's work contributes to the revival of a long-standing hypothesis concerning the development of Alzheimer's. For 30 years, a handful of researchers have been pursuing the idea that pathogenic

CURIOUS COINCIDENCE: Hippocampal section of human brain with Alzheimer's disease that shows costaining of biofilms and amyloid-β.



COURTESY OF HERBERT ALLEN

microbes may serve as triggers for the disease's neuropathology. Most came across the connection serendipitously, as Allen did, and some have made it their life's work, in spite of scathing criticism and related challenges in attracting funding and publishing results.

"There have been all these observations over time," says Miklossy. Although she says she's been dismissed as an "idiot" and denied funding, she continues to pursue spirochetes as an instigating factor in Alzheimer's disease. "I'm a physician who believes in the Hippocratic Oath," she says. "We have to do everything we can."

And the Alzheimer's field seems primed for a fresh look at a theory that might account for the disease's pathogenesis. Researchers still cannot say with confidence which features of the disease, such as neuroinflammation, tau tangles, and amyloid plaques, are involved in disease progression and thus would make effective targets for treatment. So far, most drugs that have made it to clinical testing have targeted the amyloid-β peptide, the main component of the amyloid plaques that characterize Alzheimer's brains. The idea is that a build-up of amyloid-β causes the neuropathology and that removing amyloid-β—by decreasing its production, impeding aggregation, or aiding removal of the molecule from the brain—will improve, or at least stall, symptoms of dementia. But so far, researchers have come up empty-handed.

Last November, for example, a Phase 3 trial of Eli Lilly's amyloid-targeting antibody solanezumab revealed no improvement in patients in early stages of the disease. This costly and crushing failure was followed up a few months later with another, when Merck halted its clinical trial of the small-molecule drug verubecestat, which blocks the enzyme that yields amyloid-β, in patients with mild to moderate disease. A trial using verubecestat in the earliest diagnosable stage of the disease is still underway.

And these are just the latest in a string of experimental drugs for Alzheimer's disease that have failed to show any benefit in clinical trials. Some blame the trials themselves for these high-profile flops. "The quality of the clinical trials has been low," says John Hardy, a molecular neuroscientist at the University College London, pointing out that a couple of the drugs didn't even make it into the brain.[4] But other researchers question the underlying theory.

As early as the 1990s, three laboratories in different countries, each studying different organisms, had each implicated human pathogens in the etiology of Alzheimer's disease.

In light of continued failures to develop effective drugs, some researchers, such as Harvard neurobiologist Rudolph Tanzi, think it's high time that more effort and funding go into alternative theories of the disease. "Any hypothesis about Alzheimer's disease must include amyloid plaques, tangles, inflammation—and, I believe, infection."

A history of microbial links

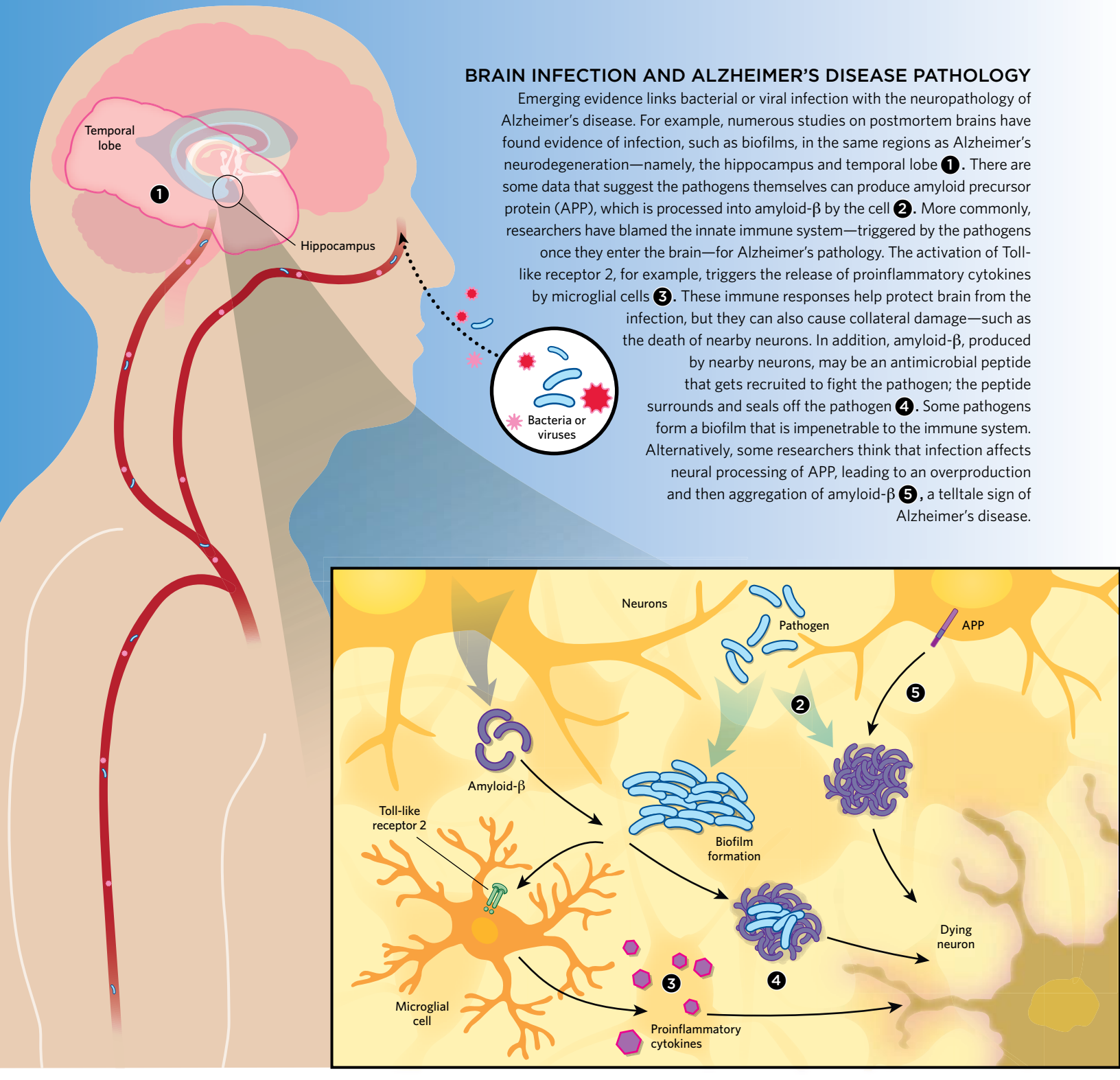
Herpes simplex virus type 1 (HSV1) can acutely infect the brain and cause a rare but very serious encephalitis. In the late 1980s, University of Manchester molecular virologist Ruth Itzhaki noticed that the areas of the brain affected in HSV1 patients were the same as those damaged in patients with Alzheimer's disease. Knowing that herpes can lie latent in the body for long periods of time, she began to wonder if there was a causal connection between the infection and the neurodegenerative disorder.

Itzhaki began looking for HSV1 DNA in the brains of Alzheimer's patients—and found it. But the viral DNA also turned up in the brains of age-matched controls. Using PCR, still a new technique at the time, was fraught with difficulty, and Itzhaki's findings were challenged as resulting from contamination. Itzhaki repeated her work with great care and consistently found that two-thirds to three-quarters of elderly people harbor HSV1 in their brains, whether they had Alzheimer's or not. So she searched for a genetic difference that might explain why only some HSV1-infected individuals develop dementia. Finally, in 1997, she reported that having both HSV1 in the brain and the apolipoprotein E gene variant *APOE4* accounted for 60 percent of the Alzheimer's cases she considered—much higher than either factor alone.[5] But most Alzheimer's researchers still dismissed her work. Itzhaki says her detractors have been set in their ways—and perhaps too wedded to scenarios involving plaques and tangles. "They don't know anything about viruses," she says, especially the fact that herpes can linger in the body and brain. "If we say the virus causes this, they imagine the scenario is fast. It's incredibly naive."

Around the same time, neuropathologist Miklossy, then at the University of Lausanne in Switzerland, was detailing the brain damage caused by spirochetes—both in neurosyphilis and neuroborrelia, a syndrome caused by Lyme bacteria. She happened upon a head trauma case with evidence of bacterial invasion and plaque formation, and turned her attention to Alzheimer's. She isolated spirochetes from brain tissue in 14 Alzheimer's patients but detected none in 13 age-matched controls. In addition, monoclonal antibodies that target the amyloid precursor protein (APP)—which, when cleaved, forms amyloid-β—cross-reacted with the spirochete species found, suggesting the bacteria might be the source of the protein.[6]

Although Miklossy says she received some positive reactions to her findings when she published them in 1993, she, like Itzhaki, also faced her fair share of skepticism. The critiques included comments that the work was "foolish, unorthodox, and crazy," she adds.

Meanwhile, in the U.S., a third line of evidence linking Alzheimer's to microbial infection began to emerge. While serving on a fraud investigation committee, Alan Hudson, a microbiologist then at MCP-Hahnemann School of Medicine in Philadelphia, met Brian Balin, who studied neuropathological processes at the Philadelphia College of Osteopathic Medi-

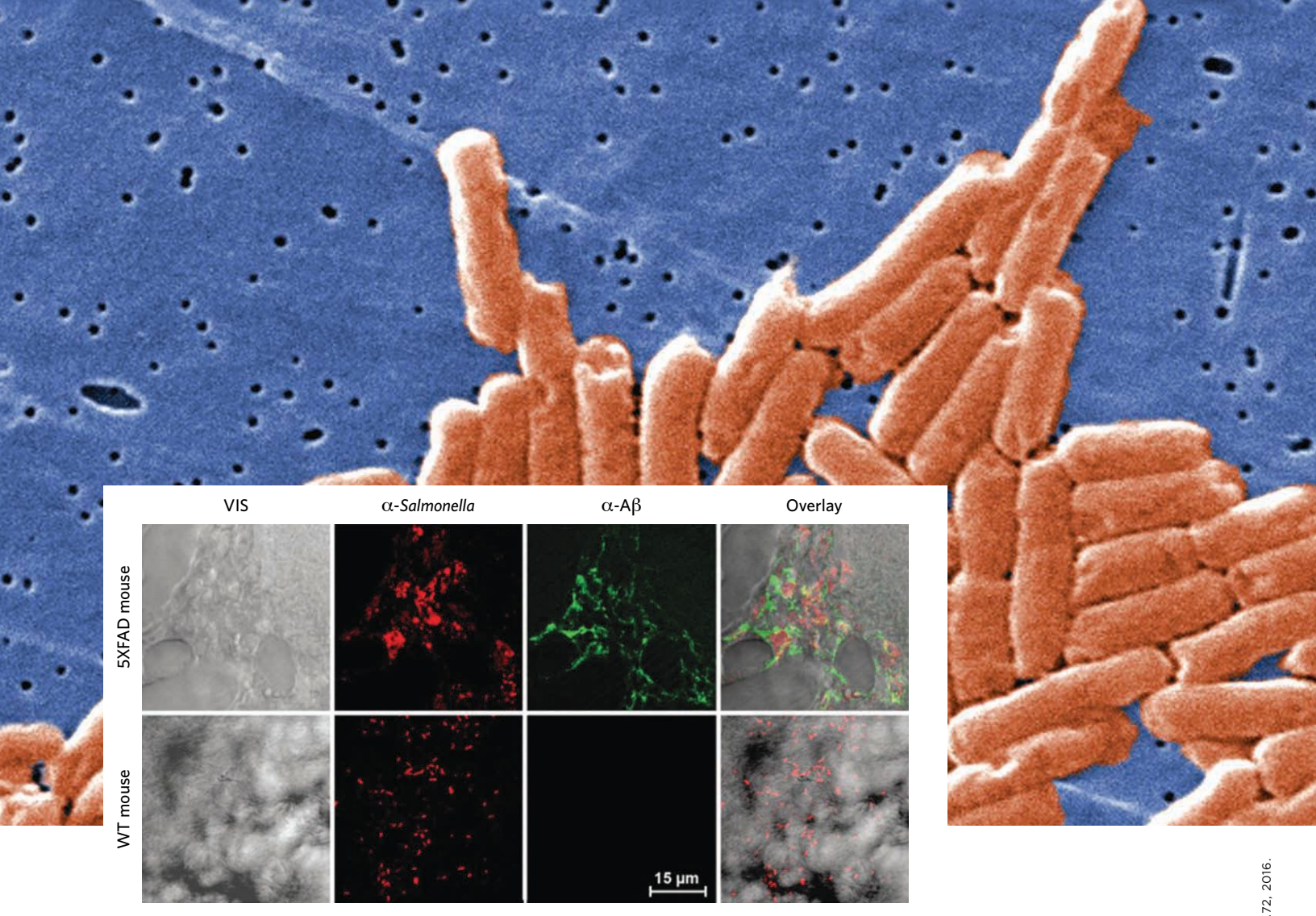


BRAIN INFECTION AND ALZHEIMER'S DISEASE PATHOLOGY

Emerging evidence links bacterial or viral infection with the neuropathology of Alzheimer's disease. For example, numerous studies on postmortem brains have found evidence of infection, such as biofilms, in the same regions as Alzheimer's neurodegeneration—namely, the hippocampus and temporal lobe ❶. There are some data that suggest the pathogens themselves can produce amyloid precursor protein (APP), which is processed into amyloid-β by the cell ❷. More commonly, researchers have blamed the innate immune system—triggered by the pathogens once they enter the brain—for Alzheimer's pathology. The activation of Toll-like receptor 2, for example, triggers the release of proinflammatory cytokines by microglial cells ❸. These immune responses help protect brain from the infection, but they can also cause collateral damage—such as the death of nearby neurons. In addition, amyloid-β, produced by nearby neurons, may be an antimicrobial peptide that gets recruited to fight the pathogen; the peptide surrounds and seals off the pathogen ❹. Some pathogens form a biofilm that is impenetrable to the immune system. Alternatively, some researchers think that infection affects neural processing of APP, leading to an overproduction and then aggregation of amyloid-β ❺, a telltale sign of Alzheimer's disease.

cine. Soon, Balin began to send Hudson Alzheimer's brain tissue to test for intracellular bacteria in the *Chlamydia* genus. Some samples tested positive for *C. pneumoniae*: specifically, the bacteria resided in microglia and astrocytes in regions of the brain associated with Alzheimer's neuropathology, such as the hippocampus and other limbic system areas. Hudson had a second technician repeat the tests before he called Balin to unblind the samples. The negatives were from control brains; the positives all had advanced Alzheimer's disease.[7] "We were floored," Hudson says.

The paper that Balin, Hudson, and colleagues wrote to announce the findings received worldwide press coverage, says Hudson, now professor emeritus at Wayne State University School of Medicine. But when the authors went to the Alzheimer's disease meeting, he says, "nobody talked to us."



