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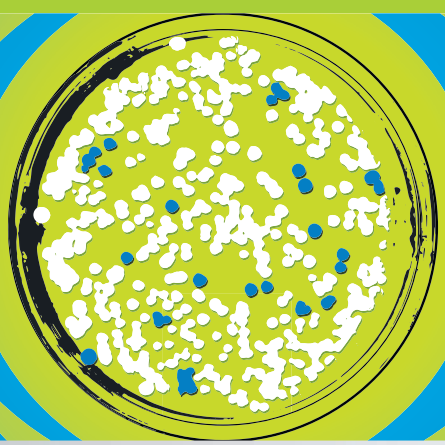

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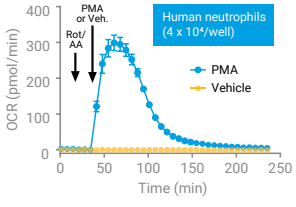

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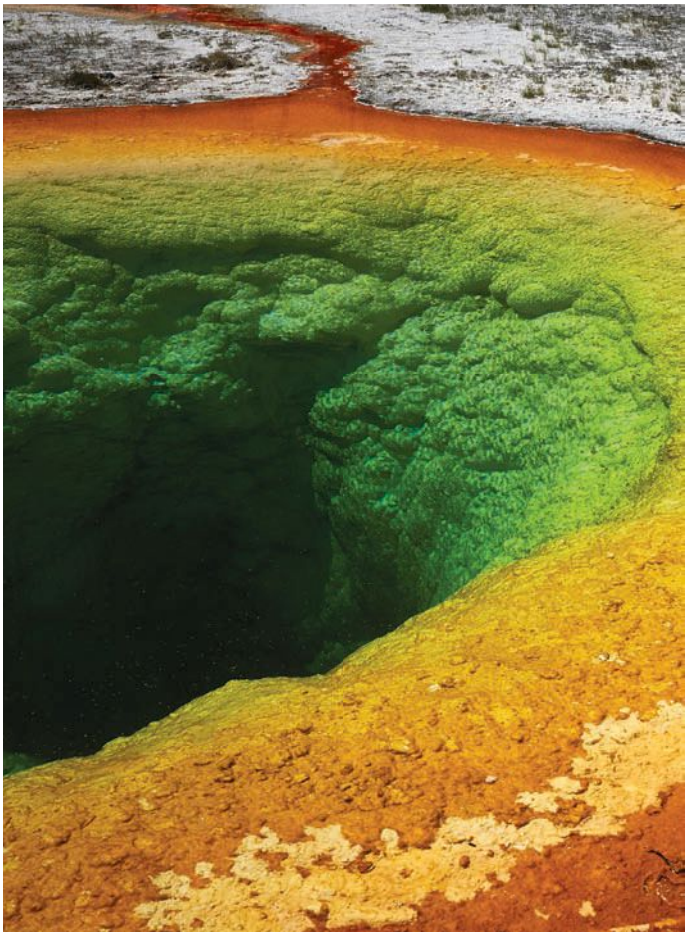

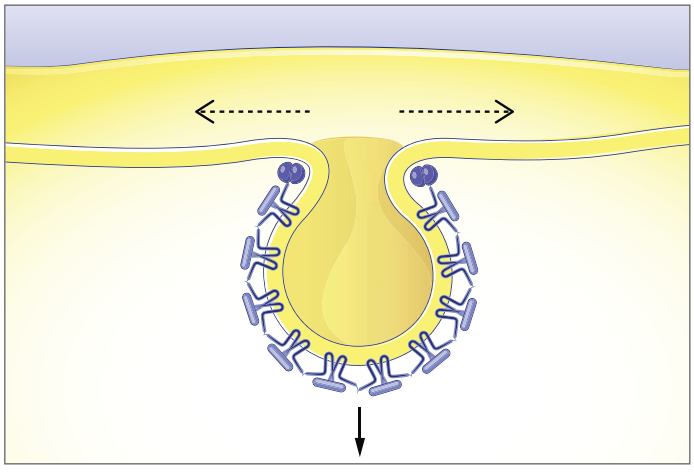

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TheScientist TOP 10 INNOVATIONS

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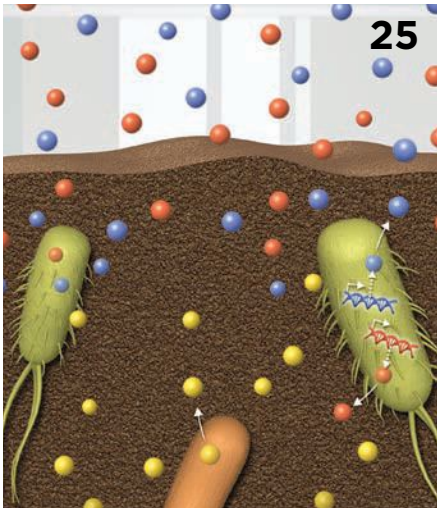

VERÔNICA FIALHO; © GEORGE RETSECK; © HANS HOCHSTÖGER

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CORRECTIONS:

The May article "The TelePostdoc" incorrectly referred to a postdoc survey carried out by *Nature*. In fact, the survey was part of a report authored by attorney Jessica Lee and colleagues at the Center for WorkLife Law at the University of California, Hastings College of the Law in San Francisco. *The Scientist* regrets the error.

The May feature "Answers in the Exome" incorrectly stated that Anne O'Donnell-Luria was a cofounder of ExAC, a database that contains data from individuals over 18 years old. In fact, O'Donnell-Luria is only a part of the current ExAC team, and this database (along with its successor gnomAD) contains mostly, but not exclusively, data from over-18-year-olds. *The Scientist* regrets the error.

PUZZLE ON PAGE 12

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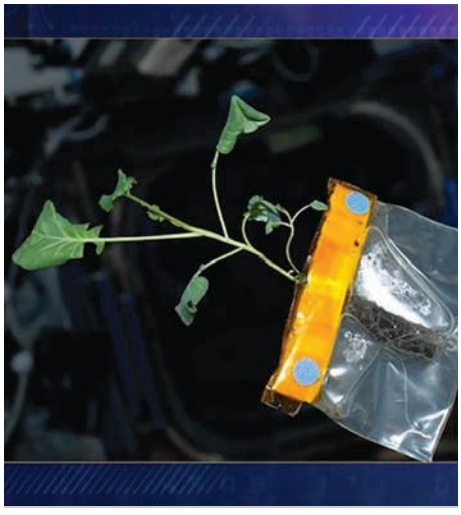

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PTSD Circuits
Watch profilee Israel Liberzon of the University of Michigan discuss his work trying to piece together the neurological routes of post-traumatic stress disorder.

VIDEO
Meet the Leechmeister
See the American Museum of Natural History curator Mark Sidall explain his fascination with leeches, which he and other scientists are using to infer biodiversity in some far-flung places.

VIDEO
Far Out Gardening
Blast off into orbit, where researchers on the International Space Station are growing plants in systems that may one day sustain astronauts travelling far across the solar system and beyond.

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- The science behind predicting changing climate vulnerability
- Paleogenetics extracts DNA from fossils
- The evolution of human language
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Contributors

While he was an assistant professor of microbiology at the University of Paris back in 1981, Patrick Forterre read a now-famous *Scientific American* article by Carl Woese. In it, Woese laid out his theory that life on Earth exists in three domains—Archaea, Bacteria, and Eukarya—instead of two, as had previously been the accepted wisdom. "I was one of the first [scientists] in France to jump on the idea," Forterre says. He began studying Archaea as head of a research group at the Institute of Genetics and Microbiology in Orsay in 1989. He says he was surprised that the idea of a third domain of life didn't catch on more quickly. "Things are quite different now, of course," he says. "In France there are [about] 100 people working on Archaea now." He also became interested in the origins of viruses, which he says deserve to be classified as living organisms. "Many people have a very reductionist view of viruses," he says. "Because of that, they don't realize that new genes can originate in viruses exactly like they originated in cellular organisms." In 2004, Forterre was named the head of the Department of Microbiology at the Pasteur Institute in Paris. Read his article "Older Sisters" on page 23.

In the early 1990s, Ben Nichols was working on his PhD at the University of Bristol in the U.K. and studying how calcium signaling affects a class of enzymes called mitochondrial dehydrogenases. He shared the lab with George Banting, a biochemist at Bristol who studies membrane trafficking pathways in mammalian cells. "It was largely from discussing and socializing with people from that lab that I started to think that cell membranes are really something interesting," says Nichols. "The properties of cell membranes as a material were not really very well understood [at the time]." He says he began envisioning cell membranes as a composite material that can't easily be artificially recapitulated. "To understand what's going on, one would need to look in cells," he says. "That's what appealed to me." At Banting's suggestion, Nichols went on to do his postdoctoral work on membrane fusion dynamics at the Medical Research Council Laboratory of Molecular Biology at Cambridge in 1996. He's still there, although he moved on to studying endocytosis—a process by which cells transport material through the membrane—and later, the role caveolae play in that process, which he discusses in his article "The Mystery of Caveolae" on page 42. Nichols says he enjoys writing about science: "It's a pleasure trying to construct an argument piece by piece so it makes some kind of coherent sense to the reader."

While he was an undergraduate student at the University of Chicago, Rob DeSalle's interests were "all over the place," he says. But when he started working at the Field Museum of Natural History, he fell in love with evolutionary biology. That was in 1976, about 20 years after the double helix structure of DNA was discovered, and a mentor advised the young DeSalle to study genetics. "That was very good advice," he says. After obtaining his undergraduate degree, he studied the genetics of fruit flies at Washington University in St. Louis. At the time, researchers were just starting to use genomics to understand the relationships among species. In the 1980s, DeSalle carried on his studies as a postdoc at the University of California, Berkeley, where he worked with biochemist Allan Wilson, who was among the first researchers to demonstrate the concept of the molecular clock. "He is widely recognized as the father of modern molecular evolutionary biology," says DeSalle. "I was honored to be able to work in his lab." In 1990, while he was an assistant professor of biology at Yale, the American Museum of Natural History asked him to serve on a search committee for a curator position. But when members of the committee saw his resume, they asked him to interview for the job himself, and they eventually hired him. "I'm a pretty lucky guy," he says. He meets with fellow curator Ian Tattersall, with whom he coauthored the article "Glorious Varieties" on page 61, about once a week to talk. "Ian is a spectacular writer," says DeSalle. "His books really give me a template for how to write scientifically."

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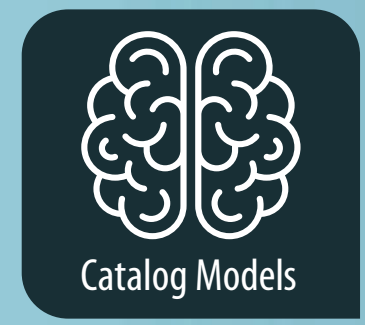

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From Little Things Big Things Grow

We should take comfort in the fact that life on Earth had such unassuming, shared beginnings.

BY BOB GRANT

ANDRZEJ KRAUZE

There was a sign hanging on the door of a microbial ecology lab at the South Carolina Department of Natural Resources, where I did my marine biology master's thesis work: "Respect bacteria—they're the only culture some people have."

This homage to our single-celled partners on this planet stuck with me over the years, and my appreciation of microbes has only grown as I've learned more and more about them—most recently, the central role archaea have played in the evolution of life on Earth.

The current thought is that millions of years ago, archaeal ancestors of eukaryotes and ancient bacterial species engaged in fortuitous (as least from the human perspective) acts of cellular hanky-panky. Engulfed by more-complex, nucleated cells, bacteria entered into a symbiotic relationship with their archaeal hosts, and the partnership blossomed. Eventually, the symbionts evolved into a new type of cell, with those encapsulated bacteria morphing into mitochondria and chloroplasts, essential engines of the eukaryotic newcomers.

And how lucky we eukaryotes are to count archaea as distant evolutionary relations. Without them, eukaryotic cells might never have come about, and the most spectacular life forms—forests full of trees, shoals of giant fish, flocks of bright birds—might be missing from our planet.

As researchers prospect ever further for archaeal species, the ancient prokaryotes, which were initially misidentified as bacteria, are turning up virtually everywhere: in the alkaline lakes and hot springs where intrepid naturalists originally found them, but also in forest soils, marshlands, ocean waters, and even the human body. Scientists recently detected members of this ancient microbial group inhabiting human lungs, colons, mouths, and skin. So I guess some people have at least two cultures.

As befits a fascinating corner of biology, research on archaea continues to surprise. Not only are the organisms turning up in unexpected places, but from every new pocket emerge new species, genera, even orders, classes, and phyla. As Amber Dance writes in "The Ancient Ones," on page 26, "The archaeal family tree has exploded in recent years, and the discoveries show no signs of slowing. . . . With these discoveries come insights into their unique and diverse biology, as well as their roles in the environment." Add to those important implications the insight archaea give researchers trying to reconstruct the evolutionary relationships among the three domains of life.

This is an unsettled issue, even though there is general agreement that the intricate endosymbiosis that brought about eukaryotes happened. Some researchers, such as those Dance interviewed for her story, conclude that sequence data point to a freshly discovered, and more recently evolved, archaeal group, the Asgard superphylum, as the branch from which eukaryotes grew. But others, including Institut Pasteur scientists Patrick Forterre, Violette Da Cunha, and Morgan Gaia, contend that eukaryotes and archaea share a more recent ancestor than that shared by eukaryotes and bacteria. As they write in "Older Sisters," on page 23, "Archaea would be our sisters and bacteria our cousins."

While this debate simmers and archaeal explorations bear fruit, I find myself, yet again, amazed not only by the complexity and history of life on Earth, but by the ability of one particular eukaryotic species to piece it all together. And to think, we humans could all conceivably trace our lineages back, through eons of evolution, to a couple of cells cooperating to make a go of it in a changing world. Those are some shared cultures I think all *Homo sapiens* can celebrate. ■

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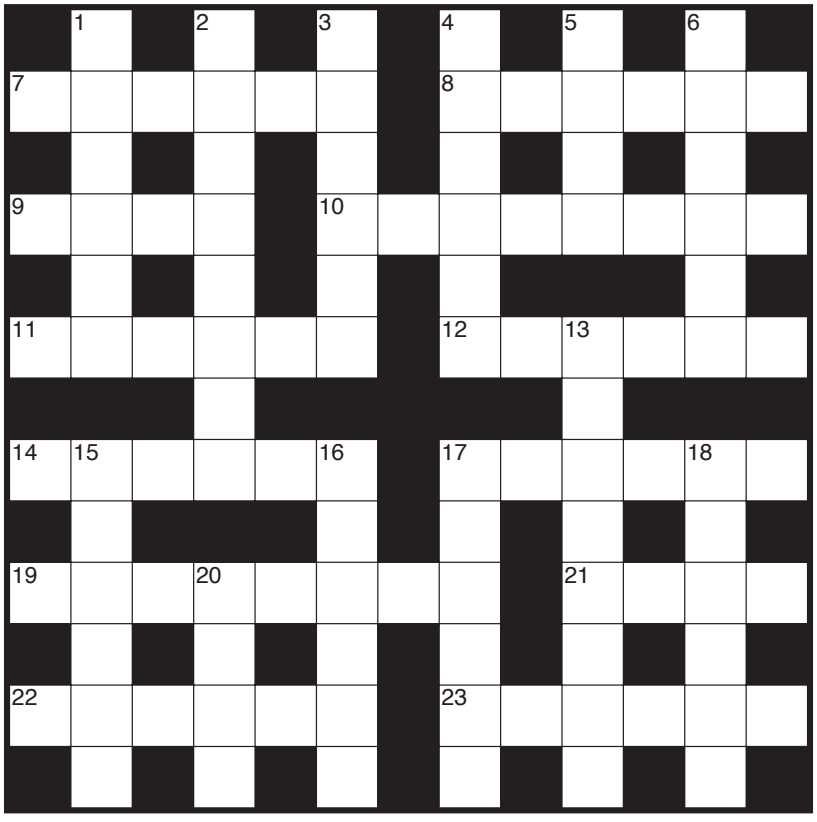
Speaking of Science



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

BY EMILY COX AND HENRY RATHVON

ACROSS

7. Lacking the normal pigmentation
8. SETI targets
9. Flying class?
10. Objects of study for Pasteur
11. Biological ranks just above families
12. Electricity pioneer Tesla
14. 12-Across, with respect to a coil or car
17. That life evolves, to Darwin
19. Legume often called a bean
21. Great white features
22. Ectotherm with a retractile neck
23. Phenomenon caused by charged solar particles

DOWN

1. Trifoliate nectar source for bees
2. "The faster you go, the shorter you are" theorist
3 PBS science series; worldly bloomer?
4. High-speed raptor
5. Farewell to Fermi or Volta
6. Fictional, like unicorns or jackalopes
13. Patellar reflex, in common parlance (2 wds.)
15. Film about teeth?
16. "The Father of Genetics"
17. Cavity housing the heart and lungs
18. Like a centripetal force
20. Chiroptera members

Answer key on page 5

To remain the world leader in advancing scientific knowledge and innovation while ensuring national security, the US science and technology enterprise must continue to capitalize on the international and multicultural environment within which it operates.

—**Rush Holt**, CEO of the American Association for the Advancement of Science, in a statement released in response to revelations that President Donald Trump's administration is considering barring Chinese citizens from participating in what *The New York Times* termed "sensitive research" at American institutions (May 1)

Surely the agreement between Mr. Moon and Mr. Kim creates an opportunity to restart talks over cooperative research.

—South Korean researcher **Ryo In-Chang**, a geologist at Kyungpook National University in Daegu, voicing hopes that the newly thawed relations between North and South Korea might encourage scientific collaboration across the border (*Science*, May 1)

GREG BREWER

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Notebook

JUNE 2018

VERÔNICA FIALHO

NOM NOM: Carrion-feeding beetles in the genus *Deltochilum* tear off pieces of meat to roll back to their nests for later.

Clean Eating

Stacks of dead pigs line a freezer at the Department of Entomology at the Federal University of Viçosa, Brazil. In the past six years, lab technician Verônica Saraiva Fialho has been stockpiling the carcasses, which she procured from the university's pig breeding farm. Around sunrise, she carts several of the bodies out to a nearby forest. Fialho, affectionately called "the Girl of Dead Pigs" by some of her friends, then leaves the pigs in the forest so she can study the beetles that eat and live on rotting flesh.

This gory work is a dream come true for Fialho. As a young girl, she used to walk in the forests near Teixeiras in Brazil with her grandfather. Whenever they came across dead animals on the ground—monkeys, dogs, anteaters—Fialho would stop, lift the carcass, and look for insects. The many kinds of ants, flies, and beetles she found there captivated her. "A carcass has no good smell and looks disgusting, but on dead bodies you see a lot of life," says Fialho. "You can see a lot of behaviors in this little system. This is amazing, at least for me!"

Fialho was particularly impressed by the beetles, and she's been studying them for several years now. Carrion-feeding beetles mostly belong to the subfamily Scarabaeinae—a group known collectively as the true dung beetles, although not all of its members feed on feces. In those species that specialize in scavenging dead flesh, adults home in on a fresh carcass and bite off small chunks of meat to feed their young in nests, which the beetles build in a variety of ways. So-called "dwellers" build their homes between carcasses and the soil; "rollers" roll the meat away to shallow nest; "tunnelers" dig nests directly under carcasses.

The behavioral diversity in these beetles is something of a mystery, but one theory is that at least some nesting strategies might help the insects avoid infection. While decomposing carcasses potentially harbor lots of microorganisms that can kill insects, scientists almost never find a beetle nest infected, suggesting that elaborate nesting behaviors such as tunneling

might help mitigate the risks of feeding on and living near rotting flesh. Still, no researcher had explicitly tested the underlying assumption that insects on a carcass face high infection risks. Nobody, that is, until the Girl of Dead Pigs came along.

In 2016, as part of her PhD work at the Federal University of Viçosa, Fialho marshaled a rotating team of assistants—undergrads, colleagues, and, on occasion, her husband—and set out to examine the risk of infection for insects ingesting rotting meat. The team selected three sites in the forest, separated by at least 150 meters, and collected soil samples both on the soil surface and up to 70 cm underground. Then they placed a dead pig at each site and returned to collect soil in the same way 7 and 30 days later. A site without a pig carcass served as a control.

The work was equal parts hard labor and painstaking attention to detail. The team spent full days preparing materials: pots and tools to collect soil, labels to mark samples, and agar plates to cultivate bacteria and fungi. Field days meant heading out to the pigs at 6 AM, returning with all the bags of soil at 4 PM, and processing the samples until 2 AM the next day. Digging under each putrefying carcass required a strong back, and an even stronger stomach. "The soil was very wet and smelled really bad," says Fialho. She became used to the stench, she adds, but the rest of the team found it disgusting.

Back in the lab after each trip, Fialho isolated insect-pathogenic fungi and bacteria from the soil samples. She found that the densities of insect-killing pathogens in the soil around each carcass was highest on the seventh day of the experiment—when the carcass had decomposed into liquefied tissue and was packed with insects. Pathogen densities were consistently highest at the carcass, and decreased the deeper into the soil Fialho dug. However, horizontal distance from the carcass made little difference: pathogen densities were consistent in the topsoil over a radius of up to 120 cm.

Fialho also exposed larvae of the mealworm—a beetle that does not live in soil and is not expected to strongly resist soil pathogens—to the soil samples and monitored how long the insects survived. Larvae died fastest when exposed to soil collected on day seven from shallow depths, she found, lending support to the idea that decomposing carcasses present a high infection risk, particularly aboveground (*Ecol Evol*, doi:10.1002/ece3.3919, 2018).

The work required a strong back, and an even stronger stomach.

"[The study] is really nice. It shows that whilst moving horizontally from a carcass doesn't affect the pathogenic risk greatly, moving vertically down does affect the risk," says Sheena Cotter, an evolutionary ecologist at the University of Lincoln, U.K., who acted as a reviewer on Fialho's paper. "That could explain why you get some insects tunneling, which is really interesting." However, she adds that more work will be needed to make the jump from correlation to causation.

Ana Duarte, an evolutionary biologist at the University of Exeter's campus in Cornwall, U.K., says Fialho's study further unravels the complex influence that pathogens exert on beetles that exploit carcasses. Duarte, who has studied burying beetles, also emphasizes the need for experiments to show cause and effect. She suggests an experiment that changes pathogen levels at different soil depths to see if beetles dig deeper or shallower. But "I can totally understand that it's very difficult to measure," she says.

VERÔNICA FIALHO

BEYOND DUNG: Though classified taxonomically as true dung beetles, many members of the Scarabaeinae subfamily, including this male *Coprophanaeus (Megaphanaeus) bellicosus*, feed on rotting flesh.

Getting a handle on these beetles' behaviors is important for understanding larger-scale changes in the insects' habitat, notes Cotter, who has discussed future collaborations with Fialho. Beetles that feed on dung and carrion disperse nutrients and affect soil properties, Cotter says. "The picture is much bigger than why are dung beetles tunneling."

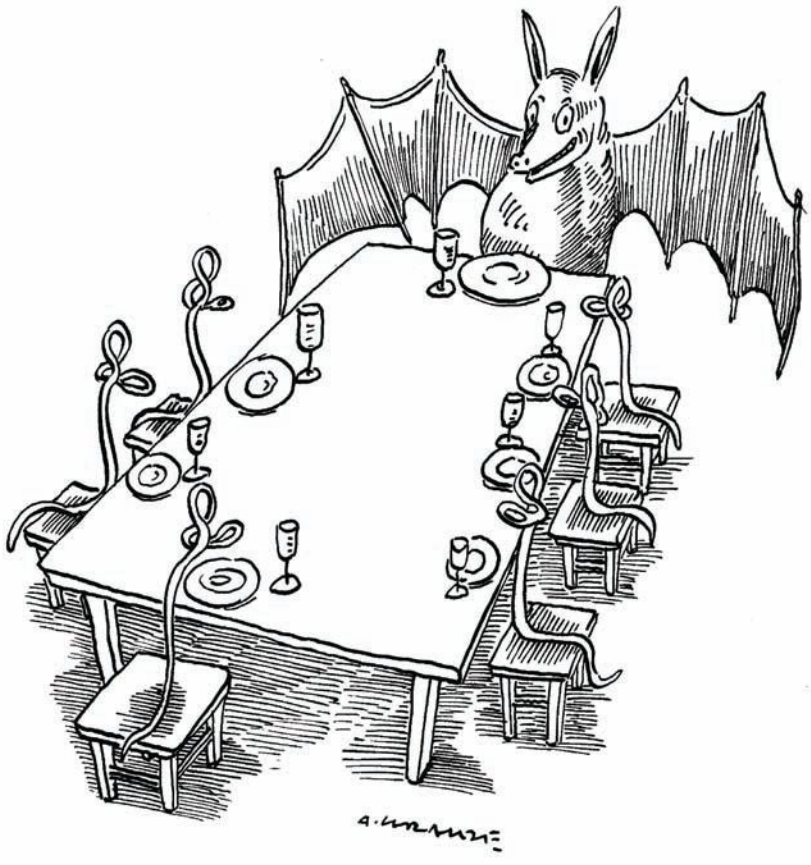
Now that she has completed her PhD program, Fialho has started experiments on other beetle species that visit her dead pigs, and is keen to spend more nights in the forest, when the insects are more active. "I have always wanted to work with these [carrion-feeding] beetles," she says. "And now that I can, I just want to stay happy, study these things, and stay fascinated."

—Yao-Hua Law

The Hosts with the Most

Bats carry and transmit some of the world's deadliest zoonotic viruses: Ebola, Marburg, Nipah, and the pathogen behind severe acute respiratory syndrome, SARS coronavirus, to name a few. What has puzzled researchers for a long time is why bats don't appear to get sick from their unusually high microbial loads. The question has been nagging Peng Zhou, a virologist at China's Wuhan Institute of Virology, for more than a decade, ever since he took part in a survey of bat populations in southern China. Zhou and his colleagues were looking for the strain of the SARS coronavirus responsible for the 2003 outbreak that sickened more than 8,000 people worldwide and killed nearly 800. "We started to think, why bats?" he says.

Other researchers have suggested that bats' super-tolerance might have something to do with their ability to generate large repertoires of naïve antibodies, or that flight ramps up the animals' body temperatures to a fever-like state that helps fight off infections. But in 2013, Zhou and his colleagues stumbled across another clue during a comparative genom-



ANDRZEJ KRAUZE

ics study of two distantly related bat species. The genes that showed some of the strongest evidence of positive selection, the team found, appeared to be related to DNA damage and innate immunity (*Science*, 339:456-60). "We thought we needed to go further and work on the molecular mechanics," says Zhengli Shi, Zhou's colleague at the Wuhan Institute and a coauthor on the study.

The team decided to focus on a protein known to lie at the center of several molecular pathways involved in the vertebrate innate immune response. STING, or STimulator of INterferon Genes, detects pieces of DNA where they shouldn't be: in the cellular cytoplasm. This free DNA can occur through infection by DNA viruses, and potentially also RNA viruses. Once activated, STING triggers rapid production of signaling proteins called interferons that help launch an immune response against infection.

Sequencing of the gene encoding STING in a handful of mammal species revealed a striking abnormality in the bat version: a mutation—responsible for a single replacement of a serine residue with another amino acid at an important phosphorylation site in the STING protein—was universal across 30 bat species. By contrast, the serine was retained in 10 other vertebrate species, including zebrafish, chickens, and nonflying mammals such as the house mouse and cow.

In a series of subsequent in vitro experiments, the researchers found that bat STING—isolated from the Chinese rufous horseshoe bat, *Rhinolophus sinicus*—produced a significantly milder interferon response compared to its mouse counterpart when treated with a known activator molecule in vitro. The team could encourage the production of higher levels of interferons if they corrected the mutation to restore the serine residue. Conversely, they observed a decrease in interferon production by human STING after they pulled the serine out (*Cell*, 23:297-301.e4, 2018).

The findings suggest that this mutation dampens bats' interferon-activating pathway just enough to stop the animals' immune systems from going into overdrive. Many viruses are so deadly to humans and other animals because they trigger an uncontrollable storm of interferons and other inflammation-inducing molecules, overwhelming the immune system, Zhou explains. But, by virtue of this mutation, bats can avoid such chaos, and instead can tolerate the same viruses. "That has a very significant meaning for bats," he says.

Despite STING's role in viral responses, Zhou believes that the mutation may be conserved in bats for a different reason, one related to another aspect of bats' livelihood. Fragments of the bats' own DNA can also be released into their cells' cytoplasm as a byproduct of the strenuous effort bats make during flight, Zhou says. This has led him to the hypothesis that the mutation originally provided an evolutionary advantage to bats by preventing their immune systems from boiling over every time the animals fly.

Emma Teeling, a bat biologist at University College Dublin who was not involved in the work, says she isn't surprised by the findings. Her own research has shown that bat macrophages will quickly mount a robust antiviral response when immunologically challenged, but compared to those of a mouse, they will then rapidly backpedal on their response by releasing anti-inflammatory cytokines (*Acta Chiropt*, 19:219-28, 2017). "What bats are very good at doing is resolving their constant inflammation," she says.

Teeling agrees with Zhou's interpretation that bats evolved anti-inflammatory responses such as this due to the metabolic demands of flight. Busy, stressed mitochondria produce a lot of free radicals that can damage DNA, which in turn can drive high levels of inflammation, she explains. "Having a much, much higher metabolic rate because of flight has driven the potential ability for bats to dampen and throttle their inflammatory response."

She adds that this may have given bats an edge not only over viruses, but perhaps also over death itself. "As you age, your level of inflammation increases," she says. "This should be even more heightened in bats because they have such a high metabolic rate." Evolving ways to dampen inflammation might have had a knock-on positive effect for longevity, and could partly explain why bats live disproportionately long lives compared to nonflying mammals of similar body size.

For now, "how flight is incorporated into this field is still just a hypothesis," cautions Vincent Munster, chief of the

virus ecology unit at the National Institutes of Health. Zhou's study is a good step toward understanding what makes bats' immune systems so special, Munster adds, "but I think the biggest challenge now is how to put this in the bigger perspective." Researchers still don't understand how bats' immune systems overall are responding in vivo to these viruses, he says, adding that STING is just one part of the story.

Shi is aware of these gaps. "We hope to get the full picture in, I don't know, 10 years or maybe 20 years," she says. Zhou, meanwhile, likes to turn the problem on its head. Rather than thinking that bats "tolerate" so many viruses, he suggests that it's more appropriate to think of the viruses choosing their hosts. His research shows how bats' immune systems have built an ideal home for viruses to thrive for a long time without killing their hosts, he says. "Think about it. . . [The bat] immune system is just perfect for viruses." —Katarina Zimmer

Bloody Clues

In 2009, a leech made headlines after it helped catch a criminal in Australia. Two burglars had assaulted and robbed a 71-year-old woman in her isolated home in the Tasmanian forest, where the bloodsuckers are widespread. The leech, which had latched onto one of the thieves to feed, plopped off at the scene of the crime in 2001. Investigators extracted the perpetrator's DNA from the invertebrate and cracked the case nearly eight years later, when the robber's blood showed up as a match after he was arrested for an unrelated offense.

But leeches aren't just useful at crime scenes. These creatures' sanguineous appetites have also come in handy for scientists, who are hoping to use them to solve another mystery: the diversity of animals, particularly those hidden deep within forest habitats around the world. "Contrary to popular opinion, [leeches] don't just suck," says Mark Siddall, an invertebrate zoologist at the American Museum of Natural History's Sackler Institute for Comparative Genomics.

Leeches are a group of segmented worms primarily found in freshwater habitats, although a few species live in the oceans and on land. Despite their reputation, many don't feed on blood, instead eating earthworms, snails, and other invertebrates. The bloodsucking (hematophagous) leeches inhabit a number of biodiversity-rich regions, such as the rainforests of Southeast Asia, Australia, and Madagascar.

Ida Bærholm Schnell, a postdoc at the University of Copenhagen, says that conversations with employees at Copenhagen Zoo motivated her and some colleagues to investigate whether they could use hematophagous leeches to identify elusive animals within biodiversity-rich regions. A zookeeper had been bitten by a leech during a jungle trek in Malaysia, and he and his coworkers wondered whether it would have been possible to find traces of his DNA within the little critter.

If scientists could find DNA from other animals within leeches' blood meals, the team realized, the worms might serve as a tool for biodiversity studies. To examine this possibility, Schnell and her colleagues collected *Haemadipsa*—a genus of hematophagous, terrestrial leeches—from the densely forested Central Annamites region of Vietnam. After analyzing the DNA in 25 of the creatures' blood meals, the researchers found that 21 specimens contained mammalian DNA sequences from various animals, including recently discovered species, such as the Annamite striped rabbit, and threatened ones, such as the serow, a goat-like ungulate (*Curr Biol*, 22:R262-63, 2012).

To their surprise, Schnell says, the researchers also learned that mammalian DNA could last in leeches for at least four months. "The leeches conserve the DNA, in a way," she explains. "They inhibit the digestion [of DNA], and they only have a very limited number of bacterial species in their gut."

This study got some members of the scientific community excited about the possibility of using leeches for biodiversity surveys, says Michael Tessler, now a postdoc at the American Museum of Natural History. The findings from Vietnam motivated him and his colleagues to conduct follow-up studies to further refine the method. "Our objective was to see whether we [could] actually put this into practice," Siddall, who was Tessler's PhD supervisor at the time, tells *The Scientist*.

For one study, Tessler, Siddall, and their colleagues gathered about 750 *Haemadipsa* leeches from various habitats, including farms, forests, and riversides, in China, Cambodia, and Bangladesh. The treks weren't for the squeamish: one particularly memorable moment for Siddall was being covered head to toe in leeches on a rainy collection day in Cambodia. "When it rains, it is glorious for leeches—you get so many of them," he says. "[After] I made the call that we were done for the day because we ran out of bags and containers to put

FOLLOW THE BLOOD: Researchers are using the DNA stored in leeches' meals to study animal biodiversity.

them in, I turned around and I had leeches going up my back [and on] my hair, my beard, and my neck."

After dissecting the leeches to isolate the parts of the digestive tract that contained the blood meal, the team extracted, then amplified and sequenced the DNA using vertebrate-specific primers. "We spent some late nights in the lab chopping up leeches" under a microscope, recalls Sarah Weiskopf, who was then a master's student at the University of Delaware.

A key observation from this analysis was that individual leeches appear to feed on a wide variety of animals, and among leech species there are no clear preferences for a particular prey (*Syst Biodivers*, doi:10.1080/14772000.2018.1433729, 2018). Identifying the feeding habits of different species is important, Siddall explains, because "if your sampling is biased, you want to know that it's biased." Although it appears that the leeches examined in the latest analysis can feed on all kinds of animals, it's possible that some species might have specific host preferences, he adds. "There's not a large body of literature to tell us what various species of leeches feed on. . . . [But] we're getting that information now."

This knowledge will be important for future leech studies, says Andreas Wilting of the Leibniz Institute for Zoo and Wildlife Research in Berlin who studies biodiversity but didn't take part in this work. In future biodiversity analyses, researchers will need to include the detection probability of the animals based on the leeches' distribution and feeding behavior, he adds. "It is important to highlight that more detections in the leeches do not always relate to higher abundance of [the identified] species in the area."

In a second study, the team compared the leech-based biodiversity survey to camera traps, one of the most popular and well-established techniques for documenting wild animals, in four Bangladeshi forests. "Camera traps are a really common way to study mammal biodiversity in the tropics, but it takes a long time and they can be expensive," says Weiskopf, now a biologist at the US Geological Survey. "So we were interested in seeing if there was another way."

Approximately 200 leeches, collected over four days, uncovered 12 different species, while 27 camera traps, set out at various stations for around nine months, identified 26. The two methods also had some qualitatively different results—for example, the leeches helped researchers identify more rhesus monkeys, while only the cameras were able to identify wild cat species (*J Appl Ecol*, doi:10.1111/1365-2664.13111, 2018).

These findings suggest that the two methods would "nicely complement each other," says Sébastien Calvignac-Spencer, a researcher who investigates the use of blowflies, another bloodsucking critter, as a biodiversity tool at the Robert Koch Institute in Berlin. "I think [both papers] are important in the sense that they will show practitioners—people who are doing biodiversity monitoring—that it makes sense to collect leeches."

However, Calvignac-Spencer, who wasn't involved in the leech studies, notes that while terrestrial bloodsucking leeches are a promising tool, they're not present everywhere. For example, they aren't found in some of the hotspots for biodiversity, including areas in Africa and South America. This is where other invertebrates, such as blowflies—which are widespread across the globe—come in handy, he adds.

Schnell notes that while the two recent papers (which she didn't take part in) both used older, low-throughput Sanger sequencing methods, "the aim is to go from one leech, one DNA sequence, to many leeches in one high-throughput sequencing analysis." In a soon-to-be published study, Schnell, Siddall, and their colleagues collected and processed more than 3,000 hematophagous terrestrial leeches from five regions around the world, including Madagascar, Australia, and Southeast Asia. Using next-generation sequencing methods, they identified a wide range of vertebrates, including mammals, birds, reptiles, and amphibians.

"Our objective is to show that the strategy works, and that it is complementary to, and in some respects better than, existing techniques," Siddall says. "[But] this is really going to have to be something that conservation organizations or forestry organizations in various countries decide that they want to do." —Diana Kwon

Cosmic Salad

Vibrant orange flowers crown a leafy green stem. The plant is surrounded by many just like it, growing in an artificially lit greenhouse about the size of a laboratory vent hood. On Earth, these zinnias, colorful members of the daisy family, probably wouldn't seem so extraordinary. But these blooms are literally out of this world. Housed on the International Space Station (ISS), orbiting 381 kilometers above Earth, they are among the first flowers grown in space and set the stage for the cultivation of all sorts of plants even farther from humanity's home planet.

Coaxing this little flower to bloom wasn't easy, Gioia Massa, a plant biologist at NASA's Kennedy Space Center in Florida, tells *The Scientist*. "Microgravity changes the way we grow plants." With limited gravitational tug on them, plants aren't sure which way to send their roots or shoots. They can easily dry out, too. In space, air and water don't mix the way they do on Earth—liquid droplets glom together into large blobs that float about, instead of staying at the roots.

Massa is part of a group of scientists trying to overcome those challenges with a benchtop greenhouse called the Vegetable Production System, or Veggie. The system is a prototype for much larger greenhouses that could one day sustain astronauts on journeys to explore Mars. "As we're looking to go deeper into space, we're going to need ways to support astronaut crews nutritionally and cut costs financially," says Matthew Romeyn, a long-duration food production scientist at Kennedy Space Center. "It's a lot cheaper to send seeds than prepackaged food."

In March 2014, Massa and colleagues developed "plant pillows"—small bags with fabric surfaces that contained a bit of soil and fertilizer in which to plant seeds. The bags sat atop a reservoir designed to wick water to the plants' roots when needed (*Open Agriculture*, 2:33-41, 2017). At first, the ISS's pillow-grown zinnias were getting too much water and turning moldy. After the crew ramped up the speed of Veggie's fans, the flowers started drying out—an issue relayed to the scientists on the ground in 2015 by astronaut

SPACED OUT: Zinnias such as this one were among the first flowers to be grown on the International Space Station.

NASA

Scott Kelly, who took a special interest in the zinnias. Kelly suggested the astronauts water the plants by hand, just like a gardener would on Earth. A little injection of water into the pillows here and there, and the plants perked right up, Massa says.

The gardening helped to boost the astronauts' diets, and also, anecdotally, brought them joy.

With the zinnias growing happily, the astronauts began cultivating other flora, including cabbage, lettuce, and microgreens—shoots of salad vegetables—that they used to wrap their burgers and even to make imitation lobster rolls. The gardening helped to boost the astronauts' diets, and also, anecdotally, brought them joy. "We're just starting to study the psychological benefits of plants in space," Massa says, noting that gardening has been shown to relieve stress. "If we're going to have this opportunity available for longer-term missions, we have to start now."

The team is currently working to make the greenhouses less dependent on people, as tending to plants during space missions might take astronauts away from more-critical tasks, Massa says. The researchers recently developed Veggie PONDS (Passive Orbital Nutrient Delivery System) with help from Techshot and Tupperware Brands Corporation. This system still uses absorbent mats to wick water to plants' seeds and roots, but does so more consistently by evenly distributing the moisture. As a result, the crew shouldn't have to keep such a close eye on the vegetation, and should be able to grow hard-to-cultivate garden plants, such as tomatoes and peppers. Time will tell. NASA sent Veggie PONDS to the ISS this past March, and astronauts are just now starting to compare the new system's capabilities to those of Veggie.

"What they are doing on the ISS is really neat," says astronomer Ed Gui-

nan of the University of Pennsylvania. If astronauts are going to venture into deep space and be able to feed themselves, then they need to know how plants grow in environments other than Earth, and which grow best. The projects on the ISS will help answer those questions, he says. Guinan was so inspired by the ISS greenhouses he started his own project in 2017 studying how plants would grow in the soil of Mars—a likely future destination for manned space exploration. He ordered soil with characteristics of Martian dirt and told students in his astrobiology course, "You're on Mars, there's a colony there, and it's your job to feed them." Most of the students worked to grow nutritious plants, such as kale and other leafy greens, though one tried hops, a key ingredient in beer making. The hops, along with some of the other greens, grew well, Guinan reported at the American Astronomical Society meeting in January.

Yet, if and when astronauts go to Mars, they probably won't be using the Red Planet's dirt to grow food, notes Gene Giacomelli, a horticultural engineer at the University of Arizona. There are toxic chemicals called perchlorates to contend with, among other challenges, making it more probable that a Martian greenhouse will operate on hydroponics, similar to the systems being tested on the ISS. "The idea is to simplify things," says Giacomelli, who has sought to design just such a greenhouse. "If you think about Martian dirt, we know very little about it—so do I trust it is going to be able to feed me, or do I take a system I know will feed me?"

For the past 10 years, Giacomelli has been working with others on a project, conceived by now-deceased business owner Phil Sadler, to build a self-regulating greenhouse that could support a crew of astronauts. This is not a benchtop system like you find on the space station, but a 5.5-meter-long, 2-meter-diameter cylinder that unfurls into an expansive greenhouse with tightly controlled circulation of air and water. The goal of the project, which was suspended in December due to lack of funding, was to show that the lab-size greenhouse could truly sustain astronauts. The greenhouse was only partially successful; the team calculated that a single cylinder would provide plenty of fresh drinking water, but would produce less than half the daily oxygen and calories an astronaut would need to survive a space mission. Though the project is on hold, Giacomelli says he hopes it will one day continue.

This kind of work, both here and on the ISS, is essential to someday sustaining astronauts in deep space, Giacomelli says. And, if researchers can figure out how to make such hydroponic systems efficient and waste-free, he notes, "the heck with Mars and the moon, we could bring that technology back to Earth."

—Ashley Yeager

Older Sisters

Is Archaea an ancient sister group to Eukarya, rather than a direct ancestor?

BY PATRICK FORTERRE, VIOLETTE DA CUNHA, AND MORGAN GAIA

In 1977, biologist Carl Woese discovered that microbes living in anaerobic conditions and producing methane had a genetic imprint very different from known bacteria species. He and his colleagues eventually suggested that researchers stop referring to such methanogens and related microorganisms as bacteria, classifying them instead as members of a new domain in a tripartite division of the living world, alongside Bacteria and Eukarya.