BEFORE AND AFTER: Transgenic mice (top row) whose brains were injected with *Salmonella* expressed high levels of amyloid-β in those same regions 48 hours later.

JANICE HANEY CARR, USCDCP; INSET REPRINTED WITH PERMISSION FROM D.K.V. KUMAR ET AL., *SCI TRANSL MED*, 8:340RA72, 2016.

Thus, as early as the 1990s, three laboratories in different countries, each studying different organisms, had each implicated human pathogens in the etiology of Alzheimer's disease. But the suggestion that Alzheimer's might have some microbial infection component was still well outside of the theoretical mainstream.

New century, new mechanisms

Last year, Itzhaki, Miklossy, Hudson, and Balin, along with 29 other scientists, published a review in the *Journal of Alzheimer's Disease* to lay out the evidence implicating a causal role for microbes in the disease.[8] The paper opens with a plea: "We are researchers and clinicians working on Alzheimer's disease . . . and we write to express our concern that one particular aspect of the disease has been neglected."

George Perry, editor of the journal and an Alzheimer's researcher at the University of Texas at San Antonio, not only agreed to publish the article, he signed on as an author too. "The *Journal of Alzheimer's Disease* promotes all sorts of different ideas," he says. "We don't care about popularity."

And, slowly but surely, Alzheimer's researchers finally seem to be giving the pathogen hypothesis a good, hard look. Harvard's Tanzi, one of the newer microbial theorists, has been a prominent figure in the Alzheimer's field for decades. He contributed to the 1987 discovery of *APP*, the first Alzheimer's disease gene. Recently, Tanzi and his colleagues showed that amyloid-β inhibits the in vitro growth of pathogenic bacteria, including *Candida albicans, E. coli,* and *Staphylococcus aureus,* suggesting the Alzheimer's-linked peptide was acting as an antimicrobial.[9]

Tanzi's working hypothesis is that microbes trigger an innate immune response, in which amyloid-β plays a key role. The peptide surrounds the site of infection to shield healthy tissue from the invaders. Too much clumping, however, can cause problems of its own—the very processes by which plaques trigger neuronal death.

A subsequent study by Tanzi's group found that amyloid-β binds to microbes and links together with more amyloid-β to entrap the invaders and keep them from interacting with host cells. Indeed, in a transgenic mouse model of Alzheimer's disease, *Salmonella* infection seeded amyloid plaques in the brain.[10] "The plaques are generated in the hippocampus and temporal cortex—the regions most susceptible to blood-brain barrier breach," Tanzi

says, suggesting that those areas are where pathogens would first gain entry. "It makes perfect sense to me."

Tanzi is well aware of the work by Miklossy and others and the criticism that they've received. Expecting to get their own dose of criticism, Tanzi says, "we wanted to do everything right, do every control. We spent eight years on this paper." But to his surprise, the backlash didn't come. "To our delight, the field looked at what we did," he says—a sign, perhaps, that the Alzheimer's research community is finally ready to consider the microbe theory.

Proponents of this idea still face skepticism, however. Elaine Bearer, a molecular neurobiologist at the University of New Mexico Health Sciences Center, received mixed responses when she began publishing and presenting her work linking HSV1 to Alzheimer's neuropathology. As is a familiar story by now, Bearer had stumbled onto the microbe theory serendipitously.

Her main research interest is how molecular motors pick up cargo in the giant squid axon, and she uses HSV1 as experimental cargo because it's known to travel in both directions along axonal transport routes. During infection, HSV1 travels from sensory nerve endings to nerve cell bodies where the virus can hole up. When activated, HSV1 travels back out to synapses, reinfects epithelial cells, and voilà—cold-sore recurrence.

In 2006, Bearer found that HSV1 uses APP to attach to axonal transport machinery.[11] And as a result, HSV1 redistributes APP within the neuron, she says. That means APP can pile up in ways that don't happen in uninfected cells. More recently, Bearer showed that "the virus does something to APP," she says. "In epithelial cells, it induces a 25-fold increase in the protein," suggesting synthesis of the protein also responds to infection.[12]

Bearer has also produced evidence that HSV1 is trapped in amyloid plaques in human brains. (She has presented this work, but not published it.) This mirrors Tanzi's findings of *Salmonella* within amyloid plaques in an animal model, and those of Allen, who found bacterial biofilms colocalized with amyloid-β in human brain tissue.

Despite the increase in evidence supporting the microbial theory of Alzheimer's disease, however, funding for such research remains difficult to procure. And scientists working in this area also continue to face skepticism from the Alzheimer's research community. University College London's Hardy, squarely in the amyloid hypothesis camp, is aware of the work of Itzhaki, Tanzi, and some of the others, but he says he's still "not convinced."

Any hypothesis about Alzheimer's disease must include amyloid plaques, tangles, inflammation—and, I believe, infection.

—Rudolph Tanzi, Harvard University

Hardy's main objections are twofold: the idea that microbes cause Alzheimer's neuropathology doesn't fully explain the hereditary aspects of the disease, and it doesn't explain the characteristic anatomical distribution of plaques and tangles in diseased brains. He thinks distribution would be more widespread in the brain with blood-borne disease. "It just doesn't ring right," he says. "It doesn't fit the epidemiology, the neuropathology, or the genetics." To get him to change his tune, Hardy says, he'd need to see more experimental evidence "to show some element of cause and effect: infect mice, infect primates, and show disease."

The microbe theorists freely admit that their proposed microbial triggers are not the only cause of Alzheimer's disease. In Itzhaki's case, some 40 percent of cases are not explained by HSV1 infection. Of course, the idea that Alzheimer's might be linked to infection isn't limited to any one pathogen; the hypothesis is simply that, following infection, certain pathogens gain access to brain, where immune responses result in the accumulation of amyloid-β, leading to plaque formation. In the meantime, with Alzheimer's patients representing a huge unmet medical need, and experimental drugs often failing in late-stage trials, even Hardy admits that there are more questions than answers at this point in terms of the causative factors in Alzheimer's. "The pathology is a mess. The brain has been diseased for a long time by the time we see it," Hardy says. "We're looking at the end product and trying to determine how it got that way."

Perry agrees: "Most of the resources in this field are spent on a few biomarkers. All the evidence shows that amyloid is important. But causality and centrality are two different things." ■

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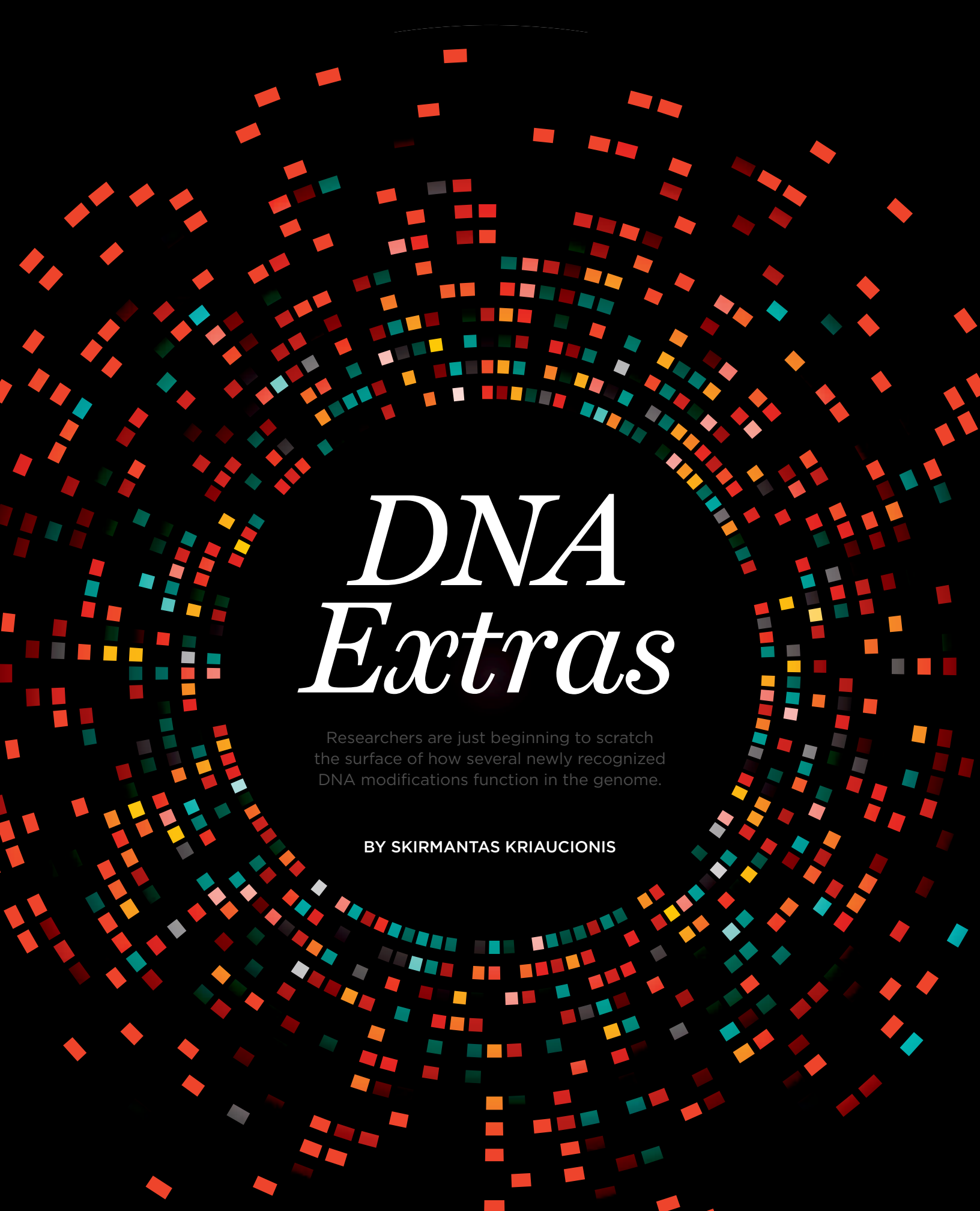


DNA Extras

Researchers are just beginning to scratch the surface of how several newly recognized DNA modifications function in the genome.

BY SKIRMANTAS KRIAUCIONIS

One day in 2006, while a postdoc in the Rockefeller University laboratory of Nathaniel Heintz, I had an unexpected eye-opener. Heintz showed me some electron microscopy images of Purkinje neuron nuclei in the murine cerebellum. They stunned me—the heterochromatin localization in the nucleus was different from anything I'd ever seen before. Rather than the dispersed, irregular patches with enrichment near the nuclear membrane typical of many cells, nearly all the heterochromatin was in the center of the nucleus, adhered to the single large nucleolus. Not only did heterochromatin organization look different, the volume of it in Purkinje neurons seemed much lower, too. Because links between DNA methylation and heterochromatin proteins were suggested in the literature, we thought that DNA methylation might be depleted in Purkinje neurons.

After nearly a year of work, I was able to isolate enough Purkinje nuclei to start quantifying DNA methylation using thin-layer chromatography. This technique usually yields a single spot per each base in the DNA that has a neighboring G. Normally, five intense spots representing the bases A, G, T, C, and methylated C (known as 5-methylcytosine, or 5mC) migrate to expected locations. In our experiments with Purkinje neuron DNA, however, we consistently noticed the presence of a sixth spot that had not been previously described. Could the spot represent a novel DNA base variant, which had gone unrecognized due to the low abundance of Purkinje neurons in the brain? (In the cerebellum, they constitute just 0.3 percent of all cell types.) After several long months of research, we identified the suspect: a cytosine base that had not only a methyl group added, but also a hydroxyl. We termed this new mark 5-hydroxymethylcytosine (5hmC).[1]

DNA MODIFICATIONS ADD TO THE TOOLKIT OF CRITICAL GENE-REGULATORY MECHANISMS.

The diversity of all life on Earth is largely encoded by a relatively simple alphabet: the standard set of four DNA bases, A, G, C, and T. But in many organisms, this alphabet can be expanded by modifications to these bases. Bacteriophages are known to incorporate modified bases during DNA replication, for example. More commonly, organisms make modifications to the DNA bases after replication by adding chemical extensions to nucleic acids. Some postreplication modifications can be stably propagated during cell division, thus adding another layer of information onto DNA, a phenomenon that serves as the founding and principal example of the field of epigenetics. While extending the DNA alphabet typically does not affect the encoding of proteins, it can influence the expression or maintenance of phenotypic traits, and thus play a role in organisms' survival and evolution.

The existence of modified bases varies throughout the tree of life, and the distribution of these variant bases may shed light on how and why these modifications evolved. Some organisms, such as yeasts and members of the order Diptera (flies, mosquitos), contain no modified bases, while others, including viruses, bacteria, plants, some fungi, nematodes, ants, honeybees, and all examined vertebrates, have modified DNA. Modifications reside typically, but not exclusively, on DNA bases. The most common way of modifying bases is the addition of a methyl mark, and across species, methylation has been found on cytosines and adenines, resulting in 5mC, N4-methylcytosine (N4mC), or 6-methyladenine (6mA). N4mC is present in bacteria, while 6mA, also once thought to be exclusively prokaryotic, was recently reported in the DNA of metazoan species, where its function still remains elusive.

In vertebrate genomes, 5mC is the most common modified base, found predominantly on cytosines that are followed by guanines (the so-called CpG context), with 70 percent to 80 percent of all CpGs in the genome containing such methylation. This epigenetic mark has been investigated for nearly 60 years, but in 2009, our work—and work done simultaneously by Anjana Rao's group, then at Harvard Medical School[2]—revealed the existence of 5hmC. The abundance of 5hmC is quite variable, ranging from less than 1 percent of 5mC in some cancer cell lines to nearly 30 percent of 5mC in Purkinje neurons. Research is now underway to understand how this DNA modification is regulated and how it differs functionally from 5mC. (See "Unmasking Secret Identities," *The Scientist*, February 2014.)

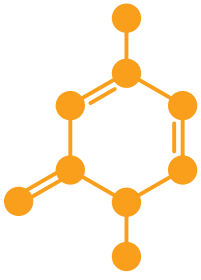
Just two years after discovery of 5hmC, Yi Zhang, then at the University of North Carolina at Chapel Hill, and Thomas Carell of Ludwig-Maximilians-Universität in Germany identified two other types of marks that can be added to cytosine, resulting in 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC).[3,4] These modifications are even rarer than 5hmC, occurring at levels nearly two orders of magnitude lower. But their discovery, along with continued research into 5mC and 5hmC, have scientists rethinking the prevalence and functions of cytosine modifications—and how they alter the basic function of DNA.

Arms race

It is widely accepted that one of the main purposes of modified DNA bases in bacteria is to defend the genome against bacteriophages. The defense strategy is based on the activity of two bacterial enzymes—a restriction enzyme that cuts the DNA at defined sequences and a second enzyme that modifies the DNA in that same sequence context to protect it from the cutter enzyme. When the genes for both of these enzymes are present in a bacterium—often found in close proximity in the genome, in what's referred to as a restriction-modification (R-M) operon—the two gene products cause no harm to its genome, as the modifying enzyme provides the necessary shield before the cutter can do its work. But when a bacteriophage injects unmodified DNA, it is cleaved by restriction enzymes, disabling viral replication.