Woese named this domain Archaea (from the Greek *archaio*, meaning ancient or original) because the microbes he studied seemed to thrive in extreme conditions akin to those of early Earth. Later on, scientists observed archaea in more-diverse environments, from oceanic water and deep sediments to forest soil and the surface of human skin. Recently, a new archaeal group named after its discoverer, Woesearchaeota, was even detected in human lungs.

Although archaea superficially resemble bacteria in terms of size and cellular organization (members of both groups lack nuclei), they are surprisingly similar to eukaryotes at the molecular level. For instance, all archaea replicate their DNA and synthesize proteins using molecular machines like those of eukaryotes. This suggests that Eukarya and Archaea belong to a same "super-domain" that one of us (P.F.) proposed calling Arkarya, and that eukaryotes share a common ancestor with archaea that existed much more recently than the separation of Arkarya and Bacteria. In other words, archaea would be our sisters and bacteria our cousins.

Many new archaea species discovered in the past decade exhibit additional eukaryotic features, such as components of the cytoskeleton, but many of these are only present in one or a few archaeal subgroups. This indicates that these features were probably all present in the ancestor common to Archaea and Eukarya before being lost in some archaeal lineages. These ancient eukaryotic features were potentially replaced by bacterial ones over time in some archaeal lineages from frequent lateral gene transfer between archaea and bacteria living in the same environments.

If this model is correct, the common ancestor of Archaea resembled eukaryotes more closely than any modern archaeon, and combining all eukaryotic features presently dispersed in Archaea should allow researchers to reconstruct the ancestor's phylogenomic profile. Assuming that these shared archaeal/eukaryotic features were present in the common ancestor of these two domains, the profile would provide a starting point to picture how eukaryotes originated and evolved. Screening for new archaeal lineages with additional eukaryotic features is therefore crucial to get more information about our origin.

Researchers are also seeking to understand the origin of the unique eukaryotic features missing in Archaea. One possibility is that some of them originated in the many lineages of large DNA viruses that coevolved with the ancestors of eukaryotes after their separation from the archaeal lineage. We suggested, for instance, that the nucleus evolved from nucleus-like factories that these viruses built in the cytoplasm of infected cells to protect their genomes (*Curr Opin Microbiol*, 31:44-49, 2016).

Over time, several researchers have proposed alternative evolutionary scenarios in which the eukaryotic features actually appeared and accumulated in some archaeal lineages before Eukarya eventually originated from a specific archaeal branch. These scenarios, which include the archaea ancestor hypothesis where an ancient archaeon merged with a bacterium, have recently been supported by universal trees of life (See THE EOCYTE TREE on next page) with Eukarya branching from Asgard archaea, which contain many eukaryotic features. (See "The Ancient Ones," page 26.) These models posit Archaea as the mother of eukaryotes, rather than a sister.

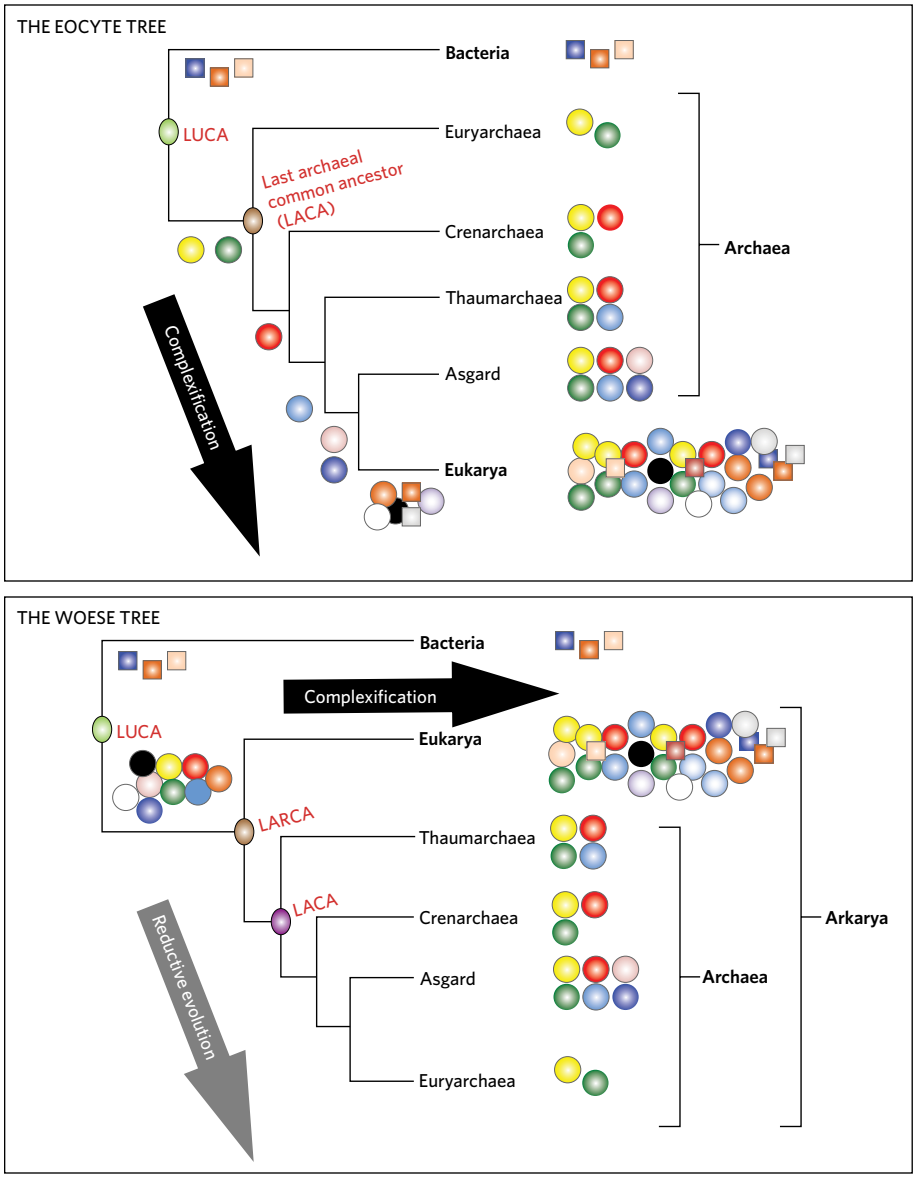
Our data come to a different conclusion. Phylogenetic trees are built using universal proteins, which are conserved in the three domains of life. Recently, we showed that the results of such analyses are strongly dependent on the sets of proteins and species used. Avoiding artifact-prone elements from our analyses, we obtained a robust universal tree that did not support the archaeal ancestry of Eukarya but the sisterhood of the two domains instead (*PLOS Genet*, 13:e1006810, 2017; *PLOS Genet*, 14:e1007215, 2018).

In our view, scenarios in which Archaea gave birth to Eukarya raise several difficult questions. For one, they imply that thousands of archaeal lineages remained similar to their ancestors during the last 3 billion years, whereas one was dramatically transformed into a new domain, the Eukarya. This seems unlikely because Eukarya exhibits many unique features absent in the two other domains. For instance, large DNA viruses that infect eukaryotes have no direct ancestors that infect archaea. Moreover, a few eukaryotic features, such as the nature of their lipids, remain more similar to those of bacteria. Deriving all these features from archaea requires proposing ad hoc scenarios that seem far from parsimonious, such as getting all of those characters directly or indirectly from the bacterium that engaged in the original endosymbiotic union.

Considering archaea as eukaryotes' ancestors also reproduces the common confusion of ignoring the evolution that takes place in two lineages after their divergence. This would be akin to considering chimps as humans' direct ancestors. Humans and chimps share a common great ape ancestor that was neither one nor the other. Similarly, the last common ancestor of Archaea and Eukarya was most likely different from all modern organisms.

Interestingly, if archaea are indeed our sisters and not our mothers, one could imagine that some common features present in Bacteria and Eukarya have been inherited from the last universal common ancestor (LUCA) of all life and subsequently lost in Archaea. The identification of these features in already known organisms or in lineages of Bacteria and Eukarya yet to be discovered would be another important step in the reconstruction of LUCA, crucial to truly understanding the origin of life itself. ■

Patrick Forterre is a microbiologist at Institut Pasteur in Paris. Violette Da Cunha and Morgan Gaia are postdocs in his lab.



PARSING BRANCHES: In the eocyte tree, the various features shared by Archaea and Eukarya (circles) appeared and accumulated progressively during the diversification and complexification (black arrow) of Archaea. Many eukaryotic-specific features originated after the separation of Eukarya from other Archaea. In this scenario, Eukarya evolved from a subgroup of Archaea beside other archaeal phyla such as Euryarchaea, Crenarchaea, Thaumarchaea, and Asgards.

In the Woese tree, which our research supports, the various features shared by Archaea and Eukarya appeared in the branch leading from the last universal common ancestor (LUCA) to the last arkaryal common ancestor (LARCA). After separation of the branches leading to Archaea and Eukarya, the former progressively lost some of these features (gray arrow), whereas new features accumulated in the branch leading to Eukarya (black arrow). In both scenarios, Bacteria and Eukarya evolved other features (squares) in parallel.

MODIFIED FIGURE COURTESY OF PATRICK FORTERRE

Sniffing Out Gene Expression

Soil scientists use a gas-producing reporter system to assess gene activity in bacteria.

BY RUTH WILLIAMS

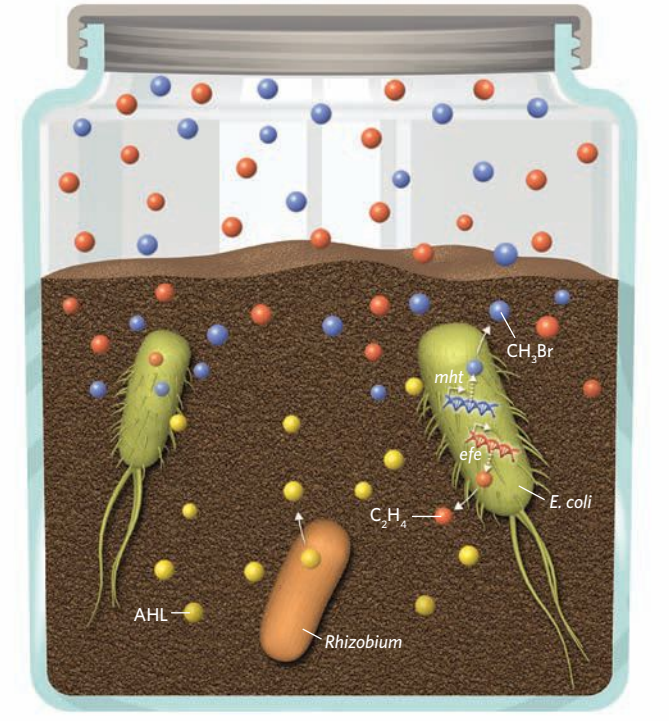
Fluorescent reporter proteins have revolutionized gene expression analysis, but their use is limited to more-or-less transparent systems, such as single cells or zebrafish larvae. For researchers studying organisms that live in soil, using these visual reporters is infeasible in any but the thinnest of samples, says environmental and synthetic biologist Jonathan Silberg of Rice University.

"We can analyze cultured microbes in exquisite detail," adds biogeochemist Caroline Masiello, also at Rice. "But the question is—does it matter at the scale of an ecosystem?" Silberg, Masiello, and colleagues have devised a new gas reporter system that can be used to detect the presence and activity of microbes in opaque samples of any size.

Ecologists often use headspace gas analysis to measure common bacterially produced gases, such as carbon dioxide and methane, without disrupting the soil or sediment sample. If bacteria could be genetically engineered to produce unusual gases, the team reasoned, they could be readily detected and analyzed by this technique, which involves quantifying mixtures of gases in closed containers.

The team identified two potential reporter genes—one from a plant, *Batis maritima*, and one from the bacterium *Pseudomonas syringae*—encoding enzymes, MHT and EFE, respectively, that synthesize methyl bromide and ethylene. The researchers then cloned the genes into a plasmid, placing *efe* under the control of a constitutive promoter and *mht* under a promoter responsive to acylhomoserine lactone (AHL), a bacterial signaling molecule key in quorum sensing. The plasmid was transferred into *Escherichia coli*.

In gas from soil samples containing the engineered *E. coli*, the team could detect both the bacteria's presence (via ethylene) and the production, or destruction, of AHL (via methyl bromide). The system, which also worked in the sediment-dwelling bacterium *Shewanella oneidensis*, could be used as-is for studying bacterial communication, or could be engineered to respond to other environmental signals, such as pollutants.



GASSY GENES: To detect microbial activity, *E. coli* bacteria are genetically engineered to produce the EFE protein constitutively and MHT in response to the bacterial communication molecule AHL. The presence of the *E. coli* in the soil, and the levels of AHL, in this example produced by *Rhizobium* bacteria, can then be detected non-disruptively using headspace gas chromatography—with the ratio of MHT-produced CH_3Br to EFE-produced ethylene reflecting the concentration of AHL.

"This is a fascinating new technique," says University of North Carolina at Chapel Hill microbial biogeochemist Carol Arnosti, who was not involved with the project. "[It] promises to revolutionize the manner in which we investigate the activities and interactions of bacterial communities in soils." (*ACS Synth Biol* 7:903-11, 2018) ■

AT A GLANCE

IN-SOIL BACTERIAL ANALYSIS	REPORTER GENES ENCODE	HOW IT WORKS	STRENGTHS	WEAKNESSES
Visualization with a rhizotron	Fluorescent proteins	Soil is placed in thin glass or plastic cassettes. Reporter-expressing bacteria, typically in association with reporter-expressing plant roots, can be visualized via fluorescence microscopy.	Yields information about spatial and temporal distribution of bacteria and processes	Soil samples must be extremely thin, so not easily scalable
Gas reporters	Methyl halide transferase (MHT) and ethylene forming enzyme (EFE)	Methyl bromide and ethylene gases produced by reporter-expressing, soil-dwelling bacteria are analyzed using headspace gas chromatography.	Provides temporal information about bacterial processes. Easily scalable	No spatial information

The Ancient Ones

Identification of new archaeal species elucidates the domain's unique biology and its relationship to eukaryotes.

BY AMBER DANCE

Every summer from 2013 to 2015, Dimitry Sorokin waded into the shallow, briny, alkaline lakes of Siberia's Kulunda Steppe. Pale carbonate minerals crusted the pools' edges, where lambs, too young to know the perils of drinking here, sometimes perished on the shores. As the water lapped at his thighs and abdomen, the stink of sulfur and methane that bubbled up from the disturbed sediments filled his nostrils. "For me, it's Chanel No. 5," says the microbiologist, who splits his time between the Russian Academy of Sciences in Moscow and Delft University of Technology in the Netherlands.

Sorokin was hoping to identify the microbes producing that heady fragrance. From his previous research, Sorokin knew that the lakes' denizens contained a gene for part of the methane-processing methyl-coenzyme M reductase (MCR) complex, but he didn't know which microbial species harbored the gene in their chromosomes.[1] He collected mud from the pools' bottoms and packed it in glass containers in an insulated, chilled box. He then returned with the samples, by car and then plane, to Moscow to culture whatever was living

COURTESY OF DIMITRY SOROKIN

ON THE HUNT: Dimitry Sorokin takes samples of anaerobic sediments from a hypersaline soda lake in southern Russia in July 2013. Analyzing the samples back in his lab in Moscow, he identified a new class of methane-producing archaea, which he dubbed Methanonatronarchaeia.

within them. What he found—in the samples from the Siberian lakes as well as those from other salty lakes around the world—was an entirely new class of archaea, which he christened Methanonatronarchaeia.[2]

"When I realized something [new] was struggling at the end of my line, something very unusual, of course I was exhilarated," says Sorokin. "I was working 12 hours, 14 hours a day, trying to get it."

> Archaea is the least known of the three groups of living organisms.
>
> —Laura Eme, Uppsala University

For researchers who study archaea, however, the excitement of finding a novel order, class, or even phylum has become just another day at the lab. While it's rare to identify new high-level taxa of the bacterial or eukaryotic domains of life, the archaeal family tree has exploded in recent years, and the discoveries show no signs of slowing. Around the turn of the century, scientists had classified just two phyla of Archaea: the heat-loving Crenarchaeota and the Euryarchaeota, which includes methanogens and halophiles. Over the ensuing decade, Nanoarchaeota, containing species just a few hundred nanometers across, was added. And a number of phyla related to Crenarchaeota joined it in a so-called superphylum known as TACK for its founding members (Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota). By 2017, the Archaea domain had further expanded to encompass four superphyla: Euryarchaeota, TACK, the eukaryote-like Asgard group named for various Norse gods, and the tiny-celled DPANN group (founded by Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanohaloarchaeota, and Nanoarchaeota), sometimes called nanoarchaea.[3]

"This is the least known of the three groups of living organisms," Laura Eme, an evolutionary microbiologist at Uppsala University in Sweden, says of archaea. "I think there is just a basic need to know what they are."

But while these microbes are relatively new to biology, their lineage is ancient. In fact, the group was named by Carl Woese in 1977 based on the Greek for "old ones." Previously, the organisms he christened Archaebacteria were considered simply prokaryotes, along with other bacteria. Today, many scientists agree that, among the profusion and variety of archaea, they have likely found the modern counterparts of eukaryotes' ancestors.

Although they escaped notice for much of the history of biology, archaea are everywhere. Contrary to the beliefs of scientists working with archaea when they were first discovered, members of the domain aren't limited to extreme environments; they just tend to be overshadowed by other, more abundant microorganisms in less dramatic habitats. It's only in harsh conditions that bacteria become less common, leaving archaea to rule. With better techniques to survey all the microorganisms in a given environment, researchers censusing Earth's microbial diversity, from deep-sea vents to the Australian Outback, continue to turn up new groups of Archaea. And with these discoveries come insights into their unique and diverse biology, as well as their roles in the environment.

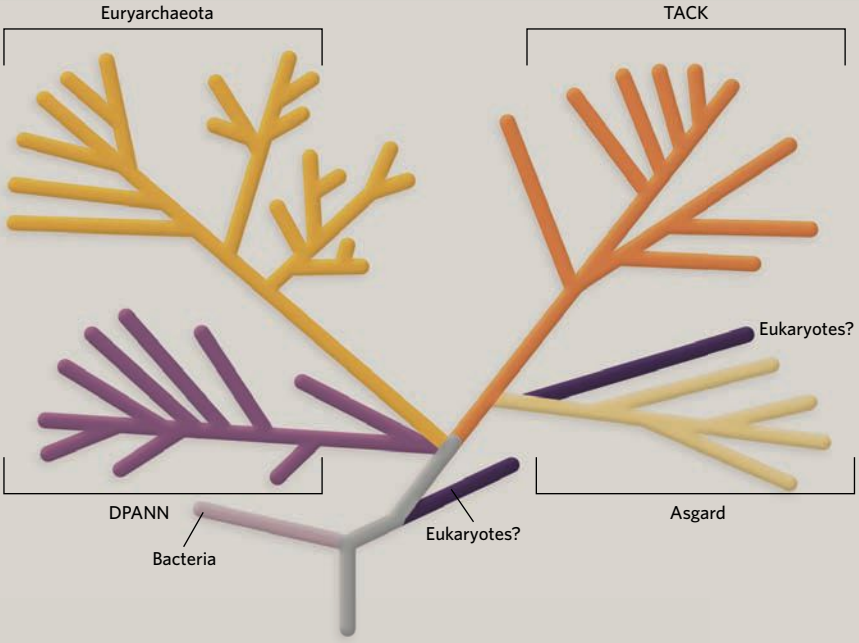
Studying the unculturables

After he brought home his mud samples, it took Sorokin a couple of years to figure out how to grow the microbes they contained and purify single-organism cultures. He read up on the metabolism of known methanogens, such as those found in the

THE SCIENTIST STAFF

AN EVOLVING FAMILY TREE

Thanks to a wealth of new genomic sequence data, the family tree of Archaea, which encompassed just two phyla 16 years ago, has exploded in recent years. It now includes more than a dozen phyla, organized into four informal "supergroups," based mostly on sequence similarities. Scientists have yet to determine precisely how novel archaea should be classified. Also in dispute is how Eukarya fit into the picture—some scientists suggest they're an offshoot of a branch known as Asgard archaea, while others suspect they diverged from Archaea earlier on. (See, "Older Sisters," page 23.) Researchers predict the tree will sprout many more branches in the years to come.



guts of termites and other animals, to find some clues to what the microbes might need. He eventually settled on a salt-saturated brew containing iron sulfide, methanol, and formate, at a pH near 10. In most cases, he says, a key ingredient was sterilized sediments from the lakes themselves. A hearty dose of luck also helped him find the right recipe, he says.

In taking this classic culturing approach, however, Sorokin says he's a "dinosaur." Today, most microbiologists seeking novel archaea have embraced speedy, inexpensive sequencing—both to evaluate samples en masse, in the case of metagenomics, and to analyze individual cells. Such next-generation sequencing has allowed researchers to sidestep the problem that the vast majority of microorganisms can't be cultured using current techniques. "We will flesh out the tree of life far more quickly than if we had been waiting for isolates," says Gene Tyson, deputy director of the Australian Centre for Ecogenomics at the University of Queensland in Brisbane.

But using sequence data to categorize newly identified species, or other taxonomic groups, is a formidable task. If microbes don't grow in culture, scientists cannot characterize the morphology and behavior of a new group. Metagenomic results also sometimes lack the 16S rRNA genes (18S in eukaryotes) that researchers traditionally use to indicate evolutionary relationships between organisms, says Eme. This results from the way computer algorithms group sequences predicted to come from the same organism. The software might cluster sequences with similar nucleotide composition—for example, how often a tetranucleotide such as ATCC appears—and 16S genes often differ in nucleotide composition from the rest of their genome.