Viruses have evolved counter-defense systems of their own. One simple way a bacteriophage evolves to avoid DNA-cutting activity is by elimination of the restriction enzyme recognition sites from its genome. In response, bacteria have evolved R-M operons that target different DNA sequences. Alternatively, some phages have evolved to protect their DNA using base modifications. In response to this tactic, bacteria have evolved enzymes that recognize and cut the modified foreign DNA, simultaneously losing their own modifications at matching sites in the genome. The consequence of this evolutionary arms race is an extensive list of R-M enzymes in bacteria with different DNA sequence preferences and a panoply of DNA modifications in both bacteria and bacteriophages.

One of the most common base modifications is methylation. The methyl group is small in size and the most neutral modification in terms of reactivity, bond participation, and influence on electron configuration of the base to which it binds. This means that methylation is able to protect bacteria against DNA cutting by restriction enzymes while having minimal consequences for the main functions of the DNA, such as transcription, replication, and mutability.



DNA METHYLATION IN MAMMALS IS INVOLVED IN SUPRESSING ACTIVITY OF VIRUSES AND PARASITES THAT HAVE INVADED OUR GENOMES.

Bacteriophages also have a variety of methylation modifications, but compared with bacteria, they possess a much more extensive DNA modification profile, with around 20 known base modifications. These include less common marks such as glucosylated 5hmC, 5-dihydropentyluracil, and hexosylated 5-hydroxycytosine. Rather than modifying DNA after synthesis, bacteriophages often produce enzymes capable of modifying the building blocks of DNA—nucleotide triphosphates—which are then incorporated randomly into the DNA during replication. Although it's not known why viruses have a more diverse repertoire of DNA modifications than bacteria, it may be due to the fact that bacteria are more complex and may suffer adverse consequences from more-elaborate modifications, such as an increased mutation rate during replication or affinity changes for DNA-binding proteins.

What can we learn about the evolution of DNA modifications in higher organisms from these bacterial and viral systems? In mammals, there is no known antiviral defense mechanism comparable to the bacterial R-M system, but intracellular strategies for combating viruses do exist. Instead of digesting foreign nucleic acids, mammalian cells have enzymes capable of mutating them. Cytosine deaminases convert cytosine to uracil (the RNA base that corresponds to thymine), eventually leading to C-to-T mutations in the viral genome. The best example of such antiviral restriction is the deaminase APOBEC3G, which is capable of inhibiting HIV infection. However, HIV evolved a protein called Vif capable of degrading the deaminase, thus maintaining viral infectivity.[5]

It is unlikely that modified bases in mammals provide substantial viral defenses in a way that is analogous to the bacterial R-M system. For one, modified bases are rare in the human genome, with just 4 percent of all cytosines being modified. Moreover, there is little overlap between methylated DNA sequences and the target sequences of some deaminases. Rather, substrate selectivity for single-stranded DNA and the fact that deaminases are usually restricted to the cytoplasm are the most likely mechanisms of preventing adverse effects of deaminases on the host DNA, which is securely locked away in the nucleus. The protection is clearly not flawless, however, as sites targeted by known deaminases are frequently mutated in cancer, suggesting that in some circumstances the enzyme can gain access to DNA and damage the genome.

Rather than participating in direct destruction of foreign DNA, DNA methylation in mammals is involved in suppressing the activity of viruses and parasites that have invaded our genomes, which are littered with remnants of these pathogens. If unleashed, such incorporated sequences could be detrimental to genome stability, but methylation is one of the mechanisms that prevents such activity.

Brakes on or off

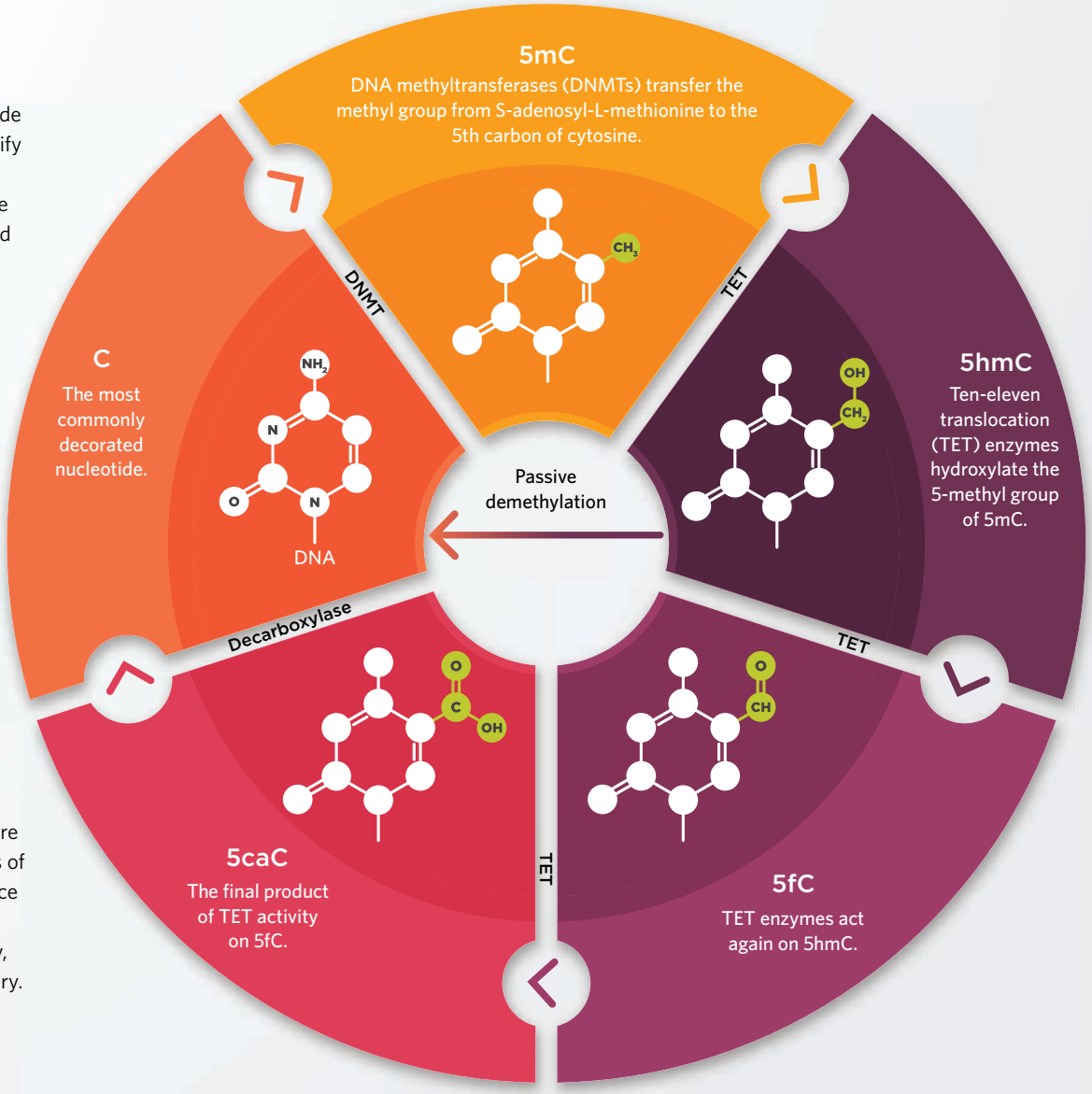
What, then, is the function of epigenetic modifications in the genomes of eukaryotes? One hypothesis is that modified bases play a role in gene regulation. The presence of 5mC modification in promoters strongly correlates with a lack of expression of those genes. During embryonic development, for example, DNA methylation is often associated with the silencing of a gene, such as during X chromosome inactivation in females. Another group of genes regulated by DNA methylation consists of imprinted genes whose expression is dependent on the parent of origin. These genes contain differentially methylated regions, which promote allele-specific expression.

DNA methylation may also regulate gene expression in a more dynamic way, possibly with environmental factors influencing the addition or removal of methyl marks to control gene activity in response to external conditions. In these cases, however, it is not known whether DNA methylation actually regulates expression. Often there is just correlation between DNA methylation and expression, which does not prove causality.

In terms of exactly how DNA methylation can prevent transcription initiation, two main mechanisms of gene silencing have been proposed: the methyl group could occlude binding of transcription activators, or it could attract transcriptional repressors. Some transcriptional repressors are known to bind 5mC and

DECORATING DNA

To expand the basic nucleotide alphabet, many species modify their DNA with the addition of epigenetic marks. Cytosine is the most commonly altered base, with methylation being the most common addition. In vertebrates, this modified based, called 5-methylcytosine (5mC), is found primarily in the CpG context—on cytosines followed by guanines. Recent research has revealed that this base can be further modified into a number of variants, including 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxyl-cytosine (5caC), though these modifications are generally rare. Researchers are still hunting for the functions of such DNA bases, but evidence points to their roles in gene regulation and DNA integrity, affecting learning and memory.



DNA modification	Found in which species/ type of organism	Found in what genomic context/cell type	Frequency in human or mouse genome	Molecular roles
5-methylcytosine (5mC)	Ubiquitous, some exceptions	Primarily CG but also found in other contexts, ubiquitous	2 percent to 4 percent of C	Represses gene expression
5-hydroxymethylcytosine (5hmC)	Vertebrates, some fungi, protozoans	Primarily CG, enriched in brain and other differentiated tissues	0.1 percent to 0.8 percent of C	Intermediate for demethylation, other roles debated
5-formylcytosine (5fC)	Vertebrates, some fungi, protozoans	Primarily CG, enriched in mouse embryonic stem cells	<0.002 percent of C	Intermediate for demethylation, other roles debated
5-carboxylcytosine (5caC)	Vertebrates, some fungi, protozoans	Primarily CG, enriched in mouse embryonic stem cells	<0.0003 percent of C	Intermediate for demethylation, other roles debated

often act on genes by recruiting histone deacetylases, resulting in a chromatin state that is less compatible with transcription.

Employing DNA modifications for transcription regulation does not come "free of charge," however. The hefty price of having 5mC in the DNA is elevated mutability, with the cytosine spontaneously mutating to thymine. Because 5mC is predominantly found in CpG dinucleotides, this has resulted in the depletion of CpGs across the methylated parts of vertebrate genomes. Thus, instead of one CpG every 16 dinucleotides (which one would expect given randomness), methylated regions in typical vertebrate genomes contain just one CpG per 100 bp (with the exception of "CpG islands," where one CpG is observed every 30 bp). CpG dinucleotides are present in four out of six codons coding for arginine, resulting in an enrichment of mutations affecting this particular amino acid in proteins.

THE DIVERSITY OF CELLULAR FUNCTIONS RELATING TO DNA MODIFICATIONS IS PERHAPS NOT SURPRISING, CONSIDERING THAT MODIFIED BASES HAVE A BROAD GENOMIC PRESENCE ACROSS VARIOUS GENES.

In addition to the footprint of DNA methylation on vertebrate genomes, researchers have identified frequent C-to-T mutations at methylated sites in genetic diseases and cancer. Last year, for example, my colleagues and I discovered that mutations at methylated CpGs are observed nearly twice as frequently as at nonmethylated ones in most cancers.[6] Interestingly, mutation frequency at 5hmC-containing sites is nearly twofold *lower* than at 5mC sites, making mutability of 5hmC equivalent to that of unmodified cytosine.

The role of 5hmC in gene regulation appears to be opposite to that of 5mC, as deduced from its location in actively transcribed regions. Several proteins of the MBD family of transcriptional repressors (e.g., Mbd1 and Mbd2) are unable to bind to 5hmC-decorated DNA, providing a possible mechanism for facilitating chromatin structure compatible with expression. But this remains an area of active investigation. Additional antisilencing mechanisms may involve 5hmC's ability to attract specific binding proteins.

Beyond its transcriptional effects, 5hmC was demonstrated to act as an intermediate for demethylation. During demethylation, enzymes known as TETs further oxidize 5hmC to 5fC and 5caC, which are subsequently removed by base excision–repair primarily triggered by thymine DNA glycosylase (TDG). (See illustration on page 51.) Demethylation can happen by a different route as well; replication of 5hmC-containing DNA results in this modification on one strand of the daughter DNA molecule. This asymmetric 5hmC site turns out to be a poor substrate for DNA methyltransferase 1, leading to the generation of unmodified DNA during subsequent rounds of replication.

Small mark, many jobs

In bacteria, modified bases influence DNA damage as well, but instead of increasing mutation rates, bacteria use such DNA modifications to enhance DNA repair. Adenine N6-methylation, for example, has been shown to direct mismatch repair after replication. The DNA methyltransferase Dam methylates adenine bases at palindromic GATC sequences, resulting in the symmetric modification on both strands of DNA. The key utility of methylation here is the ability to make parental and daughter DNA strands distinguishable after DNA replication, as only parental strands will have the modification before the symmetrical state is re-established. During base mismatch repair, MutH endonuclease confers strand specificity by cutting the unmethylated strand, which initiates repair using the parental (methylated) DNA strand as a template.

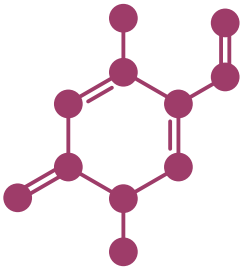
Whether DNA modifications play a role in mismatch repair in eukaryotes is less clear. Despite the fact that the majority of DNA methylation in replicating cells is found in the symmetrical CpG sequence and could indicate parental origin of newly replicated DNA, strong evidence to support the idea that DNA methylation guides mismatch repair is lacking. Some reports were able to observe methylation-guided repair in mammals, but others not.[7]

Methylation also appears to play a role in DNA replication in bacteria. Once again, the mechanism is based on the appearance of asymmetrically modified DNA—in this case, Dam-deposited adenine methylation at the origin of replication after DNA synthesis—with the parental strand containing the modification while the daughter strand does not. This asymmetrical methylation is recognized and bound by SeqA protein, suppressing the reinitiation of replication origin before one round of replication is finished. This provides a time window for the complete replication of bacterial chromosomes once per cell cycle, until Dam outcompetes SeqA to re-establish symmetrical modification, which enables replication origin for subsequent division.

In contrast to bacteria, the majority of eukaryotic species do not have clearly definable or strictly sequence-dependent replication origins. Instead, replication initiates at regions coinciding with a number of features such as promoters, DNase I accessible regions, and CpG islands. Methylated CpG islands replicate later than unmethylated ones, suggesting that DNA modifications could have a function in replication, though the significance of this is still unclear. And the fact that mouse embryonic stem cells do not display major replication defects after genetic elimination of all DNA methyltransferases argues against a major role of DNA modifications in replication.

Touch of the mind

Observations in human cells and in mice suggest that modified DNA bases may be more important to the normal function of the nervous system than of any other tissue. A number of intriguing publications have documented that neuronal cells have unusual profiles of DNA modifications. For starters, 5hmC is nearly threefold more abundant in the brain than in any other organ. The extreme example is in Purkinje neurons, where nearly a third of modified cytosines are in the 5hmC state, which is tenfold higher than in any non-neuronal cell type. Moreover, neuronal cells have the most abundant non-CpG methylation, which is close to the level of methylated Cs in the CpG context. Is it possible that evolution invented yet another function for DNA modifications in neuronal cells? Perhaps the best starting point would be to think about how unusual a neuronal cell is, compared with all the other cell types in a multicellular organism.



Neuronal cells connect in networks, enabling learning and memory. The stability as well as plasticity of neural networks is therefore critical for behavior, and the longevity of some neuronal cells (e.g., those involved in the coordination of movement) could therefore be under strong selection. Neuronal cells are also metabolically active and quite large—human motor neurons of the spinal cord have axons that extend to 1 meter in length, and the majority of neurons have other neuronal projections that measure on millimeter and centimeter scales. Combining enhanced metabolism with longevity is not easy, as oxidative phosphorylation in the mitochondria can generate DNA-damaging reactive oxygen species. Thus, the unusual DNA modification landscape of neurons may favor chromatin with elevated resilience to mutations. Alternatively, the cells' high metabolism and associated requirement for enhanced gene expression, without any need to replicate DNA (differentiated neurons do not divide), may have selected for the use of DNA modifications for more efficient transcription.

A third possibility is that neurons benefit from a more-accurate regulation of transcription, enabling "transcriptional memory." A number of reports indicate that, in addition to the synaptic mechanism of memory, transcription plays important roles in an organism's ability to consolidate and store memories. In animal models, deletion or overexpression of DNA methyltransferases (DNMTs) and TET oxygenases in post-mitotic neurons results in defects in neural plasticity and memory consolidation. In addition, neuronal stimulation induces changes in DNA modifications. These results indicate that DNA modifications regulate the expression of some genes in neuronal cells that are critically important for normal nervous system function.[8]

When all goes wrong

Defects causing stark disruption of DNA modification dynamics lead to extreme phenotypes. In mice, deletion of DNA methyltransferases Dnmt1 or Dnmt3b, or of all three TET families of dioxygenases, results in lethal developmental defects. In humans, mutations in DNA modification–related proteins are also known to cause disease. Germline mutation of *DNMT3B*, for example, causes immunodeficiency–centromeric instability–facial anomalies (ICF) syndrome, a rare genetic disorder characterized by immunodeficiency and facial deformities. Mutations in the 5mC-binding protein MECP2, on the other hand, cause a neurological disorder known as Rett syndrome, which presents as numerous verbal and physical disabilities. Somatic *TET2* and *DNMT3A* mutations are observed in a number of blood cancers, including acute myelogenous leukemia (AML) and chronic myelomonocytic leukemia (CMML).

Altogether, these loss-of-function observations do not demonstrate a particular trend that could link one phenotypic trait to DNA modifications. Instead, they reflect the idea that DNA modifications add to the toolkit of critical gene-regulatory mechanisms. This is well supported by numerous studies demonstrating the importance of DNA methylation in a wide variety of processes, ranging from the activation of T cells in the immune system to memory formation in the brain.

It is thus clear that DNA modifications are key to proper development and function of those organisms in which they exist. These epigenetic factors offer additional options for genome management. In bacteria, DNA modifications are a critical part of immune defense. In mammals, modifications play a key role in gene regulation. Finally, there is some evidence to suggest that DNA modifications affect the mutability of DNA, as well as its repair in certain species.

The diversity of cellular functions relating to DNA modifications is perhaps not surprising, considering that modified bases have a broad genomic presence across various genes. Such an expanded alphabet has presumably undergone positive selection to drive the evolution of organisms to survive and pass on their genomes through the millennia. ■

Skirmantas Kriaucionis is an associate professor at the Ludwig Institute for Cancer Research, University of Oxford, U.K.

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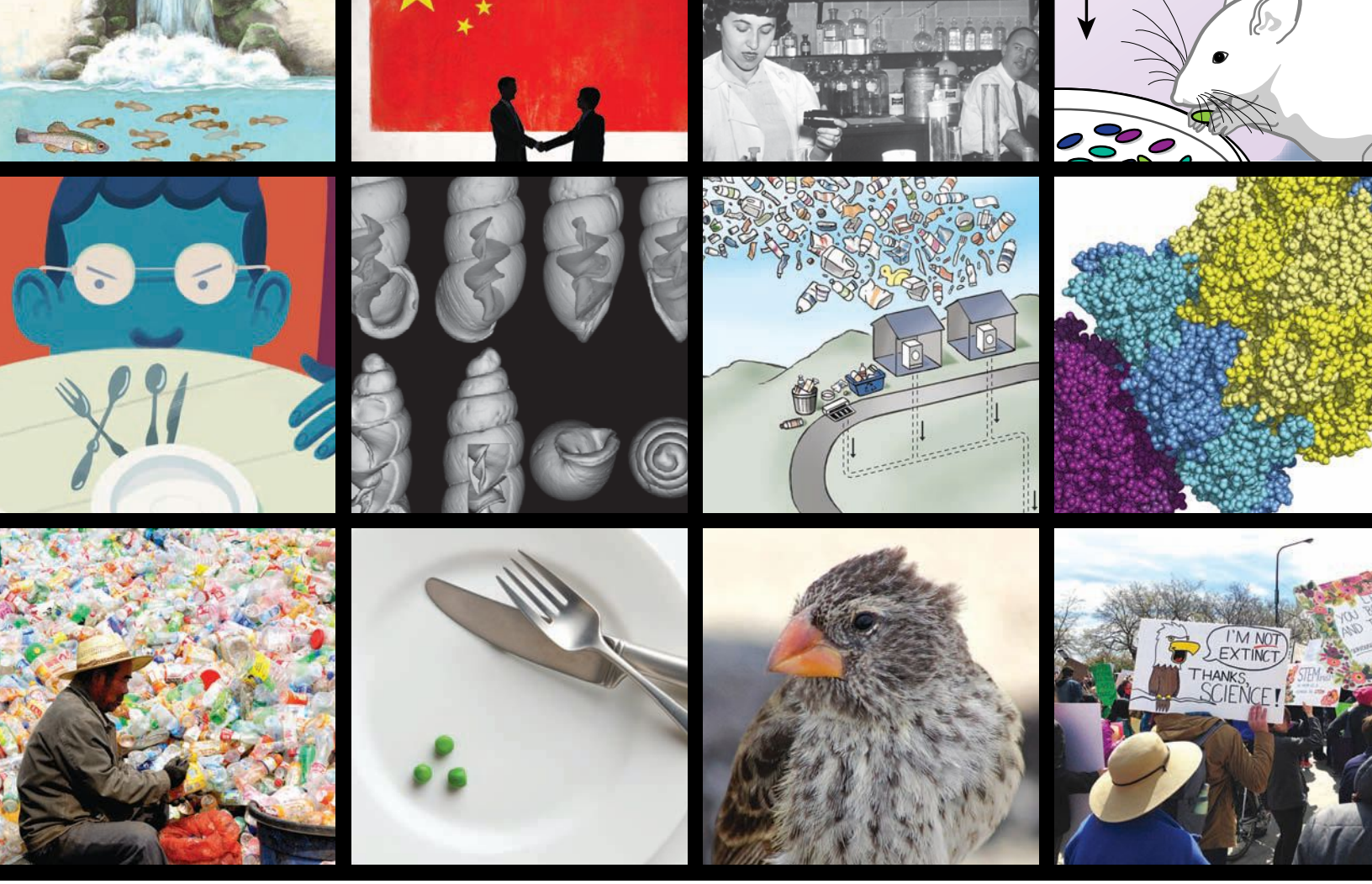

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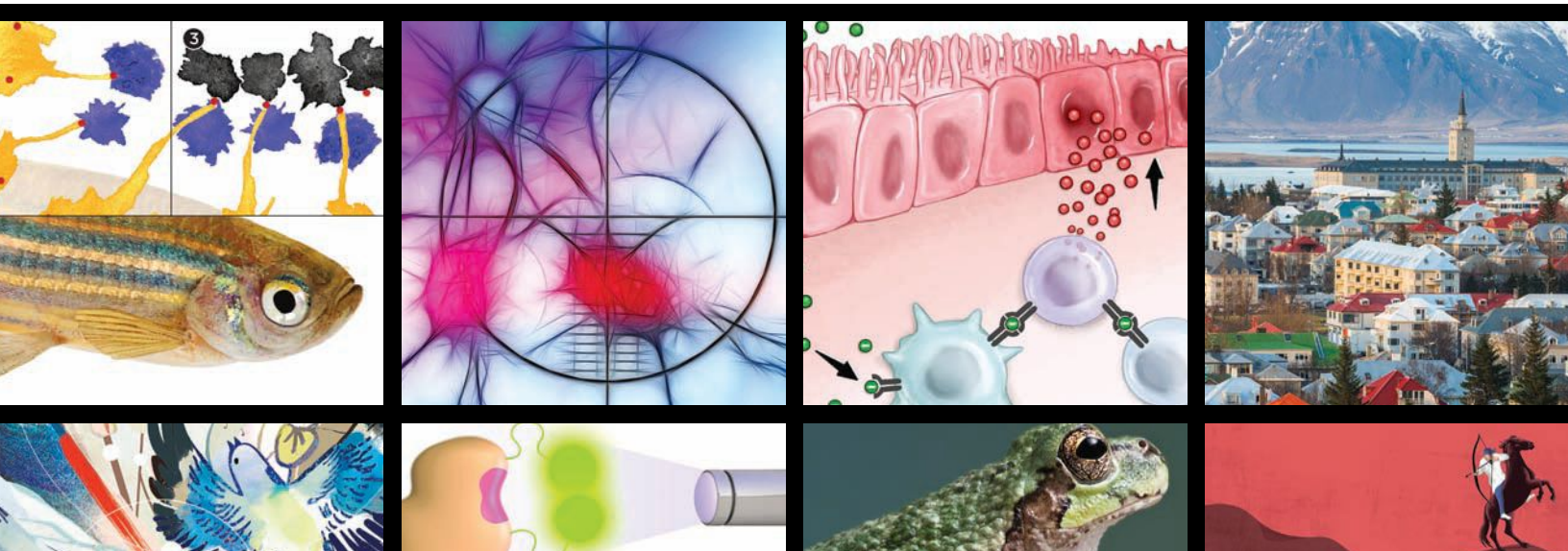


The Literature

EDITOR'S CHOICE IN CELL BIOLOGY

Hair of the Fly

THE PAPER

S. Loubéry et al., "Sara phosphorylation state controls the dispatch of endosomes from the central spindle during asymmetric division," *Nat Commun*, 8:15285, 2017.

Central to normal development are steps in which stem or progenitor cells divide asymmetrically to form daughters with different fates. But what determines these divergent paths? A recent study by Marcos Gonzalez-Gaitan and colleagues at the University of Geneva found that phosphorylation is key to preferentially directing certain cellular vesicles called endosomes to one of the daughter cells, enabling asymmetric division.

To study asymmetrical cell division, many researchers look to the sensory organ precursor cells (SOPs) that form hairs on the backs of fruit flies in a series of three cell-division steps. First, an SOP divides asymmetrically into cells known as pIIa and pIIb. The pIIa cell then divides again to form an outer hair cell and a socket, while pIIb divides twice more, ultimately producing a neuron and its sheath.

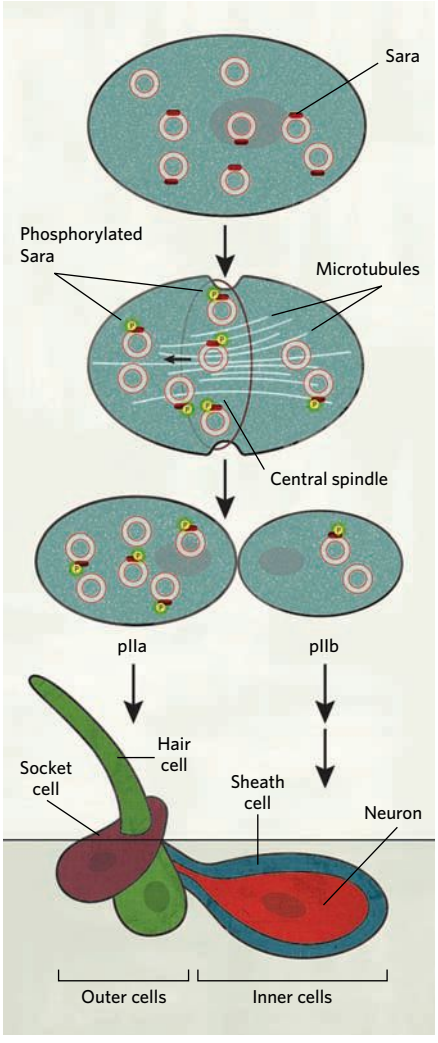
Gonzalez-Gaitan's group had previously found that while most endosomes are split evenly between the two daughter cells during asymmetric cell division, those that contain signaling molecules Notch and Delta and have a surface protein called Sara mainly end up in pIIa. Prior to that, the Sara endosomes are ferried along microtubules to a structure in the middle of the dividing cell known as the central spindle. But it remained unclear how the endosomes were able to break free of the spindle and begin their migration toward the side of the mother cell that becomes pIIa.

To decipher this part of the process, the group used immunoprecipitation to suss out factors that interact with Sara. The researchers found that a phosphatase was interacting with Sara, and that "on Sara there are three sites of possible phosphorylation," says Alicia Daeden, a graduate student in Gonzalez-Gaitan's lab. Further experiments revealed that Sara's phosphorylation state dictated the Sara endosomes' asymmetric distribution, with about 80 percent going into the pIIa cell, she says. When the team generated mutants that had only one wild-type version of Sara, and thus less of the functional protein, the Sara endosomes were distributed more evenly—closer to a 60/40 distribution between the daughter cells—and the flies' backs were nearly bald.

Matilde Cañelles López, who studies lymphocyte development in mice at the Institute of Parasitology and Biomedicine López-Neyra in Granada, Spain, says Gonzalez-Gaitan's group managed to "very nicely see in living cells how the endosomes move and go into one cell," causing the daughter cells to take different paths. The results dovetail with a hypothesis her own group developed based on their work with knockout mice, she says: that asymmetric distribution of endosomes during cell division is key to development.

"It's easy to start speculating that something like loss of this function could, for example, cause some of the tissues to become tumor prone," says Pekka Katajisto, a stem cell biologist at the University of Helsinki, if aberrant divisions result in two stem cells instead of one stem cell and one differentiated cell. However, he adds, the results likely don't apply directly to mammals, which lack the Sara protein.