Until the microbiology community develops a standardized naming system, it falls to discoverers—along with their computational tools—and publication reviewers to hash out the quickly evolving archaeal taxonomy. For example, in 2013, 22 researchers proposed the DPANN superphylum, based on single-cell sequencing data.[4] But there's tremendous species diversity within DPANN archaea, suggesting they might not be a single group at all. It's possible, says Thijs Ettema of Uppsala University in Sweden, that computer phylogeny algorithms lump them together simply

Supergroup	History	Characteristics
Euryarchaeota	Carl Woese and colleagues divided the Archaea into two "kingdoms," Euryarchaeota and Crenarchaeota, in 1990.[1]	Includes halophiles and methanogens. Members of the order Thermoplasmateles are acidophiles and thermophiles.
TACK	Scientists proposed the TACK name in 2011 to encompass the phyla Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota;[2] more phyla have been added since.	Includes thermophiles. Thaumarchaeota participate in nitrogen cycling. Some are also methanogens.
DPANN	The first phylum named was Nanoarchaeota, in 2002, for a tiny deep-sea vent organism that didn't fit into Euryarchaeota or Crenarchaeota.[3] In 2013, researchers proposed linking it with the taxa Diapherotrites, Parvarchaeota, Aenigmarchaeota, and Nanohaloarchaeota.[4] New phyla have been added since.	At least some are small in size, with small genomes lacking genes for key proteins in metabolism and other processes. Some may rely on a symbiont or host organism to survive.
Asgard	The first discovered were Lokiarchaeota, which were initially thought to be members of the TACK superphylum.[5] The group now contains a handful of phyla, all named for Norse deities.[6]	Genomes encode several proteins similar to those found in eukaryotes.

1. *PNAS*, 87:4576-79, 1990; 2. *Trends Microbiol*, 19:580-87, 2011; 3. *Nature*, 417:63-67, 2002; 4. *Nature*, 499:431-37, 2013; 5. *Nature*, 521:173-79, 2015; 6. *Nature*, 541:353-58, 2017

because they're so peculiar compared to everything else.

"[Archaeal taxonomy is] kind of like the Wild West," says Brett Baker, a microbial ecologist at the University of Texas at Austin. "There's going to be a huge debate about how we define a phylum."

The Asgard superphylum, described by Ettema, Baker, and their colleagues in 2017, is also generating some disagreement due to its implications for the evolution of eukaryotes. The research started out innocuously enough, as the scientists assembled the genomes of an archaeal group found in sediments near an Arctic deep-sea vent known as Loki's Castle.[5] But they saw something strange. "We started finding all these eukaryotic genes," Ettema recalls, including genes that seemed to encode eukaryote-like cytoskeletal proteins, small GTPases, and the ESCRT machinery involved in membrane-based processes such as autophagy and lysosome-based protein degradation.[6] These so-called "eukaryotic signature proteins" typically don't have homologs in either bacteria or archaea, says Ettema. "I realized either this is something really interesting, or this is some freaky artifact."

He and his colleagues tested the samples for evidence of true eukaryotes, such as their 18S rRNA, and came up empty-handed. Convinced that the microbes they identified were indeed archaea, the researchers reported their results in 2015, dubbing the new phylum Lokiarchaeota for the vent site.[7] Since then, Ettema's and Baker's teams have found three sister phyla to Lokiarchaeota, all containing genes formerly thought to be specific to eukaryotes: the Thorarchaeota, found in the dark, tannic acid–stained White Oak River estuary of North Carolina;[8] the Odinarchaeota, found in hot springs such as those in Yellowstone National Park;[9] and the Heimdallarchaeota, discovered in marine sediments.[9] The researchers grouped these together as the Asgard superphylum.

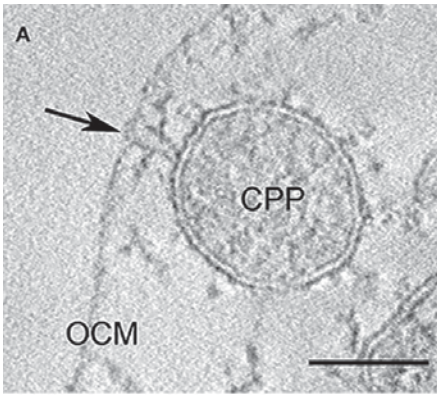
Based on their interrogations of the asgardians, Ettema, Baker, and colleagues concluded that these archaea fit neatly into the late Lynn Margulis's longstanding hypothesis of endosymbiosis—the idea that eukaryotes arose when one microbe engulfed another. The Asgard archaea would seem to be descendants of the original host that swallowed a bacterium, and at that time already possessed some of the genes scientists would come to associate with eukaryotes. This contrasts with the older idea that archaea and eukaryotes sprang from a common ancestor and evolved as two distinct, parallel lineages. (See "Older Sisters" on page 23.) The Lokiarchaeota provided the turning point, says Ettema: "Suddenly we come up with this phylum that seems to turn everything around." He notes that Lokiarchaeota's name references not only the discovery site but also the Norse trickster god for that reason.

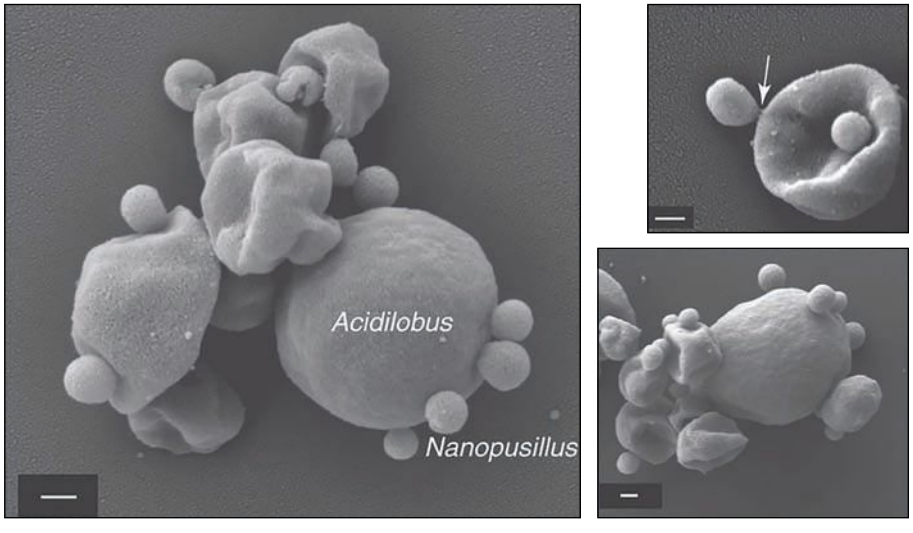
Eme, who works in Ettema's lab, says she's now analyzing about 60 new Asgard genomes, looking for more eukaryote-like features and trying to piece together how the group evolved. "Now that we have more representatives of each of the lineages . . . we are starting to be able to make more-confident claims," she says. "There's so much to understand from these archaea."

Signs of symbiosis

While genomics has already yielded unprecedented insights into the diverse domain of Archaea, scanning the microbes' DNA reveals only part of the story. To fully understand archaeal biology, says Tyson, "we need to move beyond simple sequence gazing."

Sorokin, by culturing his Methanonatronarchaeia (members of the phylum Euryarchaeota), found that they reduce methyl groups to methane. But the microbes do so via a nontraditional pathway, observed in only a few other methanogenic archaea. They collect electrons from formate or hydrogen to donate to compounds that are methylated on the first carbon in the molecule, thus releasing the methane. As for how the Methanonatronarchaeia manage to survive the high-salt lakes, Sorokin determined that they pack their cytosols with potassium ions, which prevents the influx of the more-dangerous sodium ions from the water.[2]





ARCHAEAL PARTNERS: Some Nanoarchaeota are ectosymbionts, living attached to other archaea. While the nature of this relationship remains unclear, some studies have found a direct connection between the cytoplasms of the two organisms. (Lower image: *Nanopusillus acidilobi* on *Acidilobus* sp. 7A cells; scale bar = 200 nm. Top image: *Nanopusillus equitans* on *Ignicoccus hospitalis*; scale bar = 100 nm.).

NAT COMMUN, 7:12115, 2016.; *FRONT MICROBIOL*, 8:1072, 2017

Meanwhile, Mircea Podar, a microbiologist at Oak Ridge National Laboratory in Tennessee, has successfully cultured a member of the thermophilic Nanoarchaeota, a founding phylum of the DPANN group, as well as their larger Crenarchaeota hosts. As ectosymbionts, these and other nanoarchaea are often found attached to the surface of their hosts, and seem to be genomic minimalists, having lost many genes over their evolutionary history. Presumably, DPANN archaea get what they need from the better-equipped hosts, as well as from the environment. A group of German researchers figured out how to culture one nanoarchaeon-crenarchaeote pair in 2002,[10] and Podar added another pair to the list in 2016.[11] He's now collecting other DPANN archaea and their hosts from around the world to investigate how the two symbiotic microbes communicate, exchange nutrients, and coevolve.

One lingering question is how the two cells are attached. Observing the contact site between *Nanoarchaeum equitans* and its larger host, *Ignicoccus hospitalis*, via electron microscopy, Podar's collaborators at the University of Regensburg in Germany recently found that some of the cytoplasm of the bigger archaeon protrudes into that of the smaller one, where a portion of the *N. equitans* cell envelope appears to disintegrate. At the same time, *N. equitans* creates a sort of stalk that pokes into the outer cell membrane of *I. hospitalis*. The result is a direct connection between the two cells' cytoplasms, through which nutrients or proteins could presumably flow from host to ectosymbiont.[12]

Another question is the nature of the species' relationship. Is the nanoarchaeon a parasite, bleeding the crenarchaeote of nutrients? Is it a commensal, benefiting from the partnership with no effect on the bigger microbe? Or does the crenarchaeote derive some benefit itself, making it a mutualistic relationship?

Experiments documenting the growth of these archaea in culture provide some hints. *I. hospitalis* grows more slowly when cocultured with *N. equitans* than when it's alone, suggesting it may be suffering due to parasitism—though the host doesn't behave as if it's stressed or defending itself.[13] "Maybe somewhere between a parasite and a commensal," Podar guesses, though he cautions that these experiments only tell how the organisms behave in lab cultures.

Archaea have emerged as the primary methane producers on the planet.

Perhaps the discovery and further study of new DPANN members will help clarify their biology. Two new archaeal phyla were recently identified in the groundwater and sediments of a contaminated aquifer near a former vanadium and uranium mill in Rifle, Colorado, by a team led by scientists from the University of California, Berkeley. They named the new groups for Woese and Norman Pace, the grandfather of cultivation-independent archaeal phylogeny. By comparing the DNA of these microbes to gene databases, Cindy Castelle, a researcher working with Berkeley geomicrobiologist Jillian Banfield, found that they lack the full complement of genes needed for several key metabolic processes.[14]

For example, one member of Woesearchaeota is missing genes involved in glycolysis, pyruvate metabolism, and the Krebs cycle, suggesting the organism may rely on a host to metabolize nutrients. The species is also missing genes to synthesize certain amino acids and nucleotides, which it might scavenge from the environment. What some Woesarchaeota do have is a gene apparently encoding a large extracellular enzyme that could help it stick to other microbes, which might, Castelle speculates, allow the species to gather the metabolites it can't make itself.

"They are really fascinating," Castelle says of DPANN archaea, which she is now finding in a variety of environments. "They are really divergent from the rest of the known archaea."

Methane makers

The more researchers learn about archaeal biology, the more apparent it becomes that these microbes play a key role in the Earth's biogeochemical cycles, transmitting carbon, nitrogen, and other elements central to life in and out of molecules. Even Castelle's Woesarchaeota, for all they lack, participate in nutrient cycling. They contain enzymes that allow them to use carbon compounds and hydrogen as energy sources to make products such as lactate and ethyl alcohol, which other microbes may then use as their own energy source.

In fact, archaea have emerged as the primary methane producers on the planet. And they've been at it for some time: researchers recently found fossil evidence of methanogenic archaea in 3.5-million-year-old rocks in Western Australia.[15]

Tyson's group at the University of Queensland started its metagenomic investigation of archaeal methanogens at Australia's coal seam gas mines, where pumps bring water up from underground to release natural gas—mostly methane—from the coal below. On two collecting trips in 2013, postdoc Paul Evans packed 0.22-micron filters, about 100 kilograms of dry ice, and other gear for a weeklong tour. A mining company guide drove him from site to site in regions with temperatures pushing 40 degrees Celsius (more than 100 degrees Fahrenheit), where Evans would hook the filter to the pump to collect microbes (and a fair amount of dirt). He stored the samples on dry ice, then isolated the DNA when he got back to the lab.

At that time, only certain members of Euryarchaeota were known to be equipped for methane metabolism. But Evans found representatives of the phylum Bathyarchaeota—part of the TACK supergroup—containing all the necessary genes, such as those encoding components of the MCR complex, just as Sorokin had found in the briny lakes of Siberia.[16] The finding spurred Tyson's group and others to seek, and discover, methane metabolism in additional

ARCHAEAL HUNTING: Oak Ridge National Laboratory microbiologist Mircea Podar and colleagues discovered two previously uncultured archaeal species in the Cistern Spring at Yellowstone National Park: *Nanoarchaeota acidilobi* and its host, *Acidilobus* sp. 7A.

archaeal groups, including the new phylum Verstraetearchaeota.[17]

While Evans and Tyson can detect the genes for the MCR complex, they can't tell exactly what the complex is doing in the organism. They can't even say for sure if the microbes are eating methane or excreting it, because the complex can either create or break down the molecule. "It depends on the context of the organism," says Tyson. Given that Evans found the Bathyarchaeota in a methane-producing mine, the researchers suspect they were making methane, but they can't be certain. Meanwhile, another research group has found that related enzymes process butane, suggesting that some archaea might be alkane generalists.[18]

Evans is working on the biology, but as with Sorokin, he's run into difficulty culturing the organisms, a common problem in microbiology. Based on the genome of the microbes and the environment in which he discovered them, Evans made his best guess as to what they might need: some sulfates, some hydrocarbons, anoxic conditions, and temperatures ranging from 30 degrees to 60 degrees Celsius. But it's slow going, he says. "You basically leave it for six months, a year, if not longer. We're waiting to see what happens."

Indeed, culturing these newly discovered organisms continues to be the primary hurdle in the field, with many archaea still never having been directly observed. When it comes to Asgard archaea, for example, "we have no clue what the cells look like and how they interact with other cells," says Ettema. The same is true for most other species.

> We have no clue what the cells look like and how they interact with other cells.
>
> —Thijs Ettema, Uppsala University

Eme predicts that studies of archaea will now move in two complementary directions, with some scientists going back to the bench to study the metabolism and life cycles of newly discovered and cultured organisms, while others continue to plumb every corner of the planet, via metagenomics, for microbes still awaiting discovery.

"I would assume that we're nowhere close to having discovered most of the diversity," she says. "I think there's going to be a lot more to be discovered." ■

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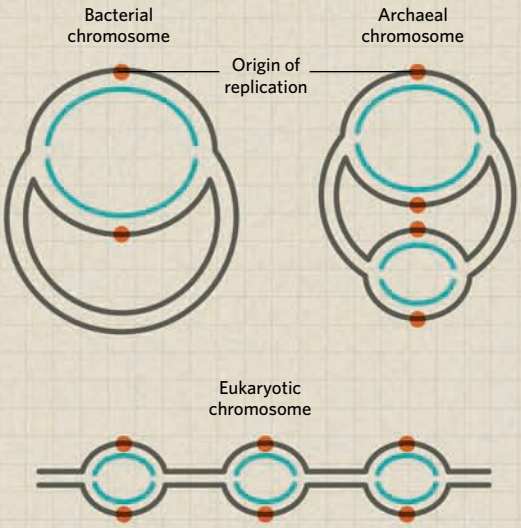
COURTESY OF MIRCEA PODAR

THE WILD BIOLOGY OF ARCHAEA

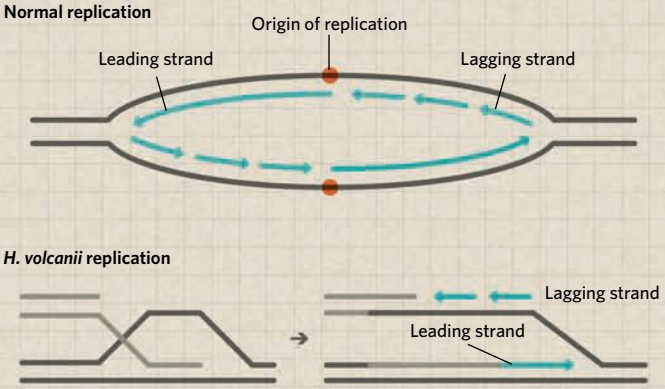
As researchers delve into archaeal biology, they are finding astonishing diversity, even among the most basic functions of life, such as how the microbes organize and copy their genomes, and how the cells divide. These observations stem from only a handful of species that can be cultured in the lab; there may be plenty more oddball examples of archaeal biology among the vast numbers of as-yet unculturables.

DNA Replication

Bacteria typically possess one chromosome with one origin of replication. Eukaryotes have multiple, paired chromosomes with numerous origins on each. Archaea straddle the divide: while they typically have one main chromosome, it often replicates from multiple origins.

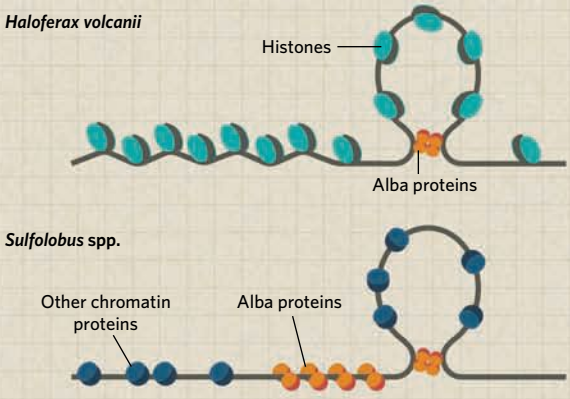


Some archaea also have the unique ability to adopt an alternate version of DNA replication initiation. Across all domains of life, DNA replication starts when initiation proteins bind the origin of replication; deleting the origins typically slows growth or halts cell division entirely. But in the archaeon *Haloferax volcanii*, deleting the origins causes faster growth. *H. volcanii* replicates its genome in a way similar to homologous recombination, in which two matching chromosomes swap strands to create a replication fork, though the details of this process are still being worked out (*Nature*, 503:544-47, 2013).



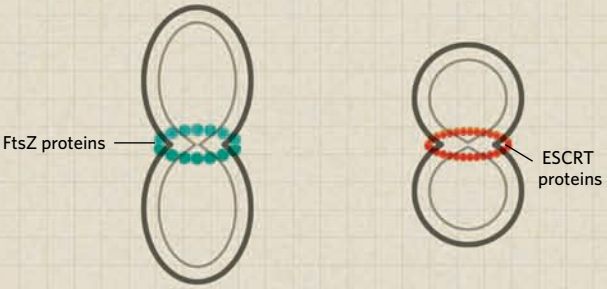
Genome Organization

Archaea can possess megaplasmids—hundreds of kilobases in size—that contain crucial genes. Some species are haploid like bacteria but many exhibit varying degrees of polyploidy. Many archaea use histones, as eukaryotes do, to organize their genomes, but some rely on alternative Alba proteins.



Modes of Cell Division

Some archaea divide via a mechanism similar to that of bacteria, using the cytoskeleton-like protein FtsZ to form a ring at the eventual division site (left). Others use homologs of eukaryote proteins, such as ESCRTs, to help separate daughter cells (right). Still others lack both of those systems, so they presumably have a distinct, as-yet-unknown mechanism, possibly relying on a form of actin.



Spotting Spillover

A step-by-step study of diseases that jump species gives subtle clues about future epidemics.

BY ASHLEY YEAGER

Down a dirt path outside of the village of Meliandou in Guinea once stood a tall, hollow tree where children used to play. Not anymore. This tree, now notorious as the potential starting point of the deadly Ebola outbreak that ripped through West Africa a few years ago, was burned after the disease sickened and killed hundreds of people over a four-month period. More than 10,000 ultimately succumbed to the disease between 2014 and 2016.

In April 2014, just a few months after the outbreak began, epidemiologist Fabian Leendertz of the Robert Koch Institute in Berlin and his colleagues went on a month-long expedition in southeastern Guinea to identify the source of the epidemic, which was suspected to have jumped from animals to humans. Fairly quickly, the team ruled out apes and other large animals as the zoonotic host for this Ebola outbreak. Numbers of grazing animals in West Africa appeared unaffected, and great ape populations may have actually increased, as the outbreak raced through Guinea, Sierra Leone, and Liberia.

Another possibility that Leendertz and his team considered was bats. Local chil-

dren commonly hunted the flying mammals, the scientists learned. In time, Meliandou villagers told Leendertz and his colleagues about the tree. Children had been seen catching bats in its hollow trunk. The villagers led the scientists down the dusty path to show them what was left of it, describing how the sky "rained" bats as the tree became engulfed in flames. Sure enough, when Leendertz's team collected soil samples from below the tree's charred trunk, the researchers recovered DNA that belonged to *Mops condylurus*, an insectivorous bat species.[1]

The evidence seemed to point to bats as the source of Ebola, Leendertz says. Emile Ouamouno, a two-year-old considered to have been patient zero in the outbreak, died a few days after contracting a fever, and probably got infected with the virus while playing with bats in the tree, he adds, "but we have no scientific proof." Indeed, the ultimate source of the outbreak remains a mystery—as is the case for many so-called zoonotic diseases.

BACTERIA CAUSE MORE ZOONOSES THAN ANY OTHER PATHOGEN TYPE.

More than 6 out of 10 known infectious diseases and 3 out of 4 new or emerging infectious diseases are spread to humans from other animals, including livestock and wildlife, according to estimates published by the US Centers for Disease Control and Prevention (CDC). Public health officials and scientists from many disciplines have been working together to understand how, when, where, and why pathogens spill over into humans, in hopes of preventing future epidemics. Key to these efforts are surveillance programs that aim to spot outbreaks early and respond quickly. Scientists are also scouring wildlife populations to identify undiscovered pathogens that could cause future zoonotic disease outbreaks.