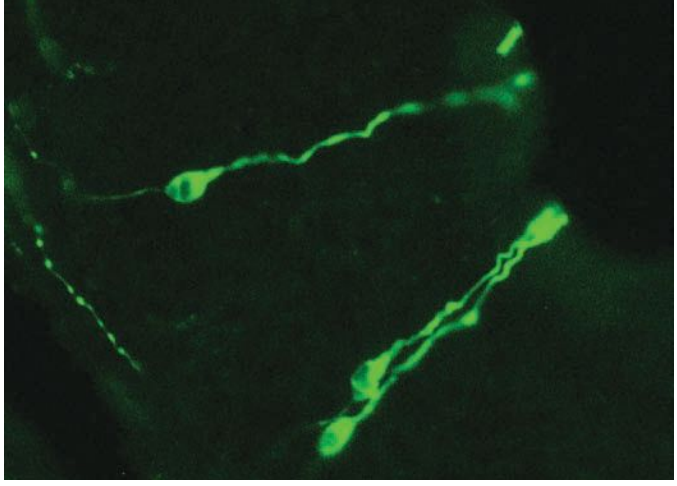
—Shawna Williams



ASYMMETRIC DIVISION: During cell division in fruit flies' sensory organ precursor cells, microtubules draw endosomes with the Sara protein on their surface to the central spindle. There, Sara is phosphorylated, causing the endosomes to detach from the spindle and travel to one side of the mother cell, with most of them moving into the daughter cell known as pIIa, where microtubule disassembly is greater. That cell divides again to form the outer shaft and socket of a hair on the fly's back, while its sibling, pIIb, gives rise to the hair's inner sheath and neuron. Without Sara, hair formation is compromised.

THE SCIENTIST STAFF

FOOD FINDER: A novel receptor identified in the nose of zebrafish helps the animals track down their next meal.



MISPLACED RECEPTORS: In the mouse olfactory neuroepithelium, vomeronasal neurons express an FPR immune receptor (green).

MOLECULAR BIOLOGY

Follow Your Nose

THE PAPER

N. Wakisaka et al., "An adenosine receptor for olfaction in fish," *Curr Biol,* doi:10.1016/j.cub.2017.04.014, 2017.

A NOSE FOR ATP

Studies have shown that fish sense ATP, the cellular unit of energy, and follow concentration gradients of the molecule released by zooplankton to track down their next meal. Testing zebrafish in the lab, neurobiologist Yoshihiro Yoshihara of the RIKEN Brain Science Institute in Japan and colleagues found that ATP appeared to activate a small number of short, pear-shape olfactory sensory neurons at the very tip of the nose.

A NEURONAL ODOR TRAIL

When the researchers hunted for ATP receptors in the zebrafish genome, they found a novel receptor called A2c. Cell culture experiments revealed that A2c was not directly activated by ATP, however; two enzymes, tissue-nonspecific alkaline phosphatase (TNAP) and CD73, first broke down ATP to adenosine, which then bound the receptor and triggered the neurons in the nose to fire. Signals from these neurons then activated a single large cluster of nerve endings, or a glomerulus called IG2, in the olfactory bulb of the zebrafish brain.

EVOLVING FOOD SENSE

A database search of available genomes showed that the A2c receptor is found in fish and amphibian species, but not in terrestrial reptiles, birds, and mammals. "The A2c receptor must serve a very fundamental function in all the aquatic lower vertebrates," Yoshihara says.

OPEN QUESTIONS

Arnaud Gaudin, a neurobiologist at Canada's Dalhousie University who was not involved in the study, pointed out that the paper only considers the amphibian group *Xenopus*, which live and hunt in water even as adults. "It would be very interesting to see whether the A2c receptor would also be found in other anuran species . . . in which tadpoles have a fully aquatic olfactory phase that is lost after metamorphosis."

—Sandhya Sekar

EVOLUTIONARY BIOLOGY

Hijacked Receptors

THE PAPER

Q. Dietschi et al., "Evolution of immune chemoreceptors into sensors of the outside world," *PNAS*, doi:10.1073/pnas.1704009114, 2017

SUSPICIOUS SIMILARITIES

Proteins known as formyl peptide receptors (FPRs) on the surface of immune cells are involved in detecting signs of infection. Previously, Ivan Rodriguez of the University of Geneva and colleagues had found that FPR-like receptors on the surface of neurons in the olfactory system of rodents can trigger the cells' activation, but it wasn't clear how immune proteins had evolved to sense smell.

RODENT INNOVATION

By comparing the genomes of multiple mammal species, the researchers homed in on several events involved in the coopting of FPRs for olfactory sensing. Twice, a duplicated FPR gene landed near a promoter sequence for vomeronasal receptors; later, the ancestor of most mouse species acquired the ability to use one of its FPRs for either smell or immunity by splicing together transcripts of different genes.

SPEEDY REPEATS

These events occurred within the last 30 million years, a relatively short period of time on the evolutionary scale, Rodriguez says. Such speedy evolution is a hallmark of chemical-detecting receptors more generally, notes Duke University's Hiro Matsunami, who was not involved in the study. The genes for the FPRs are surrounded by many relatively unstable repeat sequences, making them prone to duplications.

INSIDE OUT

Rodriguez's group is still working to determine just what the immune system receptors are doing in the nose, but he thinks FPRs may underlie rodents' ability to detect illness in their compatriots. Regardless, Matsunami points out that the immune and olfactory systems share a common goal: to survey the environment—whether internal or external—for signs of danger. "They end up using the same kind of genes for their common purposes."

—Shawna Williams

COMINGSOON | Mining the Tumor Microenvironment: Advanced Tools and Protocols for Tumor-Cell Signaling

The tumor microenvironment forms a complex, privileged zone where conditions are permissive for unchecked tumor progression. Therein, both cancer and stromal cells exhibit aberrant growth and survival signaling, making pathway analyses ever-more difficult. Advanced tools have enabled deeper, more thorough investigation into how the tumor microenvironment has adapted to evade the immune system, and how we might counteract those adaptations. *The Scientist* is bringing together a panel of experts to discuss the interplay of cells within the tumor microenvironment and to share their latest methods and findings. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.

GARRY P. NOLAN, PhD
Rachford and Carlota A. Harris Professor
Department of Microbiology & Immunology
Baxter Laboratory for Stem Cell Biology
Stanford University School of Medicine

GAGAN DEEP, MD, PhD
Associate Professor
Department of Cancer Biology
Wake Forest School of Medicine

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COMINGSOON | Parkinson's Disease: The Search for Biomarkers

In the absence of new diagnostic tests for Parkinson's disease (PD), the diagnosis has long been one of exclusion, ruling out other causes of tremor, bradykinesia, and rigidity. With the dawn of biomarker-based molecular diagnostics, a new race has begun to identify molecular signatures of disease pathology in noninvasively derived tissue samples, including blood, urine, and saliva, in addition to radiographic or magnetic scans. Scientists have begun to sort through the molecular traces associated with PD patients to find telltale signs of disease onset and progression. *The Scientist* is bringing together a panel of experts to share their experience with biomarker discovery and validation, as well as their predictions for this as-yet-untapped market. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.

BRIT MOLLENHAUER, MD
Assistant Professor, Department of Neuropathology
University Medical Center, Goettingen, Germany
Paracelsus-Elena-Klinik, Kassel, Germany

HUGO VANDERSTICHELE, PhD
Cofounder and Chief Integration Officer
ADx Neurosciences
Founder, Biomarkable bvba

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Simmering, low-level inflammation throughout the body is responsible for many disease processes, ranging from osteoarthritis and cardiovascular disease, to digestive disorders and neurodegeneration. The bioactive molecules, known as cannabinoids, found in plants of the *Cannabis* species, have been shown to possess powerful anti-inflammatory attributes, and research into their mechanisms of action, efficacy, and tolerability are underway. To explore the potential for cannabinoid-based and/or endocannabinoid-targeted therapeutics in the realm of human disease, and particularly diseases with an inflammatory component, *The Scientist* is bringing together a panel of experts to discuss their research. The session will also offer insight into the rewards and challenges of studying a biomedical application for a well-known, but controlled, substance. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics related to their research.

ANTON REINER, PhD
Professor, Department of Anatomy and Neurobiology
University of Tennessee Health Science Center

YANNICK MARCHALANT, PhD
Assistant Professor, Departments of Psychology and Neuroscience
Central Michigan University

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TOPICS TO BE COVERED:

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- Targeting the endocannabinoid system for therapeutic effects

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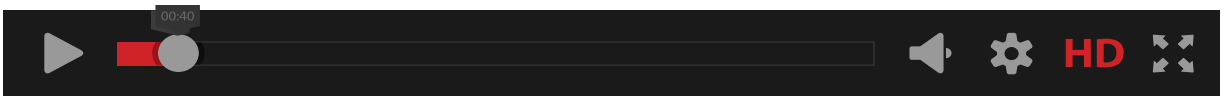
ON**DEMAND** | Recent Advances in Immunotherapy: Directing Cells to Address Disease

To target diseases such as cancer and HIV, scientists are now successfully programming the immune system to seek out and destroy the agents of disease, even when those agents are the body's own cells. Immunotherapy has demonstrated remarkable efficacy in treatment of previously undruggable diseases, leading to calls for standardization and additional research. *The Scientist* brings together a panel of experts to review the current knowledge base, share their recent work, and discuss the future of immunotherapy.



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LEENA GANDHI, MD, PhD
Director, Thoracic Medical Oncology
NYU Langone Medical Center
Associate Professor, Department of Medicine
NYU Perlmutter Cancer Center

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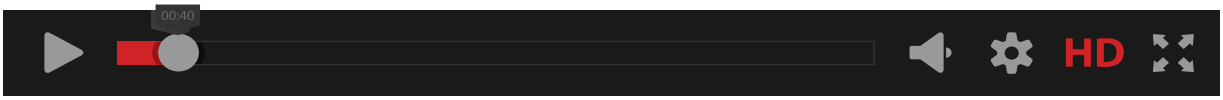
Communication between cancer cells has long been a target of drug discovery efforts, but the conversations between cells can be convoluted and confused by signaling-pathway crosstalk, feedback loops, and other complex interactions. Recent advances have led to the elucidation of unexpected interactions between cells during the oncogenic transition, and these interactions are specific to the tumor microenvironment. To explore the advances in understanding cancer signaling, *The Scientist* brings together a panel of experts to discuss their research and share insights into targeting these pathways with anti-cancer therapeutics.



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Associate Professor, Department of Experimental Oncology
University of Alberta

WILLIAM WEISS, MD, PhD
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Motor Man

Ron Vale has spent a career studying how molecular motors transport cargo within cells. He's also developed tools to help scientists communicate their findings.

BY ANNA AZVOLINSKY

In 1983, Ron Vale was three years into an MD/PhD program at Stanford University, and he already had four publications under his belt. During his first two years, spent in medical school, Vale worked with neuroscientist Eric Shooter. "These were not very influential papers, but they taught me how to start to ask a question, to start and complete the experiments, and how to write a scientific paper," says the University of California, San Francisco professor. "Having these papers . . . basically gave me a guarantee of a PhD degree, even though I had officially just begun the PhD part of the program," Vale says. "Now, I really wanted to do something that was bigger, riskier, and exciting."

Vale became intrigued by microscopy movies generated by his lab neighbors James Spudich and visiting professor Michael Sheetz showing myosin-coated beads moving along the actin cables of purified skeletal muscles. Myosin is an adenosine triphosphate (ATP)–powered motor protein whose motion along actin filaments generates muscle contractions. The two were trying to reconstitute, in vitro, the basic motility that occurs within muscle fibers. Vale, Sheetz, and Spudich wondered whether myosin movement also might account for the dynamic movement of organelles such as mitochondria and transport vesicles along the long giant axon of squid. "I was inspired by the strong visual impression made by Sheetz's and Spudich's movies and could imagine a similar mechanism working in axons," he says.

> "I don't think that anyone in the field thought that motors would be just floating around in the cell."

Vale was studying how nerve growth factor interacts with its receptor at axon terminals and wondered how molecular signals traveled from the axonal terminal to a nerve cell body across a long axonal distance. "There was little known about axon transport. . . . It seemed like an interesting problem to work on."

With Sheetz, Vale discussed ideas to test whether myosin-coated beads would move within axons, using the squid giant axon—which can be as wide as 1 mm, more than 100 times the width of a human axon—as a model. Serendipitous events and many hundreds of hours of laboratory work resulted in Vale, Sheetz, and their collaborators Bruce Schnapp and Tom Reese developing methods to study and visualize transport by molecular motors, including in vitro reconstitution assays, and the discovery of a novel motor protein—which Vale dubbed kinesin—that moves along microtubules by using the energy derived from ATP hydrolysis.

Here Vale discusses how El Niño steered him to Woods Hole and the collaboration that led to the discovery of kinesin; his passion for preprints in biology; and his project to deliver lectures by the best biologists to anyone with Internet access.

VALE VENTURES

Science interests, artistic roots. Vale was born in 1959 in Hollywood, California, where Michael Jackson was one of his elementary school classmates. His mother was an actress and his father wrote screenplays for movies and television." Neither of my parents had the opportunity to go to college because of World War II and the circumstances of their lives, but what impressed me was how incredibly well-rounded, curious, and self-educated they were," says Vale. During his childhood, Vale's mother frequently took him to the Natural History Museum of Los Angeles County, the planetarium, and other science exhibitions, which, he says, sparked his interest in science.

Getting hooked. As a high school sophomore, Vale conducted a circadian rhythms experiment in his parents' basement using bean plants, designing a device that would measure the plants' movements. His guidance counselor Ella Hogan, who was also a neighbor, noticed his appetite for science and contacted the University of California to find a professor willing to supervise Vale's extracurricular research. For the rest of high school, he worked in the plant physiology lab of Karl Hammer at UCLA. The guidance counselor also told Vale about the Westinghouse (now Regeneron) Science Talent Search, a research competition for high school seniors. For his circadian rhythms project, Vale was selected as one of 40 students in the U.S. to attend the semifinalists' meeting in Washington, DC. "It was eye-opening to meet all of these other kids interested in science and speak to scientists about your work. That's what really hooked me on science."

Great role models. In 1976, armed with a full scholarship, Vale entered the University of California, Santa Barbara (UCSB), as a student in the College of Creative Studies, where curricula were designed for independent study. Even before arriving at UCSB, Vale had sought out Beatrice Sweeney, a plant biologist who worked on circadian rhythms, to ask if he could work in her lab. "She was an amazing person, in her 60s and still doing tough circadian rhythm experiments herself, coming in throughout the night to take samples. It was just so obvious how much she loved science. She was quite inspirational to me," says Vale. In the summer, Vale worked on the

YUXIAO WANG

RON VALE

Professor, Department of Cellular and Molecular Pharmacology
University of California, San Francisco
Investigator, Howard Hughes Medical Institute
Founder, President, Chairman of the Board, iBiology

Greatest Hits

- With Michael Sheetz, Thomas Reese, and Bruce Schnapp, discovered that the bidirectional transport of organelles within axons occurs along microtubules
- With Michael Sheetz and Thomas Reese, purified and identified a novel motor protein that he named kinesin, which moves cargo along microtubules and allows for transport within cells
- Established that an individual kinesin molecule pulls itself and its load along a microtubule and that the kinesin motor protein is able to take many steps along the microtubule without detaching
- Using *Xenopus* egg extract, discovered the first microtubule-severing factor
- Developed the first single-molecule fluorescence motility assay for a motor protein
- Founded iBiology.org, ASAPbio.org, and IndiaBioscience.org, as well as other public-service and educational efforts

epidermal growth factor receptor in C. Fred Fox's lab at UCLA. "I was this freshman who showed up in his lab and, instead of giving me mundane lab tasks, he gave me my own project and, in retrospect, a remarkable amount of independence," Vale recalls.

Undergraduate scientist. Back in Santa Barbara for his final year, Vale wrote to Duke University's Robert Lefkowitz (a 2012 Nobel laureate in chemistry) and worked in his lab over that winter and spring. "It was a big and super-exciting lab that was doing the Nobel Prize–winning work of purifying the β-adrenergic receptor. I learned a lot from seeing this exciting chase for a major goal, and Bob was fantastic and extremely generous. He treated me more like a colleague than an undergraduate," says Vale. Although his work in the Fox lab resulted in a 1984 first-author paper in which Vale showed that a plasma membrane fraction can inhibit cell proliferation induced by epidermal growth factor, his work in the Lefkowitz lab resulted in the first paper on which Vale was lead author, a 1982 publication on the interactions between insulin and its receptor.

VALE, VALIDATED

Missing squid. Vale entered Stanford University's MD/PhD program in 1980. In 1983, just as he was beginning the PhD part of the program, he and Sheetz decided to test whether movement of myosin along actin filaments within the squid giant axon was the source of the organelle shuttling that had been recently observed by Robert Allen, Scott Brady, Ray Lasek, and colleagues. Vale and Sheetz planned to use squid supplied by Stanford's Hopkins Marine Station. But that spring, neither researchers at the station nor commercial fisheries were catching any squid. Only later, it emerged that 1983 was an El Niño year that left the Pacific Ocean along the coast of California too warm for the squid, which had swum off to cooler waters.

"Impetuously, we decided to do the work at the Marine Biology Laboratory (MBL) in Woods Hole, Massachusetts, and within two weeks had set up shop there," says Vale. When they got there, they were introduced to novel video-based contrast-microscopy imaging techniques being developed by researchers Robert Allen and Shinya Inoue at the MBL, which was "kind of the center of this microscopy revolution at the time," says Vale. He and Sheetz then teamed up with Bruce Schnapp and Thomas Reese, who had built a video-enhanced contrast electron microscope for their axon experiments. In two *Cell* papers published two years later, the team showed that organelles could move bidirectionally, not on actin, as Vale had hypothesized, but rather on a single microtubule.

In the summer of 1984, his last before a scheduled return to medical school, Vale challenged himself with reconstituting the microtubule-based axonal transport system, breaking apart the components and trying to put them back together again. He was able to make microtubules from purified tubulin and purify axonal organelles from squid. To Vale's surprise, when mixed together, the organelles by themselves had no ability to move on the microtubules. Adding back additional soluble proteins from the axon allowed the organelles to move along the tubules. "I thought that the motor would be on the organelles and others thought they would be on the microtubules. I don't think that anyone in the field thought that motors would be just floating around in the cell." The discovery that the cytoplasm contained soluble, free-floating motor proteins came about by accident: while doing what he thought was a control experiment, Vale observed that this soluble cell fraction bound to a glass cover slip could move microtubules along the glass surface. He also quickly showed that these soluble motors could attach to beads and cause them to move along microtubules. "That study really told us a lot about how that whole transport system was organized. It also gave us an in vitro microtubule-based motility assay, which the field has been using for 30 years."

Two-way traffic. In the winter of 1984, Vale took a leave of absence from medical school and stayed on at the MBL, purifying the motor protein and using the new assays to test the protein's function. "We discovered these assays two weeks before I was supposed to go back to medical school. It was really down to the wire for me to figure out what to do with my future," says Vale. "Woods Hole is so deserted in the winter, it was like doing science in the 19th century. It was really just you and the science, with no distractions." During that winter, along with Reese and Sheetz, he identified the previously unknown motor protein, which they dubbed kinesin. Vale credits the name to his mom and her friend, a scholar of classical Greek who told him that *kine* is Greek for movement. The team further probed the activity of kinesin and found that it moves in one direction, towards the N-terminus of a microtubule, and that another motor protein, later discovered to be a cytoplasmic dynein by Richard Vallee, moves in the opposite direction.

VALE VOICES

Pet projects. In 1986, at the age of 27, Vale set up his own laboratory at the University of California, San Francisco, giving up the idea of finishing his MD degree. For his first 10 years as a professor, Vale continued to perform experiments, and published eight first-author papers. "I wanted to be at the lab bench with everyone else. It was important to be part of the chase, because that's what motivated me personally." In 1991, Vale even published a sole-author paper in *Cell*, when he discovered the first microtubule-severing factor while trying to do organelle transport assays using *Xenopus* extracts. Then in 1996, while on sabbatical in the lab of Toshio Yanagida, who had developed single-molecule microscopy, Vale developed the first single-molecule fluorescence motility assay for a motor protein.

Shifting gears. After figuring out much about how kinesin works, including working out the protein's crystal structure in 1996, Vale's lab shifted focus to dynein—a motor protein discovered in 1963, almost 20 years before kinesin—and among the largest proteins encoded in the genome. Dynein was less studied than kinesin because of its intractable size. In 2006, Vale's lab figured out a way to express and purify the large protein from yeast and showed, using single-molecule assays, how the protein moves. His lab also studies T-cell signaling, using reconstitution systems, microscopy, and biochemistry. Additionally, his laboratory has made several contributions in RNA biology, mitosis and cell division, and microtubule-binding proteins.

Biology for the people. In 2006, Vale started iBiology, a collection of freely accessible online videos that feature leading biologists, who explain concepts and talk about their research. "The idea for the project came to me when I flew to India for the first time and gave a talk to 150 people in a country with a population of 1.3 billion. The way we communicate science in oral form is different from written communication. I wanted people all over the world to have the ability to hear leading scientists talk about their work, not just the small proportion within elite institutions." Vale is increasingly devoting more time to the project and expanding its scope to include science education.

Science ambassador. Vale also started the Young Investigators' Meeting (YIM) in 2009 to give junior scientists in India the opportunity to build a network and find mentors and resources. He started a website and organization called IndiaBioscience.org. "YIM is about the science, but also provides insights into career development and how to develop the skill set for running a laboratory, for which there are plenty of resources in the U.S. but fewer in India. The country is in an important transitional moment where its economy is growing and so is its scientific enterprise. India needs to invest time and resources into building a scientific culture and supporting young scientists," he says.

Changing tides. In 2015, Vale founded ASAPbio, an organization that promotes the use of preprints to accelerate scientific publication. "I think it's really had an impact because two years ago, biologists really did not know what preprints were. Now, the concept has taken off, and preprints have grown considerably and have caught the attention of funding agencies and scientists. It has been really gratifying to see the evolution of developing a more open culture of sharing scientific data. Preprints don't replace traditional peer review, but they can work alongside publishing as a way to get results out there faster," says Vale. "A big motivation for this effort is to help young scientists, because their papers can be stuck in review for a long time, and publications are the way scientists can demonstrate productivity. I am amazed how the preprint culture in biology is advancing. It shows that if you put an issue in front of the scientific community and engage the community in open discussion and debate, the culture in science can change in positive ways." ■

Kate Rubins: Astrovirologist

Astronaut, NASA. Age: 38

BY AGGIE MIKA

It was early morning in Kazakhstan on July 7, 2016, when virologist Kate Rubins donned her spacesuit and rode a battered elevator hundreds of feet up the side of an icy rocket—the colossal structure "creaking and moaning" from its load of cryogenic fuel. She entered the new Soyuz spacecraft and endured a rumbly, bumpy launch, headed to the International Space Station, 400 kilometers up.

Rubin's intense training regimen did little to mentally prepare her for the "controlled explosion" that was the launch, she says. During the next 115 days on the station, after mastering how to pipette water globules in zero gravity and how to keep her equipment from floating away, Rubins cultured cardiomyoctes and, using a portable handheld sequencer, became the first to person to sequence DNA in space.[1]

The switch from running a laboratory on Earth to performing experiments in space may seem like a formidable career leap. But for Rubins it was a natural progression, totally in line with her penchant for adventure and her "willingness to assume risk," says her PhD coadvisor David Relman of Stanford University.

Prior to joining NASA, "Kate spent years in a spacesuit, doing science with joy, enthusiasm, and incredible effectiveness," says Relman, referring to her graduate work studying smallpox, which required her to wear a protective body suit while working in a biosafety level 4 lab.

In graduate school, Relman tasked Rubins with investigating responses to the life-threatening infections caused by pox, Ebola, and Marburg viruses. She measured gene-expression patterns following infection in macaques and human cells[2] to identify distinguishing features that could be useful for earlier diagnosis. Rubins was the first to characterize genome-wide smallpox immune responses in a primate model[3] and traveled to the Congo to help the US Army develop a framework for studying monkeypox in children, says Relman.

After earning her PhD in 2005, Rubins continued her virology work and studied other African viruses as a fellow/principal investigator at the Whitehead Institute for Biomedical Research, bypassing a traditional postdoc and establishing her own laboratory. In the midst of her fellowship, Rubins answered a call from NASA for astronauts, taking a chance to live out a childhood dream. "When I was a kid, I really wanted to be an astronaut, a biologist, and a geologist . . . simultaneously," she says, chuckling. As an adult, however, she didn't think NASA would take her seriously.

But they did. After a lengthy selection process, Kate trained with the Navy where she learned how to fly supersonic fighter jets, how to survive harrowing situations such as an underwater helicopter crash, and how to complete a free-flyer capture—apprehending an incoming spacecraft using the station's robotic arm—which Rubins describes as akin to pulling a friend out of a car "when you're both going 17,500 miles an hour."

In space, Rubins performed two space walks to install key equipment on the space station. NASA astronaut Jeff Williams, her companion on these walks, says she knew how to handle hazardous situations. "I've been on board with over 50 people. Kate was among the best of them for first-time flyers."

At present, as an astronaut and NASA's former deputy director of human health and performance, Rubins is living two out of her three childhood dreams. "People actually do this as a career," Rubins says. "An astronaut is a real job, not just something you say you want to be when you're a kid." ■

ROBERT MARKOWITZ

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Ready, Set, Grow

How to culture stem cells without depending on mouse feeder cells

BY AMBER DANCE

Stem cells require just the right sort of coddling to stay in their pure pluripotent, dividing state. In the lab, the nanny role is often taken on by mouse embryonic fibroblasts (MEFs), lining the culture dish as a "feeder layer." However, these feeders have their downsides, so scientists are developing other options.

Exactly what makes MEFs or other feeder lines good nannies is a bit uncertain. They seem to offer stem cells two main supports: one is a cozy surface to lie down on, with other cells to contact and the extracellular matrix (ECM) the fibroblast feeders produce; the second consists of growth factors and other molecules secreted by the feeders into the cell-culture medium.

However, feeders also create complications, forcing scientists to culture not one but two finicky cell types, then separate them later when the time comes to harvest the stem cells for analysis. And feeder cells can vary from batch to batch, confounding experiments.

One popular option is to switch to Matrigel, a protein goo derived from cancerous mouse cells. But Matrigel, too, can vary by batch. And in the case of clinical applications for stem cells or their derivatives, there's an ongoing worry that mouse cells might transmit unknown pathogens, or that their proteins might activate the immune system of a person receiving them. Scientists agree that for the clinic, products must be "xeno-free," lacking in any components from nonhuman animals.

Here, *The Scientist* profiles several approaches for avoiding MEFs, or ditching feeders altogether.

HUMANS ONLY, PLEASE

Lucie Germain cultures skin cells to make grafts for burn patients. Without feeders, grafts would likely fail within a couple of months of placing them on the patient, says Germain, Canada Research Chair in Stem Cells and Tissue Engineering at Laval University in Québec City, Canada. Worried about what regulators would think of mouse feeders, her lab switched to human feeders (*Int J Mol Sci*, 14:4684-704, 2013). They obtained the human fibroblasts from a foreskin removed during circumcision of a newborn, which, Germain notes, limits the risk that the cells might carry a virus.

As with mouse feeders, Germain's team irradiated the fibroblasts so they would stop dividing. Otherwise, they'd outgrow the stem cells and overrun the culture. Their human feeders form a stable layer for weeks after irradiation. Mouse feeders, in contrast, lift off the flask floor after a week or so. While the human cells support skin stem cells well, there's a bit of a time delay. Germain can seed mouse feeders at the same time as the skin cells, but it works better to seed the human layer a week before adding the stem cells.

Derrick Rancourt, a professor at the University of Calgary in Canada, also uses human foreskin fibroblasts as feeders (*Stem Cells Dev*, 17:413-22, 2008). To inactivate their cell division, he treats the fibroblasts with mitomycin-C or radiation. The foreskin cells make key factors that maintain pluripotency, he says: both the leukemia inhibitory factor (LIF) required by mouse stem cells and the basic fibroblast growth factor (FGF) needed by human ones. He typically supplements the media with more LIF or FGF, and also adds Rho-associated protein kinase (ROCK) inhibitor to the human cultures, which prevents the cells from dissociating and undergoing a form of apoptosis.

One advantage, Rancourt adds, is that while mouse fibroblast stocks tend to senesce after just a few passages, his human lines keep on growing and dividing. That means he has a larger supply of cells to mitotically inactivate and use as feeders. "We've gone over 100 pas-

sages with these human foreskin fibroblasts without any sign of senescence," says Rancourt. "It's kind of crazy that people are still stuck on mouse fibroblasts." Those who stick with mouse cells are likely just comfortable with the protocols they're used to, he says. For those without ready access to hospital tissues, the cells are available from Millipore Sigma (FibroGRO Xeno-Free Human Foreskin Fibroblasts, $458/vial).

Although the human feeders match human stem cells in species, Rancourt notes, they would be of a different genotype. While he says it ought to be relatively straightforward to separate the feeders from any stem cell–derived transplant tissues, Rancourt is certain regulators will want proof that this is so, to avoid worry that one person's feeder cells or their pathogens could contaminate a patient's stem cells and cause disease or rejection. To circumvent this issue, he's working on a method to create matching feeders derived from the stem cells themselves.