Other researchers are working to identify biological factors that affect the probability of interspecies pathogen leaps, such as the immunological response of infected animals and the pathways of human exposure, efforts that could help scientists predict when and possibly where future zoonotic outbreaks might occur. By mapping the distribution of mammal species that host zoonotic pathogens, for example, disease ecologist Barbara Han of the Cary Institute of Ecosystem Studies in Millbrook, New York, and her colleagues have been working to identify regional hotspots at the greatest risk for spillover. "Everyone wants to know how well we can do in terms of prediction with zoonotic diseases, probably more so now after Ebola and Zika," Han tells *The Scientist*.

But even with a growing understanding of how species leaps happen, along with new data pointing to regions where spillover might occur, precise outbreak predictions aren't guaranteed. Some scientists say that such pinpoint prediction isn't even possible.

Searching for reservoirs

When hunting for the source of a zoonotic disease outbreak, scientists first work to identify the species that harbor that particular pathogen. These species are called reservoirs. Half of all carnivores are reservoirs of zoonotic disease, as well as some 20 percent of nonhuman primates and roughly 10 percent of rodent and bat species.

For many zoonotic pathogens, the main nonhuman reservoirs are relatively well known: dogs, foxes, bats, and cats for rabies; rodents for Lassa and hantaviruses; bats for Hendra and Marburg virus; and ticks, sheep, deer, rodents, and other small mammals for *Borrelia burgdorferi*, the bacterium that causes Lyme disease. For other pathogens, however, identifying the reservoirs has been more challenging, Leendertz says. And if scientists can't find them, it's hard to devise public health policies to prevent human infection.

There have been hints that Ebola virus lurks in populations of large African wildlife, with an ongoing epidemic possibly circulating in great apes and other animals such as forest antelope in the Democratic Republic of the Congo (DRC), the Congo, Gabon, and perhaps elsewhere. Before the outbreak of Ebola in the Congo in the early 2000s, researchers documented a die-off in great apes. There was also an outbreak of the virus recorded in chimpanzees in Taï National Park in Ivory Coast. So before their recent expedition, Leendertz and his colleagues thought maybe humans had contracted Ebola in the latest outbreak from eating infected bushmeat, specifically, larger animals such as antelopes and apes. "People going into the forest have a higher likelihood to find more dead animals," which they often take home and eat, Leendertz says.

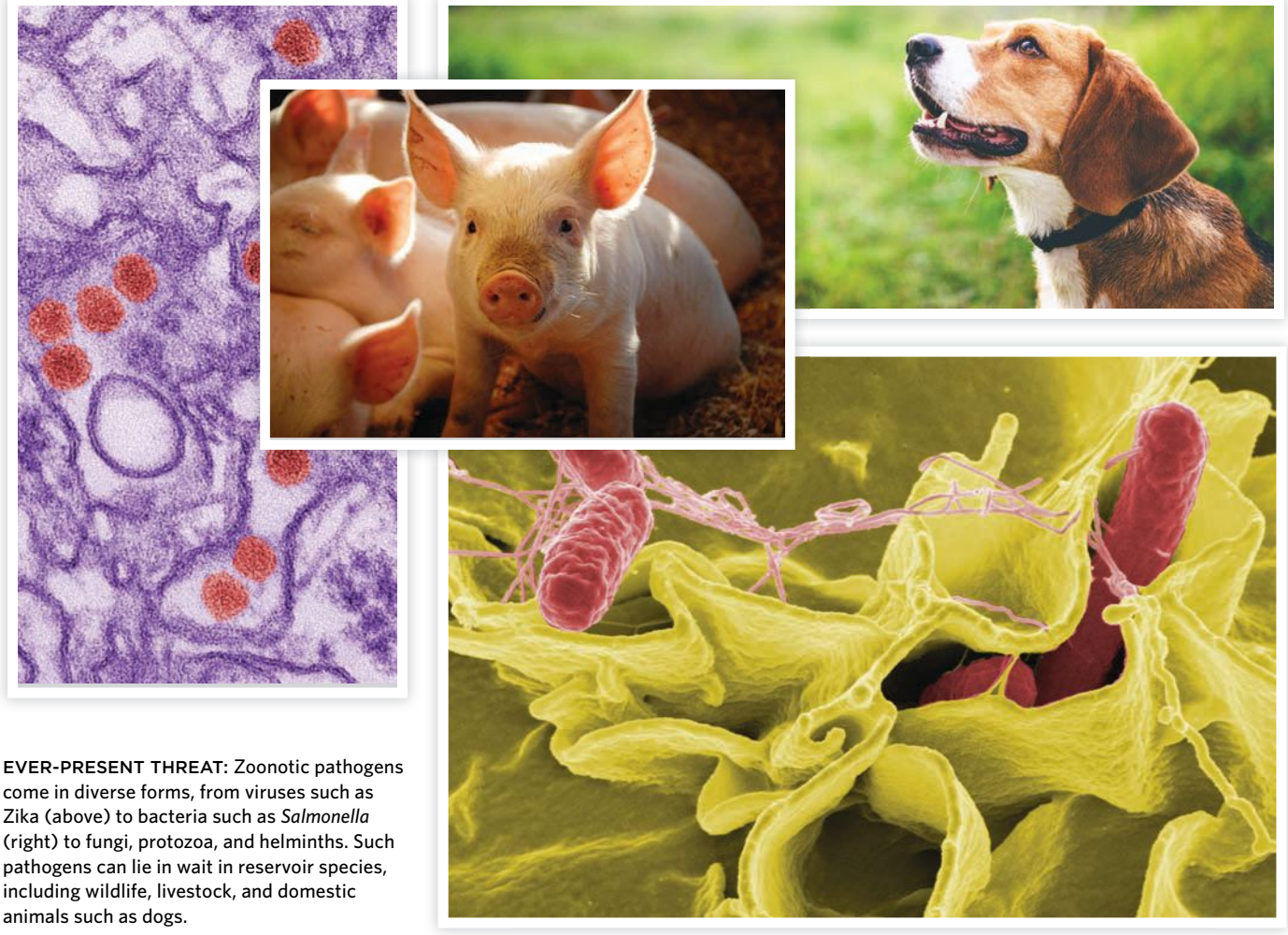
But almost as soon as Leendertz arrived in Guinea, he realized the "large wildlife" hypothesis didn't hold water: the ape populations in the forests of southern Guinea didn't appear to be declining. In addition, the region around Meliandou is forest-free. "It's just flat fields," Leendertz says. "It is a long way to reach the first forest where you could expect larger wildlife . . . too far to have any likelihood of fresh bushmeat arriving in the village," he explains. "The large wildlife hypothesis could quite clearly be excluded." Then the scientists learned about the bat-filled tree that stood just 50 meters from Meliandou.

The idea that insectivorous bats could be a reservoir for Ebola isn't far-fetched. Bats are particularly notorious for carrying zoonotic diseases, with more associated viruses than any other mammalian species. And researchers have successfully linked Ebola's relative, Marburg virus, to a bat reservoir. In 2007, researchers isolated Marburg virus from Egyptian fruit bats (*Rousettus aegyptiacus*) living in a DRC gold mine near where 154 individuals, most of them miners, had been infected.[2] Five years later, another set of scientists found that in Python Cave, a popular attraction in Uganda's Queen Elizabeth National Park, roughly 2.5 percent of the well-studied *R. aegyptiacus* colony was actively infected with Marburg virus at any given time.[3]

In the early 2000s, Eric Leroy of the International Center for Medical Research in Gabon and his colleagues set out to identify Ebola's reservoir. They collected and screened blood and tissue samples from more than 1,000 bats, birds, and rodents in Gabon and the Congo for traces of Ebola and found viral RNA fragments and antibodies against the virus in three bat species (fruit bats, not insectivores, in this case). However, the viral load—the quantity of the virus in the blood and tissue of each bat—was too low to isolate and replicate the virus, so the team couldn't definitively say that the bats were the reservoir of Ebola.[4] More than 10 years later, researchers still don't know which species carry the virus, Leendertz says. "We are truly still fishing in the dark."

Even when researchers successfully identify one possible reservoir of a zoonotic disease, that doesn't mean there aren't more. Since 1976, scientists have considered the Natal multimammate mouse (often called the African soft-furred rat) to be the sole reservoir of Lassa virus, which causes a sometimes fatal illness characterized by fever, headaches, vomiting, muscle pains, and potentially permanent hearing loss. "People get infected when the rodents come into houses and raid grain supplies," explains Raina Plowright, an infectious disease ecologist and wildlife veterinarian at Montana State University. The mice leave virus-infected urine or feces in the house, and people contract the pathogen when they touch the excrement or inhale virus particles lingering in the air. But even as many individuals heed public health directives to

THREE OUT OF FOUR EMERGING INFECTIOUS DISEASES ARE SPREAD TO HUMANS FROM OTHER ANIMALS.



EVER-PRESENT THREAT: Zoonotic pathogens come in diverse forms, from viruses such as Zika (above) to bacteria such as *Salmonella* (right) to fungi, protozoa, and helminths. Such pathogens can lie in wait in reservoir species, including wildlife, livestock, and domestic animals such as dogs.

ZIKA VIRUS: CDC GLOBAL/FLICKR; SALMONELLA: WIKIMEDIA COMMONS/ROCKY MOUNTAIN LABORATORIES, NIAID, NIH; PIG: © ISTOCK.COM/NEILYIMAGERY; DOG: © ISTOCK.COM/PEKIC

store food in rodent-proof containers and take out the trash, the virus "spills over and kills thousands of people every year in West Africa," Plowright says, suggesting there may be other reservoirs that transmit the virus, possibly in different ways.

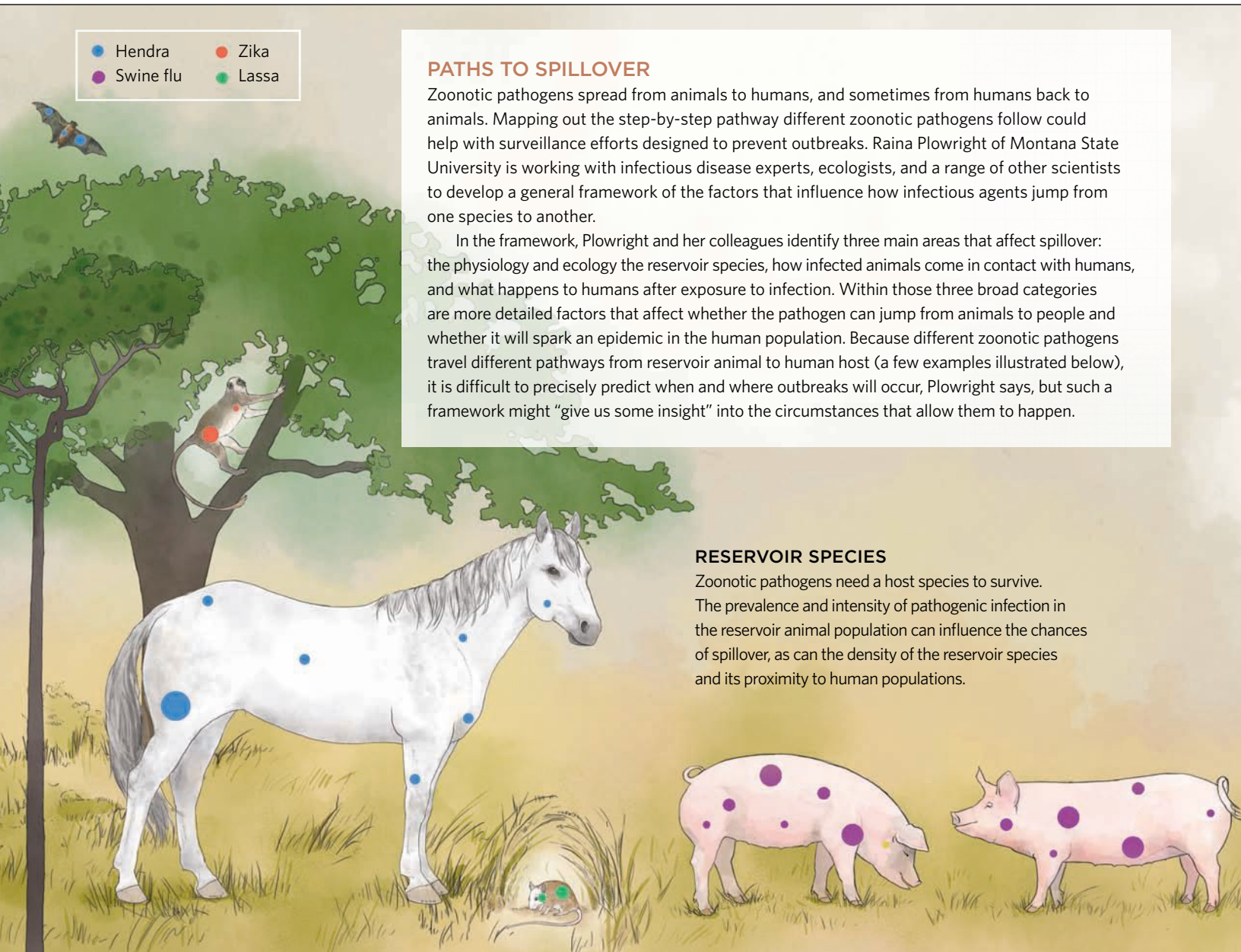
Indeed, in 2016 Elisabeth Fichet-Calvet of the Bernhard Nocht Institute for Tropical Medicine in Germany and her colleagues published findings showing that the African wood mouse (*Hylomyscus pamfi*) in Nigeria and the Guinea multimammate mouse (*Mastomys erythroleucus*) in both Nigeria and Guinea also carry the Lassa virus.[5] *H. pamfi* prefers the woods over fields, *M. natalensis*'s preferred habitat, and therefore the details of transmission to humans might differ, Fichet-Calvet and her colleagues note in their paper.

"It's just amazing that something that's such a huge burden on health . . . we know so little about," Plowright says.

Genetics, ecology, and other factors

In addition to some contact with a reservoir species—for example, via its meat, excrement, saliva, or blood—for zoonotic diseases to spill over into humans, the pathogen must be a generalist. "With exposure comes the chance an animal virus will jump to humans," says Edward Holmes, an evolutionary biologist and virologist at the University of Sydney in Australia. "Then, it's a question of whether that virus can infect human cells, and whether it can replicate to sufficient levels and have a route of transmission to the next host." The same is true for nonviral zoonoses.

Being capable of infecting many different host species gives zoonotic pathogens a higher chance of survival. With a variety of hosts and environments to explore, bacteria, viruses, and other infectious agents can invade new niches. This concept helps explain why zoonotic pathogens leap from animal to animal before jumping to humans, as in the case



PATHS TO SPILLOVER

Zoonotic pathogens spread from animals to humans, and sometimes from humans back to animals. Mapping out the step-by-step pathway different zoonotic pathogens follow could help with surveillance efforts designed to prevent outbreaks. Raina Plowright of Montana State University is working with infectious disease experts, ecologists, and a range of other scientists to develop a general framework of the factors that influence how infectious agents jump from one species to another.

In the framework, Plowright and her colleagues identify three main areas that affect spillover: the physiology and ecology the reservoir species, how infected animals come in contact with humans, and what happens to humans after exposure to infection. Within those three broad categories are more detailed factors that affect whether the pathogen can jump from animals to people and whether it will spark an epidemic in the human population. Because different zoonotic pathogens travel different pathways from reservoir animal to human host (a few examples illustrated below), it is difficult to precisely predict when and where outbreaks will occur, Plowright says, but such a framework might "give us some insight" into the circumstances that allow them to happen.

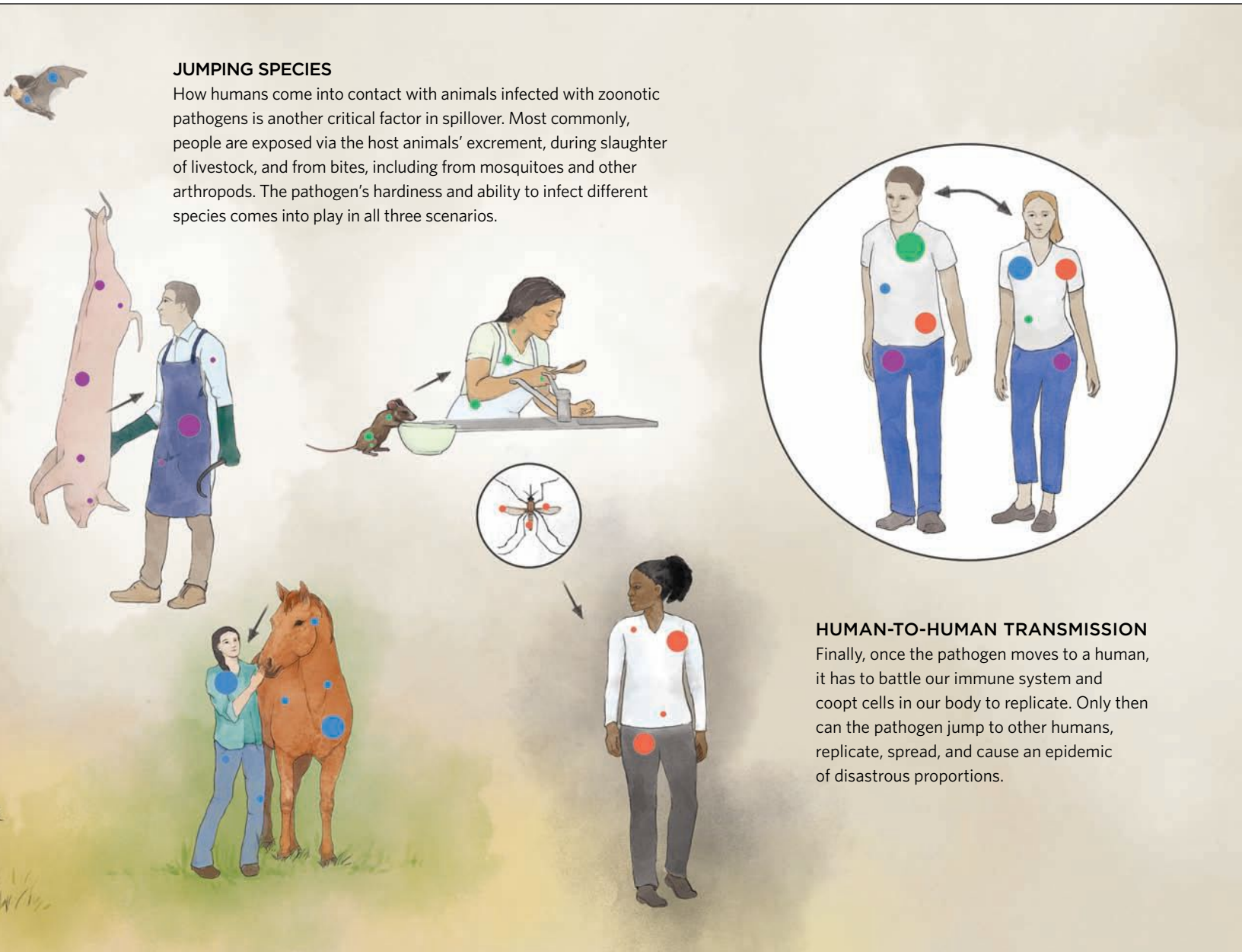
RESERVOIR SPECIES

Zoonotic pathogens need a host species to survive. The prevalence and intensity of pathogenic infection in the reservoir animal population can influence the chances of spillover, as can the density of the reservoir species and its proximity to human populations.

of Hendra virus, which lurks in bats, is then transmitted to horses, and then to humans. But for that transmission to be successful, the virus has to replicate to levels that cause infection in each of the animals involved. And what allows that to happen isn't well understood.

Host immunity is likely one factor affecting susceptibility to infection and the likelihood of carrying a pathogen for long enough to transmit it to others; and researchers have documented immune differences among species. Humans infected with immunodeficiency virus (HIV), for example, tend to have greater proliferation of T cells than sooty mangabeys chronically infected with the related simian immunodeficiency virus (SIV). HIV-infected people also have a higher expression of genes activated by cell-signaling proteins called interferons than monkeys with SIV.[6,7] The muted immune response in monkeys may prevent death of infected cells, allowing the virus to stay in the body and continue to replicate, and therefore have a chance to

RESEARCHERS ESTIMATE THAT 631,000 TO 827,000 UNIDENTIFIED VIRUSES EXIST THAT HAVE ZOONOTIC POTENTIAL.

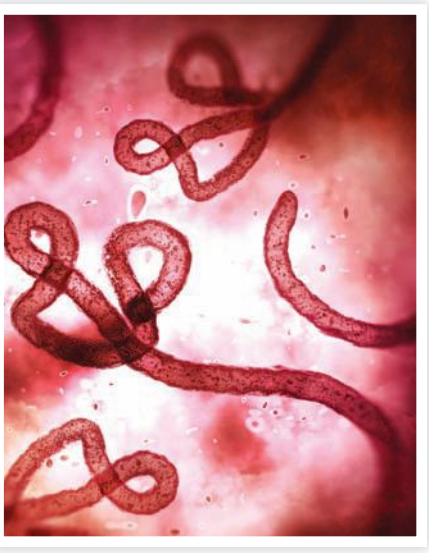


JUMPING SPECIES

How humans come into contact with animals infected with zoonotic pathogens is another critical factor in spillover. Most commonly, people are exposed via the host animals' excrement, during slaughter of livestock, and from bites, including from mosquitoes and other arthropods. The pathogen's hardiness and ability to infect different species comes into play in all three scenarios.

HUMAN-TO-HUMAN TRANSMISSION

Finally, once the pathogen moves to a human, it has to battle our immune system and coopt cells in our body to replicate. Only then can the pathogen jump to other humans, replicate, spread, and cause an epidemic of disastrous proportions.