KILL THE FEEDERS

Binata Joddar, an assistant professor at the University of Texas at El Paso, came up with another method to avoid using live feeder cells altogether during her postdoc in the lab of Yoshihiro Ito, chief scientist and director of the Nano Medical Engineering Laboratory at RIKEN in Wako, Japan. The researchers wondered if a fixed feeder layer could support stem cell growth. The cells would be dead, saving the scientists the cost and effort of culture. But their surface proteins and extracellular matrix would remain—they'd be like the jarred, preserved specimens in museum collections, reasoned Joddar.

She succeeded, fixing human dermal fibroblasts in 2.5 percent formaldehyde or glutaraldehyde, washing them well, and adding human induced pluripotent stem cells (*J Mater Chem B*, 3:2301-07, 2015). The fixed cells are tightly bound to the bottom of the dish; Joddar says they don't come off easily even if she tries to dislodge them with a rubber scraper. She's even reused fixed feeder layers, though she wouldn't do that more than once. It's important, Ito adds, that the cells be fixed gently, so the cell membranes remain fluid (*Sci Rep*, 5:11386, 2015).

Another option Joddar is exploring is to remove the feeder cells altogether, leaving behind only the ECM. One can do this by freezing the cells or adding detergent, says Rancourt, who has also experimented with decellularized feeders. Because the feeders were dead, he had to supplement the culture media with basic FGF (*Stem Cells Dev*, 19:547-56, 2010).

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GO SYNTHETIC

Outi Hovatta began a crusade to wean her lab, at the Karolinska Institute in Stockholm, from feeders in 2000. In 2011, she finally succeeded. Hovatta, now an emerita professor, and colleagues defined just two factors needed to maintain pluripotency: the ECM protein laminin and the cellular adhesion protein E-cadherin. They synthesized the laminin in human cells and used a mixture of laminin and commercially supplied E-cadherin to coat culture dishes before adding the stem cells (*Nat Commun*, 5:3195, 2014; *Nat Protoc*, 9:2354-68, 2014).

Scientists have several commercial options for chemically-defined stem cell underlayers and media. Hovatta's collaborators founded a company, Biolamina of Stockholm (in which Hovatta also holds a stake), that sells diverse forms of laminin for €45–69 (US$51–79) per 100 micrograms. Vitronectin is another popular coating (for example, CellAdhere Vitronectin solution from Stem Cell Technologies at $272/0.2 mL vial). Commercial media with defined components and no animal serum include ESGRO-2i (Millipore Sigma, $197/200 mL) and Essential 8 (ThermoFisher Scientific, $209/500 mL) or Essential 6 (ThermoFisher, $175/500 mL).

Many scientists worry that those costs could easily rise beyond the reach of a small academic lab. In response, Hovatta says that her methods require fewer personnel and less time to culture the cells. According to her calculations, a lab using her protocols could grow 300 times more stem cells at the same cost and time investment as they would with feeders, making it cost-effective.

Another group found a way to avoid the time-consuming step of coating the culture dishes. Sara Pijuan-Galitó, then working in the laboratory of Cecilia Annerén at Uppsala University in Sweden, discovered that the ECM protein inter-α-inhibitor (IαI), found in human serum, activated the same pathway LIF does in mouse ES cells (*J Biol Chem*, 289:33492-502, 2014). Pijuan-Galitó, now a postdoctoral fellow at the University of Nottingham in the U.K., simply added IαI to Essential 8 media, where she suspects it engages integrins involved in cell adhesion, and plated her cells (*Nat Commun*, 7:12170, 2016). "It makes culturing stem cells so simple," she says. It's important to avoid any bovine serum albumin (BSA) in the cultures, adds Annerén, as that seems to hinder the process.

Pijuan-Galitó has succeeded in culturing 16 different mouse and human stem cell lines with IαI. Unfortunately, this ingredient is not yet commercially available. Pijuan-Galitó isolated it from the by-products of a company's process for purifying Factor VIII, a treatment for hemophilia, from blood.

SUSPENDED ANIMATION

Stem cells typically prefer a place to lie down, but growing them as monolayers means that scientists wishing to scale up their culture systems, for production of recombinant proteins or therapeutic uses, are limited to thin sheets of cells in dishes that take up a high volume of incubator space. Therefore, some researchers are working on ways to lift the stem cells off the petri dish bottom and grow them in three-dimensional suspension culture. The trick is to still give them something to attach to—either each other, or a suspended surface such as beads.

Rancourt typically uses spinner flasks for this purpose, which he says is an "entry-level" setup. A suspended stir bar swirls the media so the cells stay floating. For example, NDS Technologies offers a few options ($176–$282 for a 100-mL flask, up to $1,208–$1,471 for 36 L).

"What we found is that mouse embryonic stem cells actually prefer the suspension-culture environment," says Rancourt, so long as he adds LIF to promote pluripotency (*Tissue Eng*, 12:3233-45, 2006). For human stem cells in suspension, he adds basic FGF, ROCK inhibitor, and rapamycin to suppress differentiation into fibroblasts (*Methods Mol Biol*, 1502:53-61, 2016; *Tissue Eng Part C Methods*, 16:573-82, 2010).

Todd McDevitt, a senior investigator at the Gladstone Institutes in San Francisco, uses a rocker to keep the cells in his culture dishes suspended. For industrial-scale, liter-order production, scientists typically use bags. Because the cells can occupy all of the media, instead of just growing on the bottom, the density of cells per mL of media is much higher, and McDevitt estimates he can save about 90 percent of his media costs compared to 2-D culture. A slight downside, he notes, is that the cells grow in little nondescript balls, making it somewhat harder to discern their health from their morphology under the microscope.

It's important to keep the cell aggregates from sticking together or growing too large, McDevitt says. If that happens, the cells in the middle may differentiate, or starve and die. Scientists can break up the aggregates with enzymes or calcium chelators such as EDTA, which weaken cell-cell adhesions. Another option to keep cell clusters distinct is to encapsulate them in a gel, such as alginate (*Biotechnol Bioeng*, 110:667-82, 2013).

One great advantage of suspension cultures, Rancourt says, is that there's enough media to nourish the cells for days at a time, unlike with adherent cultures that require daily care. "You set it, you forget it," he says. ■

Baby on Board

Many scientific conferences offer child care options that allow researchers to bring their families along for the trip.

BY KERRY GRENS

Back in February, biomechanics researcher Eva-Maria Collins brought her husband, three-year-old son, and infant daughter to the Biophysical Society's annual meeting in New Orleans. Collins, whose lab is based at the University of California, San Diego, was being honored for the 2016 paper of the year in *Biophysical Journal*—a study describing how *Hydra* open their mouths (apparently, it involves ripping through epithelial tissue).

The night before, no one in the Collins family had slept much. "The kids had a very rough night," she recalls. Given the subsequent crankiness, Collins and her husband decided to divide and conquer; he would take their son and she would handle the baby. Later that day, Collins took the stage, daughter strapped to her torso in a baby carrier, and delivered her presentation. "Besides me bouncing, I think you cannot tell there's a baby," she says. "She just sleeps."

But while Collins may pull it off with aplomb, bringing a baby to meetings isn't always a solution for parents of young children. Fortunately, conference organizers recognize that child care is important. "There are a lot of conferences that now acknowledge that people have families and lives outside work," says Kristi Casey Sanders, the director of professional development at Meeting Professionals International, a trade association. "It's actually a selling point [to attendees] if they can bring their families with them."

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On site, out of mind

Last November, tens of thousands of researchers filed into the San Diego Convention Center for the annual Society for Neuroscience (SfN) conference. Among them was Amir Eftekhar, who studies spinal cord rehabilitation at the National Center for Adaptive Neurotechnologies in Albany, New York, and who had brought along his one-year-old son. Eftekhar says he and his wife, also a neuroscientist, decided to bring the baby because, having moved to the States recently from the U.K., they didn't have grandparents or other relatives near home to watch him while they traveled. On some days they took turns, one spouse parenting while the other attended the conference, but when both had a packed schedule they took advantage of on-site day care facilities.

"It was roughly $100 per day, which is pretty reasonable," he says. "We would take him again. . . . The day care was fantastic."

SfN has been hosting babysitting on site since 2009. Parents can drop off kids ranging from 6-month-old babies to 12-year-old tweens. The number of families taking advantage of the service has varied over the years, from a high of 50 in 2011 to a low of 33 at the most recent meeting. "For Neuroscience, 20 percent of our [day care] attendees are coming for the first time, which means child care could be instrumental in helping them attend," says Dana Kiffmann, the general manager of KiddieCorp, the company that provides day care for SfN and numerous

> **It's actually a selling point if attendees can bring their families.**
>
> — Kristi Casey Sanders
> Meeting Professionals International

other professional conferences around the country each year.

According to Emily Ortman, SfN's media and communications manager, the society subsidizes 45 percent of the babysitting costs for parents. This varies per conference, says Kiffmann, as conference organizers decide how much of the tab they are willing to pick up. The annual meeting of the Society for Molecular Biology and Evolution (SMBE) stands out among science conferences by paying full freight for attendees' on-site child care, according to SMBE president Laura Landweber of Columbia University.

SfN and other large conferences have also begun to offer accommodations for nursing mothers, dedicating private space for breastfeeding and refrigerators for storing milk. Recently, the American Association for Cancer Research (AACR), which for years has provided both on-site day care and privacy for nursing mothers at its large annual conference, has started to offer such services at the organization's smaller meetings (it hosts about 50 a year). "That's something I've seen change in the last two to three years," says Pamela Ballinger, the senior director of meetings and exhibits for AACR. "At big meetings, it's commonplace."

Off-site resources

For smaller conferences, dedicating a facility to babysitting is not typically feasible—costs may be prohibitively high, or there isn't enough demand. But conference organizers aren't leaving parents out in the cold. At a minimum, they might direct attendees to the hotel concierge to find care providers, or compile a list themselves.

While children are not a frequent sight at Keystone Symposia—specialized meetings that take place at vacation hot spots—there's an uptick in families around spring break, says Heidi Daetwyler, Keystone's director of meeting management. Sometimes the host resort has babysitting available, but other times parents need to find care providers themselves. To make it easier, in 2015 Keystone developed an online bulletin board for researchers looking for day care, modeled after a roommate bulletin board attendees use to find people to share a hotel room. Parents can ask for and offer advice on finding babysitters, or arrange to watch kids for one another or to share the expense.

Cost can be a significant deterrent for parents to bring kids—or to travel at all if they can't leave kids behind.

Cost can be a significant deterrent for parents to bring kids—or to travel at all if they can't leave kids behind. In 2015, Cell

Conference	Approximate attendance	On-site day care?	Other resources
American Association for Cancer Research	22,000	Yes, ~$12/hour	
American Society of Human Genetics	8,000	Yes, $90/day	
Cell Symposia	250–400	No	Two to four $500 Elsevier Family Support Awards are available.
Ecological Society of America	4,500	Yes, $94.50/day	Child care providers are given a meeting badge; conference provides transportation to external science camps for older kids (when available).
EMBO/EMBL symposia	400	Yes, €100 (US$114) for the duration of the conference	Members of the EMBO Young Investigator Programme can receive up to €500 (US$560) for child care expenses when traveling to any conference, not just those sponsored by EMBO.
Experimental Biology	14,000	No	Website offers a list of external day care providers.
Gordon Research Conferences	150	No	Discounted lodging and meals for kids (free for children under 4 years old); information about camps and babysitting; message board coming in fall 2017
Keystone	150–800	No	A message board for parents facilitates finding day care or sharing child care.
Society for Neuroscience	30,000	Yes, $100/day	

Symposia began offering monetary awards to parents, two to four awards of $500 each per meeting—originally the brainstorm of Anne Granger, a scientific editor at *Cell Metabolism* and the mother of two small children. "Because travel awards do not typically cover child care–related expenses, we saw a great opportunity to better support young scientists and created the Elsevier Family Support Awards to help offset the costs of child care for early-career researchers (students, postdocs, and young investigators within their first five years) attending any of our Cell Symposia," Granger wrote to *The Scientist* in an email. "A highlight of this award is that both women and men can apply."

SMBE also offers travel awards to cover child care, handing out $1,000 each to about 50 attendees each year. Parents can choose how to spend it, such as on airfare for their kids or for covering day care costs back home.

Baby on board

For parents who, for one reason or another, cannot leave their children in the care of others, conferences have different policies for bringing children into presentations or poster sessions. SfN, for instance, has a rather lenient policy, allowing kids in sessions and in the meeting hall, as long as they are with a guardian. "SfN also supports women breastfeeding in the session rooms so they can continue to experience the full meeting and care for their infants," Ortman wrote in an email to *The Scientist*. Keystone meetings have a similar policy—as long as kids aren't being disruptive, they are welcome to join their parents.

Ballinger says AACR has strict rules for the exhibit floor: no children under 12 are allowed, primarily for safety reasons but also so presenters are not interrupted. But, she notes, the meeting has fully comprehensive, on-site day care options—and it's discounted to about $12 an hour, a bit more for little ones under six months.

Other meetings do not have codified rules about bringing children into sessions, which may imply flexibility. Collins says that in her experience organizers have always been welcoming. In fact, when Collins presented on her top 2016 paper, the editor-in-chief of the publication—*Biophysical Journal*—tweeted a picture of her on stage with her daughter. The post earned hundreds of likes and numerous positive comments.

Collins's advice to others needing to bring children along: just ask. "The one thing I realized is that before I started asking people whether it was OK, I would worry way too much about what people would think," she says. "In most cases, the organizers have been really trying to make it possible to bring the infant." ■

CRISPRing Mammoths

Can the latest gene-editing tools help researchers bring extinct species back to life?

BY BRITT WRAY

There may come a day when woolly mammoth–like proxies with imposing curled tusks and that iconic, shaggy mane will traipse again through their ancestral stomping grounds in the Siberian tundra. The woolly mammoth went extinct after the last holdouts on Wrangle Island, off the northern coast of far eastern Siberia, died off between 3,600 and 4,000 years ago. For now, however, the promise of this futuristic vision lives in labs at Harvard Medical School—and the cells in petri dishes are a long way off from assembling into a complete animal. Researchers are nowhere close to recreating fully formed mammoths, and, thus far, scientific efforts to resurrect the extinct beasts have been rather incremental. But that hasn't kept Harvard Medical School researcher George Church from predicting that he and his colleagues, who collaborate on a de-extinction project known as the Woolly Mammoth Revival, will create a hybrid woolly mammoth-Asian elephant embryo as early as 2019. And CRISPR-Cas9, a gene-editing technology that Church's lab played a role in developing, may be the key to speeding the eventual return of the ancient animal.

I explore this and other tales of de-extinction in my book, *Rise of the Necrofauna*.