BAT FEVER: The flying mammals are notorious for harboring pathogens with zoonotic potential, such as Ebola virus (shown here), Hendra virus, and Marburg virus. In fact, bats have more associated viruses than any other mammalian species.

jump to a new host without the monkey succumbing to infection.

Researchers have also seen similar differences between monkeys that develop acquired immunodeficiency syndrome and those that don't.[8] "The innate immune response is different," immunologist Judith Mandl of McGill University in Montreal tells *The Scientist*. Monkeys that develop AIDS produce more interferons and continue to produce them as the infection lingers, but monkeys that don't develop AIDS somehow shut down the interferon response. "That first response to infection sets the scene for what happens after," Mandl says—though scientists don't yet understand what causes this difference in the first place.

Tools for studying the immune responses of animals other than humans, apes, and mice are limited, but researchers are now beginning to probe these factors in other potential reservoir species. For example, interferons may also play a role in bats' incredible tolerance to viral infection: specifically, a mutation in the stimulator of interferon genes (STING), a signaling molecule that detects DNA floating in the cell cytoplasm. When STING senses free DNA in other mammals, the body generates interferons that spur an immune response to infection. But in bats, the mutation dampens interferon signaling and the consequent immune response to infection. (See "The Hosts With the Most" on page 16.) And rodents appear to be able to tolerate hantavirus thanks to regulatory T cells that reduce effector T-cell response to the infection as it progresses, allowing the virus to persist. As a result, the virus continues to circulate in the body until an opportunity arises to move to another host, potentially humans.

Ecological factors offer other insights into the risk of zoonotic spillover. In the case of Hendra, for example, 20 years of data suggest that sudden shifts from long, dry spells to sudden wet ones can increase the prevalence of the virus in flying foxes (bats of the genus *Pteropus*). In a shift from dry to wet conditions, eucalyptus and gum trees, which produce flowers that bats feed on, suddenly put all of their energy into growth rather than reproduction. Fewer flowers means less nectar for bats. Facing starvation, the bats will search for food around farms and other places with livestock and humans, all while excreting the virus at high levels.[9] Horses then contract the virus and spread it to humans. "Anecdotally, veterinarians have said there's Hendra when it's raining," Plowright says.

Tracking bats' behavior is lending weight to this idea. In recent years, for example, the northeastern Australian coast faced a long, dry El Niño that then changed to a wetter La Niña. In 2017, when Plowright and her colleagues were in Australia to track Hendra, the bats "were emaciated, just starving, and they were abandoning their young," she says. "And we thought, 'Well, we think we're going to have a Hendra event,' and we had a Hendra event."

Sighting future outbreaks

A primary goal of zoonotic disease prediction is identifying where the next big outbreak will occur. But such prediction requires data, which the Cary Institute's Han and her colleagues found to be lacking. "If we're going be able to predict anything, anything in any market—weather or stock prices or real estate, anything like that—you need a background level of information," Han says. For animal-borne diseases, "there was no baseline of where these things lived, and which species carried what, and so we realized we just needed to map everything," she says.

Combining data on species diversity and zoonotic disease hosts, she and her colleagues identified the tropics, specifically South America and Eastern Africa,

and some parts of Europe and the subarctic as hotspots for zoonotic disease outbreak.[10] "One of the things that really jumped out to me was this pattern of where the carnivores are located," Han says. "There's lots of arctic carnivores [including foxes, wolves, and polar bears] and . . . we noticed that they carried more diseases than we would expect." With climate change, the speed at which warming is happening in the Arctic is faster than elsewhere, and changes to the environment would bring changes to the disease dynamics there. "What's going to happen when things thaw out, and humans start to make better use of that land that's accessible since there's no permafrost? That whole system is going to be really interesting to keep an eye on," she says.

On a more local scale, understanding the factors that affect transmission could help scientists prevent outbreaks. In Kenya, for example, researchers have tied Rift Valley Fever with El Niño rainfall. The disease is caused by a virus that can infect livestock, which can then pass the pathogen on to humans. Mosquitoes, which rely on moisture to reproduce, prey on both livestock and humans and can also transmit the virus between species. In the past few years, researchers, veterinarians, and farmers have worked together to build a surveillance network to report symptoms of the virus in farm animals. Reports of miscarriages and hemorrhagic disease among livestock were highest in months with the highest rainfall, Harry Oyas of the Kenya Ministry of Agriculture and his colleagues wrote in April in *PLOS Neglected Tropical Diseases*.[11] The early warning system developed in Kenya could prevent spillover of the Rift Valley Fever virus into humans, the authors say.

The program in Kenya is one of many monitoring programs developing around the world. In addition, researchers have launched several surveillance initiatives to detect and discover pathogens that have the potential to cause pandemics. A few of these projects, such as the University of California, Davis–based PREDICT program, have been capturing and testing zoonotic disease reservoir species to identify the pathogens they carry and determine their likelihood of jumping to humans. Some such efforts are focusing on known threats, such as MERS, SARS, and Nipah virus, but there are also pathogens that could have zoonotic potential that haven't yet been identified.

EVEN WITH A GROWING UNDERSTANDING OF HOW SPECIES LEAPS HAPPEN, PRECISE OUTBREAK PREDICTIONS AREN'T GUARANTEED.

Focusing on viruses, which have caused devastating epidemics in the last few decades, a team of researchers recently analyzed virus-host interactions, the history of viral zoonoses, and patterns of viral emergence, estimating that 631,000 to 827,000 unidentified viruses exist that have zoonotic potential.[12] As a preemptive strike, the researchers have built on the PREDICT model and developed the estimated $1 billion Global Virome Project, which will collect blood samples from rodents, nonhuman primates, bats, and other wildlife to discover the majority of unknown viruses, with the aim of predicting the next pandemic—before it hits humans.

Not everyone is on board with the idea of predicting zoonotic disease outbreaks, however. The whole idea of pandemic prediction is "foolish," Holmes tells *The Scientist* by email. "There are no generalities that can be used to make accurate predictions," he says. Just because "you see a virus in animals does not mean it will spread in humans." Rather than trying to predict the future, he supports "real-time proactive surveillance of the human-animal interface to see what we're being exposed to." Then, he says, with a globally coordinated response and research network, any emerging infection "can be stamped out quickly." ■

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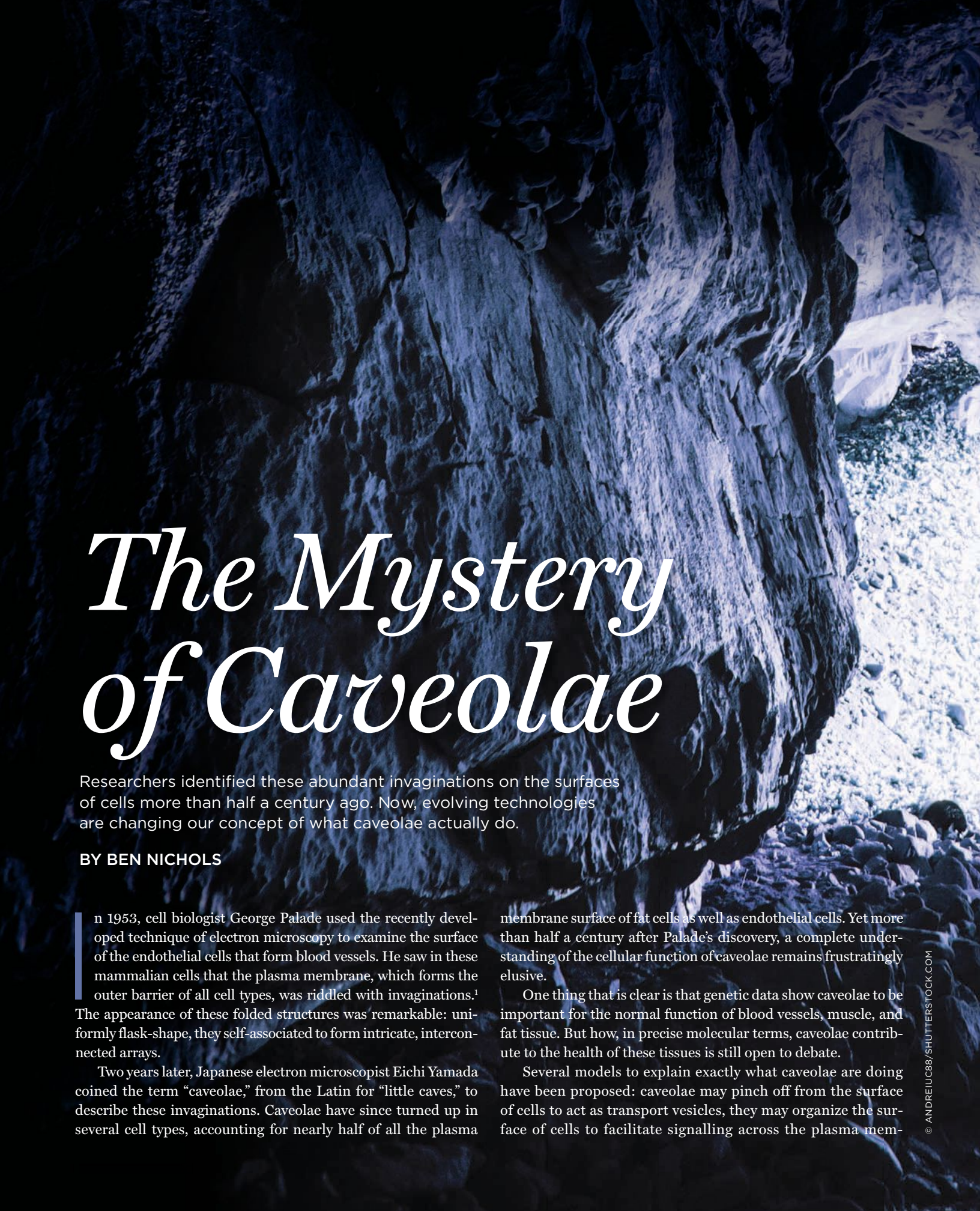


The Mystery of Caveolae

Researchers identified these abundant invaginations on the surfaces of cells more than half a century ago. Now, evolving technologies are changing our concept of what caveolae actually do.

BY BEN NICHOLS

In 1953, cell biologist George Palade used the recently developed technique of electron microscopy to examine the surface of the endothelial cells that form blood vessels. He saw in these mammalian cells that the plasma membrane, which forms the outer barrier of all cell types, was riddled with invaginations.[1] The appearance of these folded structures was remarkable: uniformly flask-shape, they self-associated to form intricate, interconnected arrays.

Two years later, Japanese electron microscopist Eichi Yamada coined the term "caveolae," from the Latin for "little caves," to describe these invaginations. Caveolae have since turned up in several cell types, accounting for nearly half of all the plasma membrane surface of fat cells as well as endothelial cells. Yet more than half a century after Palade's discovery, a complete understanding of the cellular function of caveolae remains frustratingly elusive.

One thing that is clear is that genetic data show caveolae to be important for the normal function of blood vessels, muscle, and fat tissue. But how, in precise molecular terms, caveolae contribute to the health of these tissues is still open to debate.

Several models to explain exactly what caveolae are doing have been proposed: caveolae may pinch off from the surface of cells to act as transport vesicles, they may organize the surface of cells to facilitate signalling across the plasma mem-

brane, and they may protect cells from mechanical damage. These different models are not mutually exclusive, and the task of unravelling the molecular mechanisms that support such diverse functions is ongoing. Given the amount of research on caveolae, it is surprising that there is not more conclusive experimental evidence of their cellular activities; relative to other subcellular compartments and structures, caveolae are still enigmatic.

Recent investigations into the formation of caveolae have provided a much more complete picture of the protein complexes that sculpt the plasma membrane into such distinctive shapes. One important implication of the new data is that the shape of caveolae is not fixed. Rather, the structures can undergo dynamic transitions between flat and invaginated states, and some of the recently identified protein components of caveolae may well be important for regulating these changes. This does not completely resolve the debate on the function of caveolae, but it does provide considerable support for the idea that caveolae act as buffers within the membrane to stop stretch forces from rupturing cells. In motile, multicellular organisms with a closed and pressurized vascular system, mechanical stretching is an important part of the functional environment of many different cell types, and defining a role for caveolae in withstanding stretch forces would open up this little-understood area of cell biology.

Four eras of discovery

We can divide the history of research on caveolae into four eras, each of which yielded different hypotheses for what these structures might be doing in cells. From Palade's initial description in 1953 until the early 1990s, observations were driven by electron microscopy, and caveolae were defined by their characteristic shape and tendency to cluster in complex arrays.

The era of the molecular biology of caveolae began in 1992, when a landmark paper from the laboratory of Richard Anderson at the University of Texas Southwestern Medical School identified the first caveolae-associated protein, which the investigators called caveolin-1.[2] This discovery of a defining component present specifically in caveolae triggered a series of biochemical studies linking the protein to a number of potential binding partners involved in cell signalling.

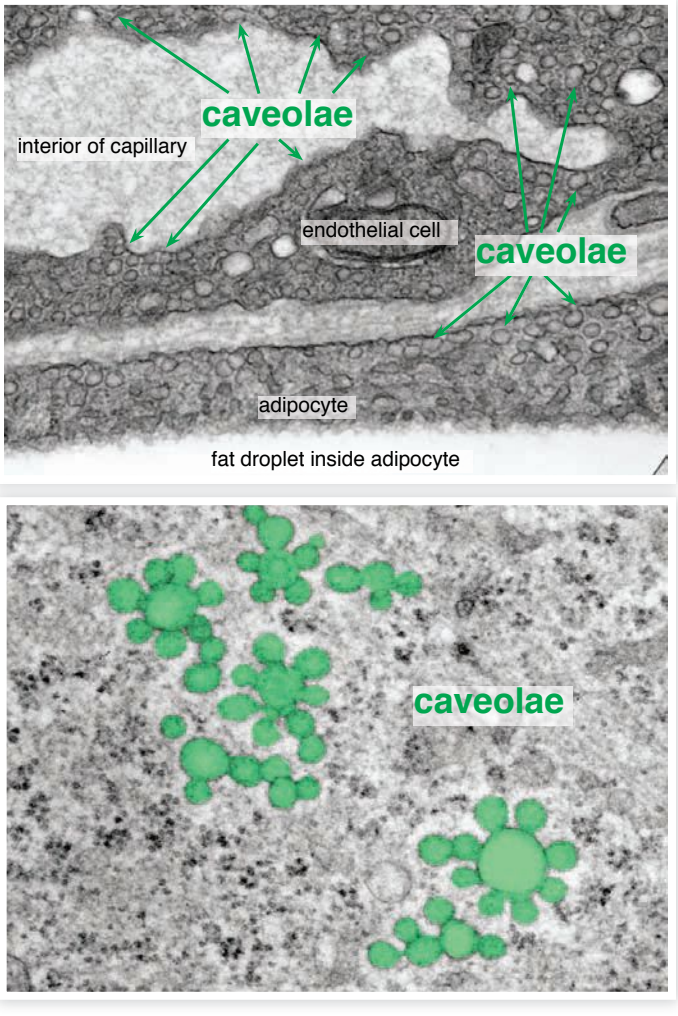
Genetics entered the fray with the 2001 development of mice lacking the gene for caveolin-1, *CAV-1*. These mice surprised the field by being relatively healthy and developmentally normal.[3,4] Most recently, close molecular structural scrutiny of caveolae has revealed new clues to their function. In 2008, the laboratories of Rob Parton at the University of Queensland in Australia and Paul Pilch at Boston University School of Medicine showed that proteins now called cavins are important structural elements of caveolae.[5,6] These findings both led to the beginnings of a detailed structural model for how caveolae assemble, and paved the way for experiments showing that caveolae are unexpectedly flexible.

The era of electron microscopy

In the initial era of caveolae research, much of the early discussion of function was informed by the abundance of these structures, particularly in endothelial cells. Why are there so many?

Controlled transport of albumin and other macromolecules between blood and tissue interstitial fluid is a crucial part of mammalian physiology, not least because it generates a concentration gradient of albumin to maintain fluid balance in the pressurized vascular system. Palade and others proposed that caveolae act as transport containers, or vesicles, carrying albumin from one side of the endothelium to the other. Many caveolae are needed to support the high flux of albumin and fluid, they reasoned.

Even at this early stage of caveolae exploration, additional functions were posited. In the 1970s Angela Dulhunty of Australian National University and Clara Franzini-Armstrong of the University of Pennsylvania, both then at the University of



CAPTURING CAVEOLAE: The structures were first imaged on endothelial cells by electron microscopy in 1953, but their function is still largely mysterious.

IMAGES BY GILLIAN HOWARD, COURTESY OF BEN NICHOLS, MRC

Rochester, carried out beautifully detailed measurements of the prevalence of caveolae in frog muscle tissue and showed that, as muscles are stretched, caveolae become less abundant.[7] The researchers suggested that caveolae flatten out to give the inelastic plasma membrane some capacity to extend as muscle cells stretch, and pointed out that this buffering action could explain both the abundance of caveolae and their propensity to form interlinked clusters.

Enter molecular biology

The discovery of caveolin-1 in 1992 as a defining protein component of caveolae was a technical tour de force, and the same paper documented striations on the surface of caveolae suggestive of a protein coat. Caveolin-1 is a membrane protein with both N and C termini in the cytoplasm and stretches of hydrophobic amino acids embedded within the lipid bilayer. Caveolin-1's discovery initially raised more questions than it answered, however.

Co-immunoprecipitation experiments, which use detergents to disrupt the membranes of cultured cells and antibodies immobilized on beads, have revealed associations between caveolin-1 and a large array of different plasma membrane receptors, leading to the idea that caveolae play a role in signal transduction. However, because caveolin-1 forms highly stable oligomers that resist extraction by commonly used detergents, co-immunoprecipitation experiments can yield false-positive interactions if solubilization is incomplete. Indeed, later research examining the structures of several signaling proteins thought to interact with caveolin-1 clearly showed that their putative caveolin binding domain is, in fact, unlikely to be accessible for interactions with caveolin, undermining the idea that caveolae act as hubs for signal transduction.[8] Much more recently, the use of chemical cross-linkers has allowed for more-stringent detergents to be used to isolate caveolin's binding partners without triggering the disassembly of protein complexes, and, tellingly, no signaling receptors were detected.[9]

Another confounding issue surrounding the initial attempts to determine binding partners was that the identification of caveolin-1 coincided with a rise in interest in plasma membrane structures known as lipid rafts. The fact that many membrane lipids and proteins, including caveolin-1, are tough to extract with detergents was interpreted as evidence that these components reside in the same region, or microdomain. This may or may not be the case, and the literature contains different views and findings addressing the issue. But it is now abundantly clear that caveolae do not contain enriched populations of many of the molecules thought to be present in lipid rafts, and are thus likely to be entirely distinct from rafts.

Genetic data surprise the field

Early hypotheses about caveolar function were challenged when, in 2001, the groups of Michael Lisanti, then at the Albert Einstein College of Medicine in New York, and Teymuras Kurzchalia at the Max Planck Institute of Molecular Cell Biology and Genetics in Germany independently reported that mice lacking caveolin-1 do not have caveolae but are apparently healthy.[3,4] Despite showing some phenotypic anomalies, the animals had no major developmental defects, and ran around their cages eating, reproducing, and generally acting normally.

Genetic data show caveolae to be important for the normal function of blood vessels, muscle, and fat tissue.

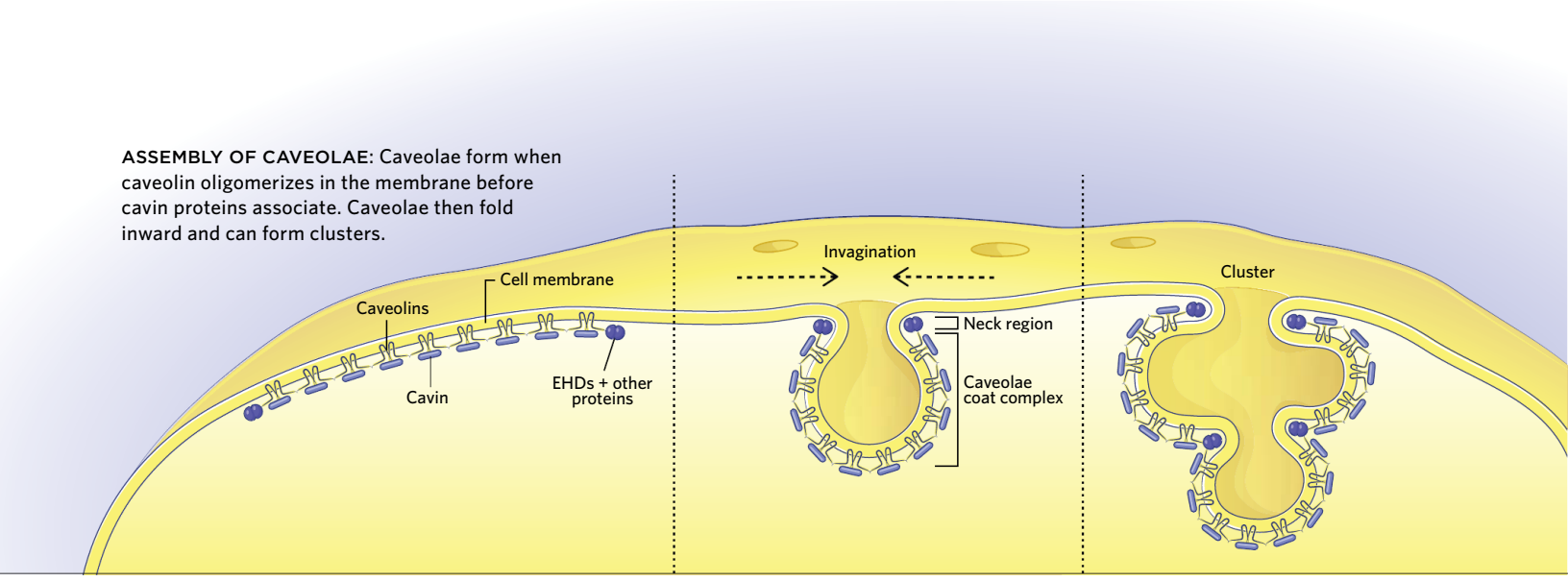
There were already hints that mice without caveolae do in fact have an array of less obvious problems, which we now know to include vascular abnormalities, lipodystrophy, muscular dystrophy, and other fat-related metabolic dysfunctions. These phenotypes show that adipose tissue, endothelium, and muscles—the very tissues where caveolae are super-abundant—do not function correctly without caveolae. Nevertheless, given the near-normalcy of the *CAV-1* knockout mice, one cannot escape the conclusion that either caveolae are not crucial components of cell signaling pathways and the machinery for regulating endothelial permeability, or that complex compensatory mechanisms exist to allow such key systems to function in the absence of caveolae. This conundrum can perhaps best be resolved from the bottom up. A detailed knowledge of the molecular mechanisms mediating the assembly of caveolae may lead to further details on functionally significant interaction partners, and thereby towards specific information on what caveolae actually do in cells.