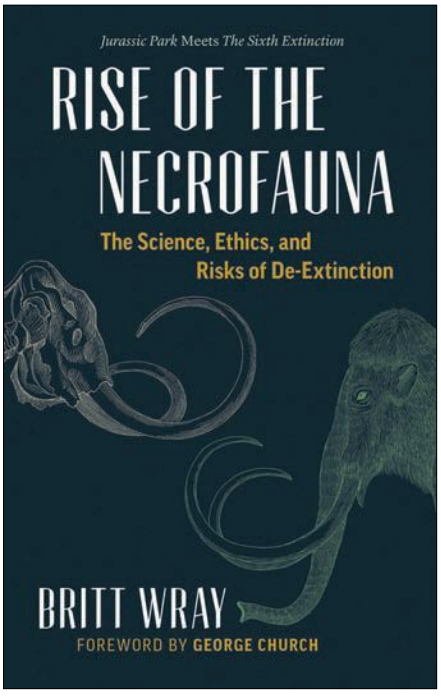
Bobby Dhadwar, a postdoctoral researcher in Church's lab, has been laying some of the groundwork for creating the engineered embryos. Since the project's early days, he has been involved in editing "background cell types" in order to test the effects certain woolly mammoth–specific genetic changes have on available cells that most resemble those of a mammoth: Asian elephant cells.

To start, Dhadwar and his colleagues identified traits that people normally attribute to the woolly mammoth, but that are missing in Asian elephants. These include an abundance of reddish-brown hair and a form of oxygen-binding hemoglobin that functions well at low temperatures. In their early experiments, the researchers went hunting in the genomes of dogs, cats, mice, and even humans with a genetic syndrome that causes unchecked hair growth all over the body in order to identify sequences that might imbue an elephant with a woolly mammoth–like pelage. But now the scientists rely on a customized bioinformatics pipeline that compares genes recovered from the ancient DNA of mammoth remains found all over the world.

Because Church's lab had a hand in developing CRISPR-Cas9, it was a natural choice for Dhadwar to use the gene-editing tool to introduce woolly mammoth–specific genetic changes into Asian elephant cells. The plan is to turn Asian elephant cells into induced pluripotent stem cells and then differentiate them into specific tissue types that will display various mammoth phenotypes of interest. This step is important because the researchers need to test their gene-editing protocol.

Dhadwar has been introducing woolly mammoth single nucleotide polymorphisms (SNPs) into immortalized Asian elephant cells. By making one genetic change per immortalized cell line, he is able to test the efficacy of each edit he makes. Eventually, Dhadwar and his colleagues will combine all of the various edits they have made into one master cell using CRISPR-Cas9. If they manage to do all of this inside of an Asian elephant embryo, then they'll be well on their way to making woolly mammoth de-extinction a success, or at least, to creating a closely related hybrid. "If we can move over just a few genes, we might not get a woolly mammoth, but at least we would get a cold-tolerant elephant," Dhadwar says.

But why does anyone want to recreate an extinct mammoth or a cold-tolerant elephant in the first place? The answer to that is part of a much wilder and woollier story. ■



Greystone Books, October 2017

Britt Wray is cohost of the BBC podcast Tomorrow's World. *Read an excerpt of* Rise of the Necrofauna*: The Science, Ethics, and Risks of De-Extinction at the-scientist.com.*

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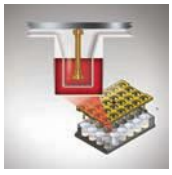

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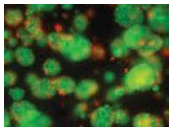

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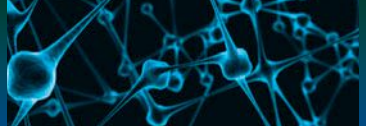
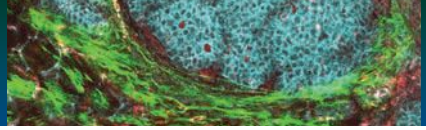
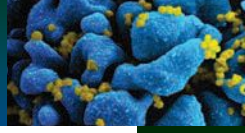

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Vectors, Pathogens & Diseases: Current Trends & Emerging Challenges (T1)
Maternal-Fetal Crosstalk: Harmony vs. Conflict (T2)
Regenerative Biology & Applications: Cell Differentiation, Tissue Organization & Biomedical Engineering (T3)
Antimicrobials & Resistance: Opportunities & Challenges (T4)
Frontiers of Serotonin Beyond the Brain (T5)
Heart Failure: Crossing the Translational Divide (A1)
State of the Brain: Genetic Dissection of Brain Circuits & Behavior in Health & Disease (A2)
T Cell Dysfunction, Cancer & Infection (A3)
Plant Signaling: Molecular Pathways & Network Integration (A4)
Natural Products & Synthetic Biology: Parts & Pathways (J1)
Tumor Metabolism (A5)
Cell Death, Inflammation & Adaptation to Tissue Stress (A6)
Organ Crosstalk in Obesity & NAFLD (J3) *joint with* **Bioenergetics & Metabolic Disease (J4)**
DNA & RNA Methylation (A7) *joint with* **Ubiquitin Signaling (A8)**
Translational Systems Immunology (A9)
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Emerging Technologies in Vaccine Discovery & Development (J5) *joint with* **Progress & Pathways Toward an Effective HIV Vaccine (J6)**
Atherosclerosis: Lessons Learned & Concepts Challenged (B2)
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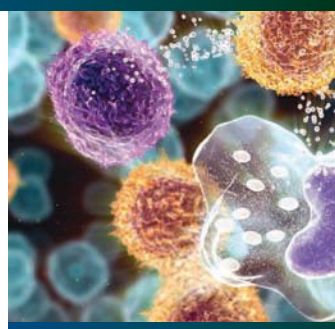
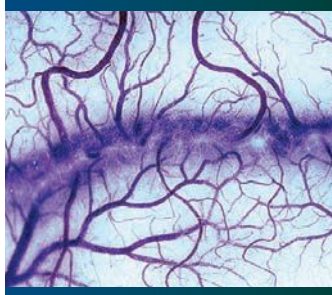
Cancer Epigenetics: New Mechanisms, New Therapies (B4)
Phosphoinositide Biology: New Therapeutic Targets Beyond Class I PI3K (B5)
Emerging Cellular Therapies: T Cells & Beyond (B6) *joint with* **Lymphocytes & their Roles in Cancer (R1)**
Mobile Genetic Elements & Genome Plasticity (B7)
GPCR Structure & Function: Taking GPCR Drug Development & Discovery to the Next Level (B8)
Regulation & Dysregulation of Innate Immunity in Disease (B9)
Antibodies as Drugs: Translating Molecules into Treatments (C1)
Noncoding RNAs: Form, Function, Physiology (C2)
Endoderm Development & Disease: Cross-Organ Comparison & Interplay (C3)
Uncomplicating Diabetes: Reducing the Burden of Diabetes-Related End-Organ Injury (J7) *joint with* **Vascular Biology & Human Diseases: From Molecular Pathways to Novel Therapeutics (J8)**
Immunological Memory: Innate, Adaptive & Beyond (X1) *joint with* **Aging, Inflammation & Immunity (X2)**
Manipulation of the Gut Microbiota for Metabolic Health (X3) *joint with* **Microbiome, Host Resistance & Diseases (X4)**
Cells vs. Pathogens: Intrinsic Defenses & Counterdefenses (C4)
Cancer Immunotherapy: Combinations (C5)
Chromatin Architecture & Chromosome Organization (X5) *joint with* **Gene Control in Development & Disease (X6)**
The Resolution of Inflammation in Health & Disease (C6)
iPSCs: A Decade of Progress & Beyond (C7)
Organs- & Tissues-on-Chips (D1)
Myeloid Cells (D2)
Therapeutic Targeting of Hypoxia-Sensitive Pathways (V1)
Pushing the Limits of Healthspan & Longevity (D3)
Tuberculosis: Translating Scientific Findings for Clinical & Public Health Impact (X7) *joint with* **HIV & Co-Infections: Pathogenesis, Inflammation & Persistence (X8)**
Mitochondrial Biology (Z1) *joint with* **Selective Autophagy (Z2)**
Precision Medicine in Cancer (E1)
Exosomes/Microvesicles: Heterogeneity, Biogenesis, Function & Therapeutic Developments (E2)
One Million Genomes: From Discovery to Health (G1)
Novel Aspects of Bone Biology (E3)
B Cells: Mechanisms in Immunity & Autoimmunity (E4)
Advances in Neurodegenerative Disease Research & Therapy (Z3) *joint with* **New Frontiers in Neuroinflammation: What Happens When CNS & Periphery Meet? (Z4)**

7 NEW TOPICS



10 NOVEL JOINT PAIRINGS



25 VENUES GLOBALLY

Conferences listed in chronological order. View details for each at **keystonesymposia.org** followed by a **/18** & the alpha-numeric **program code** (e.g., **www.keystonesymposia/18A1**). Registered attendees of one meeting in a joint pair may attend sessions of the other at no additional cost, pending space availability, and can take advantage of the joint poster sessions and social breaks.

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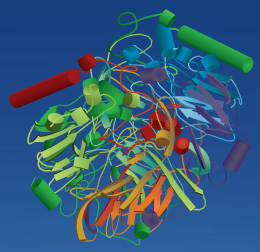

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Discovery of the Malaria Parasite, 1880

BY SHAWNA WILLIAMS

The idea that bad air rising from swamps caused malaria had a good run: at least two and a half millennia, from the time of the ancient Greeks until the mid-19th century. But as Louis Pasteur and Robert Koch popularized the germ theory of infection in the late 1870s, scientists began searching for a bacterial species responsible for the disease. Two scientists even reported having found the culprit, dubbed *Bacillus malariae*, in the Pontine Marshes near Rome.

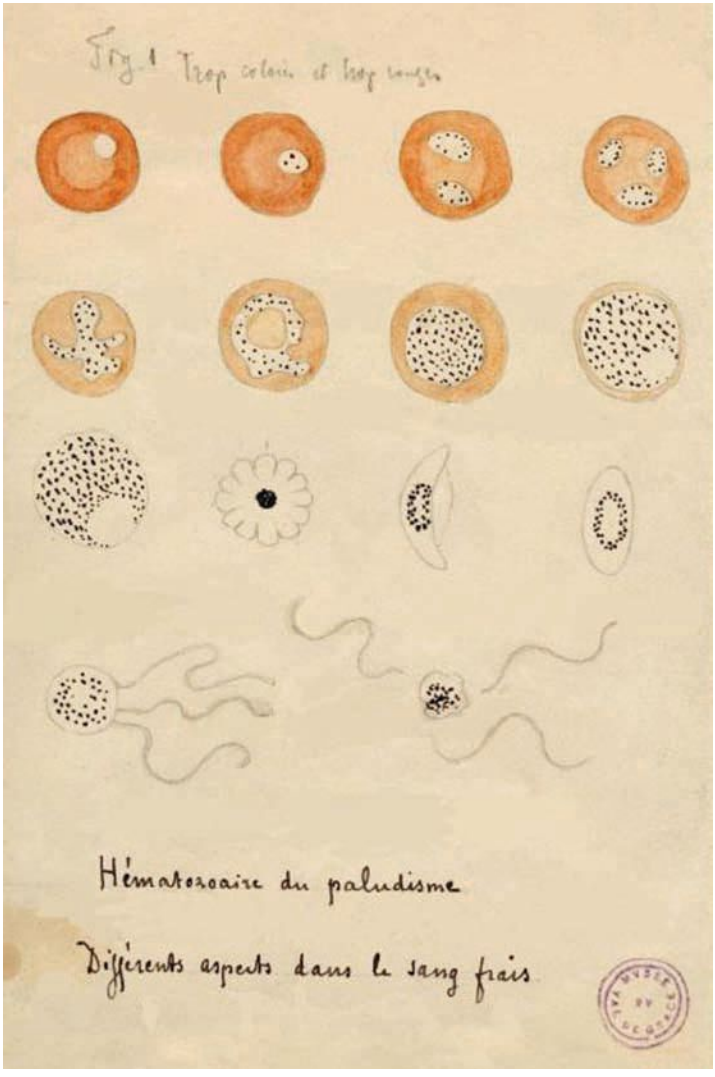
But in a military hospital in Algeria, French doctor Charles Louis Alphonse Laveran was taking a close look at a distinctive, granular pigment found in the spleens and other tissues of malaria victims and in the blood of infected people. In November 1880, he trained a light microscope with a maximum magnification of 400x on a drop of fresh blood from a malaria patient. Inside the red blood cells, he saw round, pigment-filled moving bodies with flagella-like protrusions.

Because bacterial flagella can't be seen with a light microscope, "that really convinced him that he was looking at an animal instead of a bacterium or a fungus," says Irwin Sherman, a professor emeritus at the University of California, Riverside. As Laveran watched, one of the moving bodies shed its wavy tails.

Given the limited power of his microscope, "it's remarkable that the drawings that you see in Laveran's paper are so essentially detailed," says Sherman. "It's testimony to his expertise as a microscopist." Differential stains had not yet been invented, but Sherman says the "highly refractive" malaria pigment inside the protozoa would have been key to Laveran's ability to detect them.

Laveran went on to examine the blood of hundreds of patients with and without malaria, and found the tiny organisms only in the malaria samples. But he faced an uphill battle in convincing other scientists that what he was seeing were not just decaying red blood cells, but protozoa, and that the single-celled organisms caused the disease. Ultimately, Laveran succeeded in persuading even Pasteur and Koch that his discovery was real, and in 1907 he was awarded a Nobel Prize for it.

Not all of Laveran's views on malaria would catch on, however. He continued to insist, for example, that the disease was caused by only one protozoan species, long after evidence emerged that there were two. (It would ultimately turn out that there are four species of malaria protozoa.) He also disliked the term "malaria," thought to be derived from the Italian term for "bad air," because he considered it unscientific and superstitious, and instead referred to the disease by its alternative French name, "paludisme." ■



INTO THE LIGHT: Laveran's illustration of the various stages of in the life cycle of malaria parasites and their telltale pigment (black dots), published in the bulletin of the Société Médicale des Hôpitaux de Paris in 1881. As Francis Cox of the London School of Hygiene and Tropical Medicine writes in a 2010 review article in *Parasites and Vectors,* Laveran "suggested a course of events that began with clear spots that grew, acquired pigment and filled the corpuscle which then burst coinciding with the fevers associated with malaria." At bottom is a male gametocyte expelling flagella-like microgametes, described by Laveran as "filiform elements which move with great vivacity."

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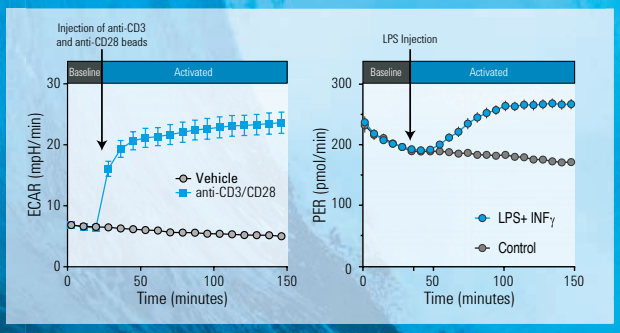

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