Caveolae and membrane stretching

Over the past decade, a series of papers from several laboratories has transformed our picture of the structure of caveolae. There was already evidence for a protein coat around the bulb of caveolae, and we now know that the components of the coat are caveolins and cavins. (See illustration on following page.)

There are three caveolin isoforms in mammals. Caveolin-1 is crucial for forming caveolae in tissues other than striated muscle, while caveolin-3 has a similar importance in muscle. Caveolin-2 appears to be less essential for forming caveolae.

There are four cavins: cavin-1 is needed for forming caveolae in all tissues, cavin-2 and cavin-3 have variable abundance across different tissues, and cavin-4 is muscle-specific. The cavins contain extended regions that are likely to join together cavins of the same or different varieties into coiled oligomers. My colleagues and I examined the purified complex of cavins and caveolins by electron microscopy, revealing it to have the size and shape of the membrane bulb of caveolae.[9] It is likely, therefore, that this caveolar coat complex (CCC) is what generates the distinctive shape of caveolae.



ASSEMBLY OF CAVEOLAE: Caveolae form when caveolin oligomerizes in the membrane before cavin proteins associate. Caveolae then fold inward and can form clusters.

The discovery of cavins and the CCC has generated tools to study the assembly and disassembly of caveolae, as the dissociation of key components from the CCC can be used as a proxy for changes in the functional state of caveolae. This approach forms the core of a 2011 landmark paper published by Christophe Lamaze and colleagues at the Institut Curie in Paris.[10] In this study, the authors showed that caveolae disassemble or flatten out under increased membrane tension. These data support the idea first promulgated by Dulhunty and Franzini-Armstrong back in 1975 that caveolae can buffer mechanical tension within the plasma membrane, and thereby prevent membranes from breaking under stress forces. Lamaze and colleagues went on to show that cells with compromised caveolin function are more likely to rupture when stretched.

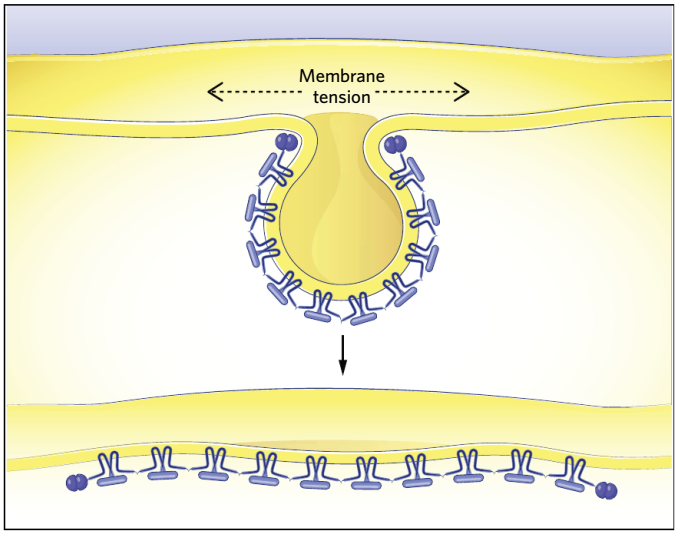
Since then, further studies have provided clear in vivo confirmation that at least partial disassembly of the CCC and flattening out of caveolar membranes is caused by increases in plasma membrane tension and that both muscle cells and endothelial cells under physiologically relevant stretch forces are more likely to suffer membrane rupture if they lack caveolae.

These and other experiments have generated a consensus within the field that caveolae protect cells from mechanical stresses that could otherwise lead to breaks in the plasma membrane. The precise mechanism of this protective effect is less clear. The simplest idea is that the folds in the plasma membrane introduced by caveolae flatten out when the cell needs to stretch, and this stops the membrane from breaking under tension. But other mechanisms for maintaining the integrity of the membrane are also possible. In 2013, for example, Matthias Corrotte and Norma Andrews of the University of Maryland, College Park used a pore-forming bacterial toxin to introduce small holes or breaks in the plasma membrane, and found that this induces local internalization of caveolae, potentially repairing membrane breaks by removing the damaged region of membrane from the cell surface.[11] And there is still a large literature linking caveolae to signal transduction, so it is possible that caveolae protect cells from membrane damage indirectly, by inducing cellular responses such as cytoskeletal rearrangement or transcriptional changes. Once again, we are left with the hope that further molecular details will generate insights into these other proposed functions.

Neck vs. bulb

While the CCC around the bulb of caveolae is increasingly well characterized, the nature and precise function of proteins found around the constricted neck region is less well understood. There are three protein families that may be relevant: dynamins, pacsins, and EHDs.

In 1998, two papers that appeared back-to-back in the *Journal of Cell Biology* claimed that dynamin-2, involved in the budding of clathrin-coated vesicles, is similarly involved in the



CAVEOLAE DYNAMICS: Data from recent studies have led scientists to suspect that caveolae may have a role in buffering changes in cell membrane tension by changing their conformation. Indeed, membrane tension causes flattening of caveolae and loss of clusters of caveolae. It is probable, but not fully proven, that cavin and caveolin proteins can switch between flat and invaginated complexes on the membrane, but other proteins known as EHDs resist these processes.

budding of caveolae.[12,13] More than 20 years later, however, our knowledge of how dynamin might act in budding remains limited.

Some publications have argued that caveolae are unlikely to bud from the membrane at all, while my group recently used genome editing to express GFP-tagged caveolin-1 at endogenous levels and showed that caveolae are internalized at a very slow rate.[14,15] The basic problem here is that there is still no extracellular cargo known to be internalized specifically by caveolae. Unless and until such cargo is identified, it remains possible that internalization of caveolae occurs not so much to deliver material (such as signaling receptors, for example) from the cell surface to the cell interior, as to control the turnover or distribution of caveolae themselves.

The shape of caveolae is not fixed. Rather, they can undergo dynamic transitions between flat and invaginated states.

EHD proteins and pacsins are also likely present at the neck of caveolae. EHD proteins are ATPases that, like dynamin, use energy from nucleotide hydrolysis to alter the curvature of the membrane to which they are bound. Only in the past year has it become clear that multiple members of the EHD protein family are recruited to the caveolae neck, where they appear to have two distinct but possibly related functions.[16] In cells lacking the EHD proteins, caveolae are much less likely to form interlinked clusters. Also, although cells lacking EHDs clearly still have caveolae, the number of caveolae drops markedly when the cells are stretched. These observations suggest that EHDs help link caveolae together in higher-order arrays and allow caveolae to maintain their shape in the face of repeated membrane stretching.

Less is known about how pacsins function in caveolae. They are not present in all of these structures, but the depletion of pacsins can cause a reduction in the number of caveolae. We speculate that the proteins at the neck control the dynamic distribution and potentially the reversible changes in membrane shape that are emerging properties of caveolae, while the basic membrane shape of the caveolar bulb is determined by the CCC.

Plenty of missing pieces

In the last few years our understanding of the parts list for caveolae has been transformed. Nevertheless, many unknowns remain, such as how cavins and caveolins fit together to make a stable, bulb-shape protein lattice. More high-resolution structural information on the CCC will be invaluable in answering this question. It will also be important, though difficult, to better understand the molecular underpinnings of the transitions in membrane shape that we infer caveolae to undergo. This will likely require establishing assays to determine the kinetics with which these morphological changes occur.

Further gaps in the molecular picture of caveolae include how cells regulate their polarized, nonrandom distribution, which suggests that they are likely linked to the cortical cytoskeleton. Why there are four different cavins and three different EHDs recruited to caveolae is also not at all clear, though it seems likely that this variety allows functional specialization of some kind.

Finally, as has been the case for the 65 years since their discovery, perhaps the biggest question pertains to the cellular function of caveolae. While we are increasingly certain that caveolae protect cells from mechanical damage, both how they do this, and what else they do, are still unresolved. More time, and more data, will be needed to unravel the mystery of caveolae. ■

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The Literature

CELL & MOLECULAR BIOLOGY

Packing DNA

THE PAPER

M. Ganji et al., "Real-time imaging of DNA loop extrusion by condensin," *Science*, doi:10.1126/science.aar7831, 2018.

How does a human cell neatly pack more than 2 meters' worth of DNA into 46 tiny chromosomes? One popular theory is that, with the help of a large protein complex known as condensin, DNA forms many compact loops.

One of the earliest pieces of evidence for this process arrived in the 1970s, when a pair of biochemists observed loops of DNA in electron micrographs of HeLa chromosomes. Researchers later pinpointed condensin as a key protein complex involved in forming these structures, yet "how it works has been completely unknown," says Christian Haering, who investigates chromosome structure and dynamics at the European Molecular Biology Laboratory in Germany. Some scientists have proposed that condensin binds to DNA and extrudes looped strands, but no one had directly observed the phenomenon.

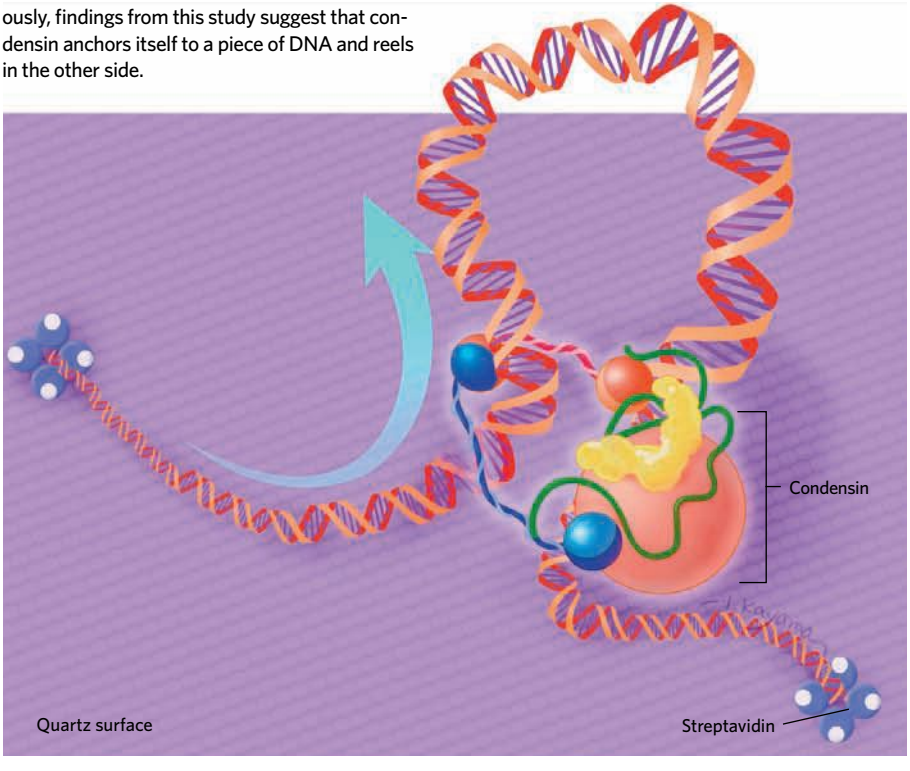
To try to catch condensin in the act, Haering and his colleagues tethered a long piece of biotin-tipped DNA to a quartz surface covered in streptavidin, anchoring the two ends of the strand in a microfluidic chamber. Then, they stained the DNA with a bright-orange dye and added a buffer, fluorescently labeled yeast condensin, and ATP. Watching through a microscope, the researchers saw that condensin gradually pulled the DNA into a loop, drawing on just one side of the strand. "A single condensin would grab on [to the strand], and like a motor, asymmetrically start reeling in the DNA," explains study coauthor Cees Dekker, a biophysicist at Delft University of Technology in the Netherlands.

"I was surprised," says Haering. "I've been very skeptical that condensin could be this loop-extruding enzyme, because if you look at its structure, it's not obvious how it could work as a motor that pushes out the DNA." He adds that the details remain a bit of a mystery, which he and his colleagues plan to tackle next.

"It was really great to see [loop extrusion] happening in real time," says Camilla Björkegren, a cell and tumor biologist at the Karolinska Institute in Sweden who wasn't involved in the work. However, she notes, it's not clear whether or how the process might work within cells, where the nucleic acid is wrapped into nucleosomes by histone proteins.

At least one scientist remains unconvinced that the new study proves the loop extrusion hypothesis. Biochemist Frank Uhlmann of the Francis Crick Institute in the U.K. who is collaborating with the coauthors on similar experiments but was not involved in this study says it's too early to rule out alterative theories of condensin's activity. For example, he and his colleagues proposed a model in which the protein complex binds to and stabilizes spontaneously forming loop structures rather than squeezing out new ones. Uhlmann says he'd like to see observations of more DNA-condensin interactions; for now, he adds, what the protein complex is actually doing remains "an exciting, open question."

—Diana Kwon



ASYMMETRY: Researchers anchored a DNA strand to a quartz surface using streptavidin and watched as the protein complex condensin latched to the nucleic acid and formed loops. Contrary to previously established computational models of loop extrusion, which predicted that the two sides of the DNA strand would be reeled in simultaneously, findings from this study suggest that condensin anchors itself to a piece of DNA and reels in the other side.

MAMMOTH APPETITE: Scientists strive to understand how large animals in northerly climes subsisted on limited vegetation.

ECOLOGY

Ice Age Paradox

THE PAPER

D. Zhu et al., "The large mean body size of mammalian herbivores explains the productivity paradox during the Last Glacial Maximum," *Nat Ecol Evol*, 2:640-49, 2018.

A PALEONTOLOGY PARADOX

During the Last Glacial Maximum, global temperatures and atmospheric carbon levels were less than ideal for vegetation to grow in the northern hemisphere, but the fossil record shows that herbivorous woolly mammoths were plentiful in unglaciated regions—a discrepancy termed the "productivity paradox."

MODEL MAMMOTHS

An international team of researchers approached the problem by modeling plant cover based on climate, the water cycle, and other variables, and incorporating the presence of large grazing animals. The scientists tested the model on a variety of modern ecosystems involving grazers, and found that its predictions of grass cover generally matched observations. For the Ice Age scenario, it was the mammoths' large bodies and relatively efficient metabolisms that allowed them to survive on sparse vegetation, says coauthor Nicolas Viovy, an informatics engineer and biogeochemist at Versailles Saint-Quentin-en-Yvelines University in France.

TOP-DOWN OR BOTTOM-UP?

Love Dalén, a paleogeneticist at the Swedish Museum of Natural History, says the paradox really boils down to whether vegetation limited the abundance of grazers or vice versa. The new study rests on the assumption that mammoths controlled the abundance of vegetation, he says, but it's still an open question as to whether that was the case.

RUMINATE ON IT

How the mammoths digested plant matter is also important in solving the paradox, says Danielle Frasier, a paleobiologist at the Canadian Museum of Nature. "I would have been interested to see how the model would differ if they included the digestive physiology of mammoths," which were relatively efficient "hindgut fermenters," she says. Viovy says the team plans to continue improving the model to account for more variables like this one. —Jim Daley

BYE BYE BIRDIE: A study suggests a weak immune response could drive pathogens toward greater virulence.

EVOLUTION

Incomplete Immunity

THE PAPER

A.E. Fleming-Davies et al., "Incomplete host immunity favors the evolution of virulence in an emergent pathogen," *Science*, 359:1030-33, 2018.

FINCH KILLER

Since 1994, an epidemic of conjunctivitis caused by the bacterium *Mycoplasma gallisepticum* has ravaged house finch *(Haemorhous mexicanus)* populations across North America. Arietta Fleming-Davies and Dana Hawley, disease ecologists at the University of San Diego and Virginia Tech, respectively, noted that many birds that had been infected remained susceptible to later infection. The phenomenon reminded Fleming-Davies of findings by other researchers that when a vaccine partially protects a host, it can drive a pathogen to evolve more virulence. (See "Do Pathogens Gain Virulence as Hosts Become More Resistant?" *The Scientist*, October 2017.)

BIRD IN A CAGE

To find out whether something similar was happening in finches, the duo and their colleagues simulated the natural epidemic in the lab, infecting captive birds with increasingly virulent strains of *M. gallisepticum* and observing the severity of their symptoms. Fleming-Davis then used the data to model infection and the evolution of pathogen virulence in the wild.

CHINKS IN THE ARMOR

The model predicted almost twice as much virulence when bacterial attackers were confronted with incomplete immunity compared to zero immunity, Fleming-Davis tells *The Scientist*. This suggests, the authors write, that a weak immune response favors nastier pathogens that can overcome defenses.

NOT THE WHOLE STORY

That idea is "an interesting concept, especially as it relates to vaccine research," says Molly Staley, an evolutionary biologist at the Brookfield Zoo, "but it doesn't quite fit with what we know" about *M. gallisepticum*. The birds' ability to resist manipulation of their immune systems by the bacteria is the primary driver of the pathogen's increased virulence in the wild, Staley explains, adding that most of the birds infected in the wild are immunologically naïve juveniles, so the responses of previously infected birds likely have only a small effect on virulence evolution. —Jim Daley

Trauma Biologist

Israel Liberzon's research has helped crack open the black box of post-traumatic stress disorder.

BY ANNA AZVOLINSKY

Israel Liberzon discovered his natural proclivity for post-traumatic stress disorder (PTSD) research when he arrived at the University of Michigan (UM) and Veterans Administration Ann Arbor Medical Center in the 1980s. There, he encountered numerous combat veterans with the condition. "The veterans tended not to like the doctors, who they thought couldn't relate to them. It was always very natural and easy for me to relate to my veteran patients, to understand where they were coming from," says Liberzon. "I didn't have the barriers of communication that other clinicians had because I had the combat experience in common with the patients."

Liberzon had served a mandatory three years in the Israeli Defense Forces starting at age 18. There, he trained as a combat paramedic and became part of an airborne unit, treating soldiers and civilians wounded in combat zones.

There is nothing more fascinating to me than the function of the brain.

He says he feels fortunate to have made personal connections with such patients. Most that he treated in the UM VA system were Vietnam War veterans, but there were also older vets who'd served in World War II and the Korean War. "It was fascinating because they were experiencing things and dealing with things that were forty years old, and they still had a profound effect on their daily life," Liberzon says. One of his most memorable patients had been a WWII pilot, who, on the way back from a bombing raid over Germany, had to use his parachute to land, and it was completely shot up on the way to the ground. "He was dealing with nightmares from this memory 50 years later."

Liberzon saw these patients as individuals, but in the aggregate they represented a major scientific question. "To me, it was one of the most fascinating questions in psychiatry, but also in psychology and neuroscience—how experiences are translated into biology, and how those biological changes can lead to psychological symptoms and behavior changes," says Liberzon.

Since his experience in the VA, Liberzon has devoted his career to trying to answer these questions. He was the first UM staffer to take on both clinical and laboratory PTSD research. His lab created the first rodent model of the symptoms and neural physiology seen in PTSD patients. And Liberzon's studies have contributed to novel and increasingly comprehensive models of the causes and effects of PTSD.

IMMIGRATION, ADAPTATION

Liberzon was born in Ukraine, in the Soviet Union, in 1958. He says that his attraction to science and medicine came from his parents, who were both rural doctors. "Being a physician was not a lucrative profession in the Soviet Union, but my parents were dedicated to helping people," he says. "That was really influential and instills expectations in you that life should be dedicated to something important and useful."

Deep in the Cold War, in 1971, the family emigrated to Haifa in Israel. They were among the first wave of Jewish families given permission to relocate from the Soviet Union. "It was just the beginning, and it was serendipitous that we were allowed to leave," says Liberzon.

The 13-year-old Liberzon adapted to Israeli culture, learning the language easily, integrating well at school, and making friends. He also relished the climate of Haifa. "The weather was terrific. We were less than a mile from the beach and spent half the year there swimming and hanging out."

In 1976, Liberzon joined the Israeli Defense Forces—a national requirement for all Israeli adults. "I am not used to talking about my military experience much," he says. "But I did realize how intrigued I was by the process of learning how to take care of people. It was an amazing discovery for me that solidified my interest in medicine."

After becoming a civilian again in 1979, he wanted to go to medical school, which in Israel is a competitive, six-year program that combines undergraduate and graduate studies. Because Liberzon's high school grades were not good enough, he first enrolled in Hebrew University in Jerusalem, studying biology, to bring his grades up. "Being in college was great! After the military, it was so easy and pleasant. I couldn't believe that all I had to do was to go to classes and do some homework," he says.

When he had completed his biology courses in the spring of 1980, he was accepted to the Sackler Faculty of Medicine at Tel Aviv University, where he started his formal medical training. Significant milestones followed: Liberzon participated in military engagements, including the Lebanon War in 1982, for which he served as the chief medic of the airborne sappers, which are combat engineers. He also got married, welcomed his first child, and had his first taste of scientific research, completing a thesis on respiratory physiology. Then he did a psychology rotation that cemented the direction of his career. "There is nothing more fascinating to me than the function of the brain," he says.

COURTESY OF ISRAEL LIBERZON

ISRAEL LIBERZON

Theophile Raphael Professor of Neuroscience; Professor of Psychiatry and Psychology; Co-director, Center for Trauma, Stress, and Anxiety, University of Michigan
Professor of Psychology, University of Michigan
Diplomat of the American Board of Psychiatry and Neurology
Past President, Psychiatric Research Society (2003)

Greatest Hits

- In rodents, demonstrated the ability of stress-related glucocorticoids to regulate oxytocin receptors
- Founded a PTSD and neuroimaging research program at the University of Michigan
- Established a widely adopted rat model for PTSD
- Using PET imaging, revealed that PTSD patients have exaggerated amygdala activation, suggesting that this part of the brain might mediate their negative responses to certain stimuli
- Formulated a novel hypothesis that the brain of an individual with PTSD has a reduced capacity to process contextual information to form an appropriate fear response

PUTTING PTSD ON THE CLINICAL MAP

After medical school, Liberzon did a postdoc in physiology at the Technion—Israel Institute of Technology in Haifa and sought a way to combine his interest in clinical psychiatry with neurobiology research. But he soon realized that the tools necessary to advance these fields were still lacking. In the 1980s, neuroimaging instruments such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) were yet to be developed. On the research side, there was also resistance to using animal models to study psychiatric disorders, says Liberzon.

Nevertheless, he decided that the best opportunity for merging clinical and basic research was in the U.S., and he joined UM's department of psychiatry as a resident in 1988. With his family about to expand with another son on the way, he says, Ann Arbor seemed like a nice place to raise kids (he later had two more children).

In 1980, the American Psychiatric Association, which publishes the Diagnostic and Statistical Manual of Mental Disorders, added PTSD as a disorder in its third edition. Yet when Liberzon arrived in Ann Arbor, UM had no PTSD research program, and there was still a debate among psychiatrists about whether PTSD was a valid diagnosis.

To help the veterans they worked with at the medical center, Liberzon and the other clinicians held individual and group psychotherapy sessions and also prescribed pharmaceuticals, including sleeping aids. But the interventions were insufficient, and there was a dearth of scientific research to back up the approaches to PTSD care because few studies on the condition were being conducted.

By the early 1990s, the psychiatry community and, importantly, government agencies responsible for awarding research funding accepted PTSD as a real and serious condition that warranted more study. Liberzon rose to the challenge. He became an assistant professor in 1992 and soon after received funding to study the neuroanatomy of PTSD.

PTSD ANIMAL MODELS

When not seeing patients, Liberzon was at the lab bench. Working with Elizabeth Young and Huda Akil in UM's Molecular and Behavioral Neuroscience Institute, Liberzon wanted to better understand the hormones and receptors underlying memory and stress responses, both of which are involved in PTSD. After reading publications suggesting that PTSD patients have faulty glucocorticoid receptor regulation, he wondered whether emo-

tional activity in the brain was abnormal as a result of changes in these receptors. Glucocorticoids are the main hormones involved in stress responses. In 1994, Liberzon was among the first to demonstrate, in rats, that glucocorticoids can also modulate oxytocin receptor activity—important for the emotional attachment between individuals. "This was important because it allowed me to begin to unravel the potential link between changes in stress response, memory, and other systems that might regulate emotional attachment behaviors," explains Liberzon.

It took about ten years to formulate this hypothesis and then get sufficient evidence that this could be what is going on in the brain of an individual with PTSD.

Liberzon next wanted to know how binding of the glucocorticoid stress hormones to oxytocin receptors might affect attachment behaviors and memory, given the role of oxytocin in both. His lab found that rodents under stress have increased glucocorticoid binding to oxytocin receptors in the brain. The study suggested that high levels of chronic stress can affect how the brain processes memories, emotions, and relationships with others.

With Young, Liberzon created a rat model of PTSD in which the animals have enhanced glucocorticoid receptor sensitivity, mimicking the neurological changes observed in clinical PTSD studies. The model has been increasingly influential due to its predictive power and is now among the most widely used in the field. Still, Liberzon says, because of the complexities of PTSD, there is no single, ideal animal model for the disorder.

A NEW HYPOTHESIS OF THE ROOTS OF PTSD

With the emergence of PET-based functional neuroimaging in the late 1990s, Liberzon, together with colleague Stephan Taylor, established a psychiatric neuroimaging program at UM to probe brain regions that function differently in people affected by mental disorders, including PTSD. In 1999, using PET, Liberzon showed that PTSD patients have exaggerated amygdala activation, suggesting that this part of the brain may contribute to the overreaction patients sometimes have to negative stimuli.

The study, according to Liberzon, was among the first to support the hypothesis that amygdala hyperactivity was at the root of PTSD. The idea was that an inappropriate fear response in those with PTSD could be due to the brain reacting intensely to negative stimuli (such as the sound of gunshots) even in an everyday, safe context (such as watching an action movie) as a result of a particularly traumatic memory.

Liberzon wasn't satisfied with this theory because it could not explain why PTSD symptoms often occurred in the absence of a fear stimulus, sometimes even during sleep. Additionally, his rodent model of the disorder didn't show enhanced fear conditioning, in which animals learn to pair a neutral cue with a negative experience such as an electric shock.

Because intense emotions are central components of PTSD symptoms in humans, Liberzon went on to focus on how emotion is processed in our brains. In a meta-analysis of fMRI data, he found that various regions of the human brain are involved with different features of emotional activity, suggesting that any of these brain regions, not just the amygdala, could be affected by a trauma and lead to PTSD.

Liberzon next tested whether abnormalities in a different part of the brain—the circuitry between the hippocampus and cortex, which modulates both the fear response and emotional systems—could explain PTSD behavior in a more comprehensive way than the initial amygdala hypothesis. Prior studies had shown that malfunctioning of this circuitry could lead to enhanced fear and impairments in determining whether something is safe or unsafe. Liberzon's work was the first to show that these abnormalities occur in PTSD. In 2012, his lab demonstrated that an exposure to a single, prolonged stress in his PTSD rodent model resulted in normal conditioning and extinction of the conditioning. But, unlike wildtype animals, the PTSD model animals displayed an enhanced fear of the stress trigger even in safe contexts, suggesting that the hippocampus-cortex connections were not functioning properly.

The lab followed up with a study in 2014 that showed the same thing in patients, namely, that those with PTSD, unlike people without the disorder, had trouble using the information about their environments to deem something safe or unsafe. The results implied that the abnormal fear response in these patients could be due at least in part to flawed signaling between the hippocampus and cortex, and provided a novel view of PTSD pathophysiology that focused the field on the importance of these circuits.

"It took about ten years to formulate this hypothesis and then get sufficient evidence that this could be what is going on in the brain of an individual with PTSD," says Liberzon.

MOVING ON

Liberzon's lab is currently studying underlying brain regions, including the hippocampus and the prefrontal cortex, and signaling pathways involved in how the brain processes both stressful and non-stressful situations in real time and how that is disrupted in PTSD. His group is also exploring how genetics combined with childhood trauma contribute to PTSD in adulthood.

Now, seeking a new challenge, Liberzon is leaving UM after 30 years. He recently accepted an offer to build a new Psychiatry and Behavioral Science Department at Texas A&M, a position he will start in August.

Liberzon is optimistic about the scientific progress in PTSD over the last 40 years. "We went from a black box of theories but no data, to accumulating a soup of information in the 1990s to begin to formulate hypotheses, to understanding the functions of single brain regions involved in PTSD in the 2000s, to now working on the neurocircuitry and molecular signatures of the abnormalities within the circuits." ■

Youssef Belkhadir: Signal Decipherer

Group Leader, Gregor Mendel Institute of Molecular Plant Biology. Age: 42

BY ASHLEY YEAGER

Growing up in Morocco, Youssef Belkhadir would look out across the wheat fields of his father's farm outside Casablanca in wonder. "I was fascinated by how plants managed to colonize the environment in such an elegant way, making the most of everything," Belkhadir tells *The Scientist*. "They are tethered in the ground and cannot escape their environment through locomotion. Their site of birth will be their site of death, and they have to deal with whatever comes their way."

Inspired by this idea, Belkhadir decided to study the molecular biology of plants as an undergraduate at the University of Paris VI-Jussieu. After receiving his bachelor's in 2001, he spent another year at the University of Paris-Sud XI earning his master's degree in plant genomics, before moving to the U.S. to join biologist Jeffery Dangl's lab at the University of North Carolina at Chapel Hill for his PhD. There, Belkhadir investigated how plants sense and defend themselves against pathogen attack using molecular machines inside each cell.[1] "Even then, Youssef was very big-thinking and tenacious," Dangl recalls.

After earning his PhD in 2005, Belkhadir joined the lab of biologist Joanne Chory at the Salk Institute for Biological Studies in La Jolla, California, as a research associate, to study how plants respond to steroid signals. "What Youssef learned in my lab was how to take a lot of knocks, and still move forward," Chory says. "At first, all of his projects failed, not because they were bad projects, but they just didn't work. Youssef just seemed to laugh through it all." Finally, success came while studying a leucine-rich repeat receptor kinase (LRR-RK), which acts as an antenna at the cell surface to bind to a steroid hormone that helps plants control their size.[2] "I got my affinity for cell surface receptors in Dr. Chory's lab," Belkhadir says. "But I didn't just want to study one at a time. I wanted to study hundreds at a time."

At the end of 2010, Belkhadir planned to tackle this problem as an independent researcher at the Centre of Excellence in Plant Energy Biology in Perth. But he never made it to Australia. While he was in California, tragedy stuck: Belkhadir found out that his father had been in a traffic accident, so he returned to Morocco to be with his family. After his father passed away, Belkhadir remained in his native country to settle his father's affairs, which kept him away from the lab bench for more than three years.

To stay engaged in science, Belkhadir cofounded a biotech company called Atlas Genomics with his own money—a venture he says taught him to be excruciatingly efficient and to outsource tasks when necessary. After a few years, however, Belkhadir chose to shut down the firm for financial reasons, partly related to the Arab Spring demonstrations that raged through 2011. It was time to return to the lab. "It's incredibly hard to come back after years away," Chory says. "But Jeffrey Dangl and I went to bat for Youssef because we knew he could do it."

When Belkhadir resumed his research in 2014, he accepted a position as a group leader at the Gregor Mendel Institute in Austria to study how plants use cell surface receptors to adapt to their environment. Just a few years later, he had successfully completed several projects: three identified the peptide ligands of three independent classes of receptors that regulate the output of plant immune responses, and another identified a receptor that ensures healthy root development. Simultaneously, Belkhadir and his colleagues developed the tools to trace 40,000 interactions among 200 LRR-RKs and discovered a complex network of surface receptors that distinguishes friendly extracellular molecules from pathogenic ones.[3]

Now, Belkhadir says, the question is: When multiple signals activate the network, how do plants compute the signals to make the best decisions to defend themselves or grow? ■

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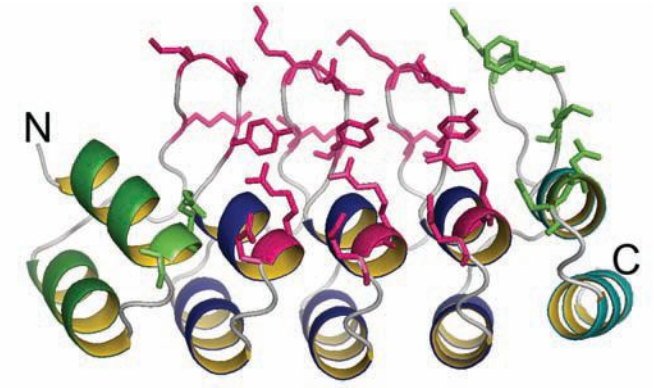
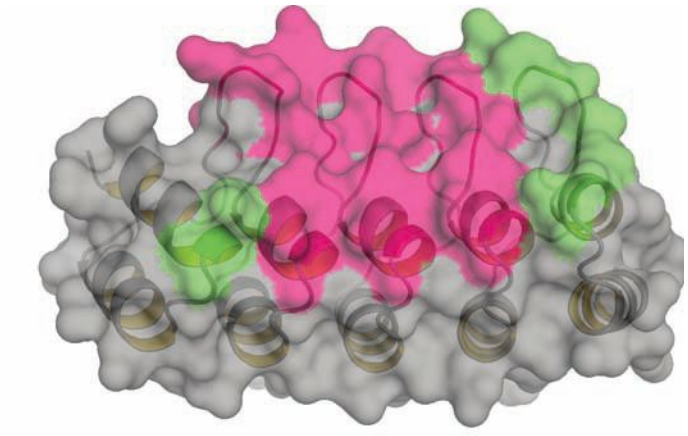
The Imitation Game

Using antibody mimics to seek out and capture your favorite proteins

BY DEVIKA G. BANSAL





PALM CLASP: DARPins, or designed ankyrin repeat proteins, consist of an N-capping repeat (green ribbon), many internal repeats whose number can be freely chosen (three shown here) (dark blue ribbon), and a C-capping repeat (cyan ribbon). The molecular model shows a classic DARPin library design.

Antibodies are the immune system's foot soldiers, the first line of defense against foreign invaders. With their unique arms that bind only to specific ligands, antibodies screen thousands of proteins to find the one that clasps perfectly.

This bit of biology also makes antibodies a powerful tool for detecting and capturing proteins in the lab. But they have some significant drawbacks. For one thing, it takes between six months and a year to develop lab-ready antibodies, and the process, which was developed in the 1970s and '80s, often involves using animal hosts, such as rabbits, to generate the molecules. Also, antibodies' unwieldy structure of light and heavy chains and their large size—most are about 150 kDa—makes it hard to fuse them with target proteins, or to use them inside live cells. What's more, antibodies often cannot be produced as genetically encoded reagents within cells of interest because they have disulfide bonds that fail to form in the reducing environment of the cytoplasm.

Another issue is that there is no way to reproduce antibodies exactly, says Andreas Plückthun, a biochemist at the University of Zurich in Switzerland. "It's the only reagent used in biology which is a black box," he says. Most monoclonal antibodies—those made from cloned, identical cells—have never been sequenced, he adds. "It's a leftover from a different time."

To get around these challenges, scientists have developed an arsenal of tools that mimic antibodies—but without the baggage. These "mimetics," such as DARPins, Affimers, and monobodies, can be produced in *Escherichia coli* and offer more control, specificity, and reproducibility than traditionally manufactured specific antibodies.

A mimetic contains a stable scaffold that holds the molecule together and a variable arm that binds specific targets. For a particular scaffold, scientists can create a large combinatorial library of mimetics with billions of variable arms to ensure good coverage against most proteins in the cell, and then screen for ones that bind specifically to the protein of interest. Producing mimetics requires a significant investment, which is why only a few research labs and companies have constructed the necessary infrastructure. But the molecules are widely available for use.

Here, *The Scientist* explores how a few of the most popular antibody alternatives stack up against conventional antibodies—and each other.

DARPINS

WHAT THEY ARE: First described in 2003, and perhaps the most widely validated antibody mimetic, designed ankyrin repeat proteins, or DARPins, are tiny proteins with masses of roughly 17 kDa—one-tenth that of antibodies. "They look like an open hand," says Plückthun, who developed them in his lab. These tiny palms offer an extended surface for interaction with target proteins, he says, and enable a lock-and-key–type binding mechanism.

For the scaffold, Plückthun and colleagues use a so-called ankyrin repeat protein, a sturdy molecule that mediates tight

ANNU REV PHARMACOL TOXICOL. 55:489-511, 2015

protein-protein interactions in nature. Because DARPins have no cysteine residues—and therefore no disulfide bonds—they can easily fold inside the reducing environments of cellular interiors. This is why researchers can make them without eukaryotic cell culture, where disulfide bonds are typically added in the acidic environment of the endoplasmic reticulum. Instead, DARPins can be produced cheaply in *E. coli*, without the need to immunize animals, within a matter of few weeks, Plückthun says. He distributes DARPins characterized in his lab at cost, or researchers can purchase them from one of several companies (such as Creative Biolabs).

"I don't see this as a cheaper alternative, but as extending the technology beyond antibodies," says Plückthun. "DARPins have become extremely robust." Members of his lab can generate binders against 96 different protein targets in parallel, and they typically generate 200–300 specific DARPins against every target. Overall, they have made and validated DARPins against 350 targets so far.

DARPins tend to be the most stable and sensitive of the mimetics, with some able to detect targets as small as 5–100 picomolar. They work extremely well for practically any protein that has folded domains. Plückthun notes, however, that antibodies may be better at detecting and binding denatured or unstructured proteins.

USES: These palm-like binders have found widespread use in research, diagnostics, and therapeutics. In the lab, they have been used to tag and chaperone target proteins, to block protein function, and to facilitate standard pull-down assays. Due to their small size, DARPins can be genetically fused with fluorescent proteins and can be expressed in a variety of compartments in eukaryotic cells and in bacteria (*Annu Rev Pharmacol Toxicol*, 55:489–511, 2015).

DARPins can do everything an antibody can, but perhaps their greatest strength is in therapeutics. For example, two DARPins can be stacked like Lego blocks, with one end that recognizes proteins on tumor cells, and another that binds to coat proteins on tumor-killing viruses. Clinicians may one day use such stacked DARPins to chaperone therapeutic viruses directly to tumor tissues, in a therapeutic approach called "oncolytic viral retargeting."

AFFIMERS

WHAT THEY ARE: Affimers are another class of mimetics that are based on either a human protease inhibitor called stefin A or a plant-based cysteine protease inhibitor called cystatin. These are small—12–14 kDa—proteins that are extremely stable in reducing environments and at temperatures higher than 100 °C.

Affimers differ from DARPins in the way they bind their targets, says Darren Tomlinson, biochemist at the University of Leeds in the U.K. who led their development. Instead of DARPins' hand-like structure, he explains, "Affimers have loop structures that can probe into the target as if with three fingers" (*eLife*, 6:e24903, 2017).

It only takes from 4–7 weeks to screen libraries of these loop-based mimetics to find specific targets, and typical Affimer binding affinity lies in the nanomolar range. Researchers keen to use Affimers can collaborate with Tomlinson's lab or purchase them from UK-based Avacta, which commercially developed them.

Affimers have loop structures that can probe into the target as if with three fingers.

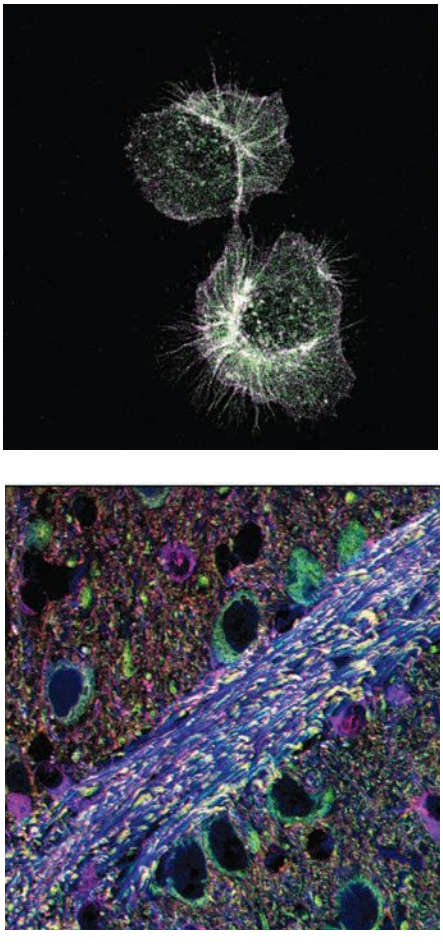
—Darren Tomlinson, University of Leeds

USES: Aside from standard antibody applications such as binding assays, Affimers can be produced directly inside cells to inhibit or activate protein function and to block specific post-translational modifications on target proteins. Affimers don't just recognize proteins: they can even bind to small organic compounds and nonprotein-based metabolites.

Due to their small size, Affimers can be employed to track single particles using super resolution microscopy, a feat that no other mimetic so far can perform. At a length of 2–3 nanometers, Affimers pose no challenges at resolutions of 20 nanometers or less in super resolution microscopy. Their diminutive size also allows them to penetrate the smallest vesicles and label the most elusive of antigens in tissue samples.

MONOBODIES

WHAT THEY ARE: Monobodies (trade name: Adnectins) are yet another class of synthetic binding proteins, which use



DARREN TOMLINSON

SUPER VIEW: Top: staining with an anti-actin Affimer (green) and phalloidin (magenta) highlights the actin cytoskeleton in insect cells. Bottom: staining with an anti-tubulin Affimer (blue), an antibody against beta3 tubulin (green), and an antibody against acetylated tubulin (red) reveals specific tubulin structures in mouse brain tissue. Both samples are imaged with super resolution microscopy.

the tenth fibronectin type III domain as the scaffold. At 10 kDa, the Fn3 scaffold is most structurally similar to natural antibodies, but with one significant difference: monobodies contain no disulfide bridges, allowing them to more easily fold in bacterial cells (*Protein Sci*, 26:910–24, 2017).

Once you have the cDNA, you know the exact sequence of your nanobody.

—Oleg Dmitriev, University of Saskatchewan

Shohei Koide, a biochemist at the New York University School of Medicine, first created monobodies in 1998. Since then, monobody scaffolds have expanded to fill two distinct libraries. One offers a flat, palm-like binding surface, and the other forms a loop, like the three penetrating fingers of Affimers, providing greater reach into the target and subsequent tight and highly specific binding. It takes three to six months to produce a set of monobodies against a target protein, depending upon complexity, Koide says. These are not commercially available yet, but researchers can get them by collaborating with his lab.

USES: "A key advantage of monobodies is they tend to hit functional sites of the target very often, although we do not program the probe to do so," says Koide. They have high affinity and high selectivity in the picomolar range, and are often inhibitors of protein function, but can sometimes act as activators. This allows researchers to use monobodies not only to mark targets, but also to modulate cellular signaling.

NANOBODIES

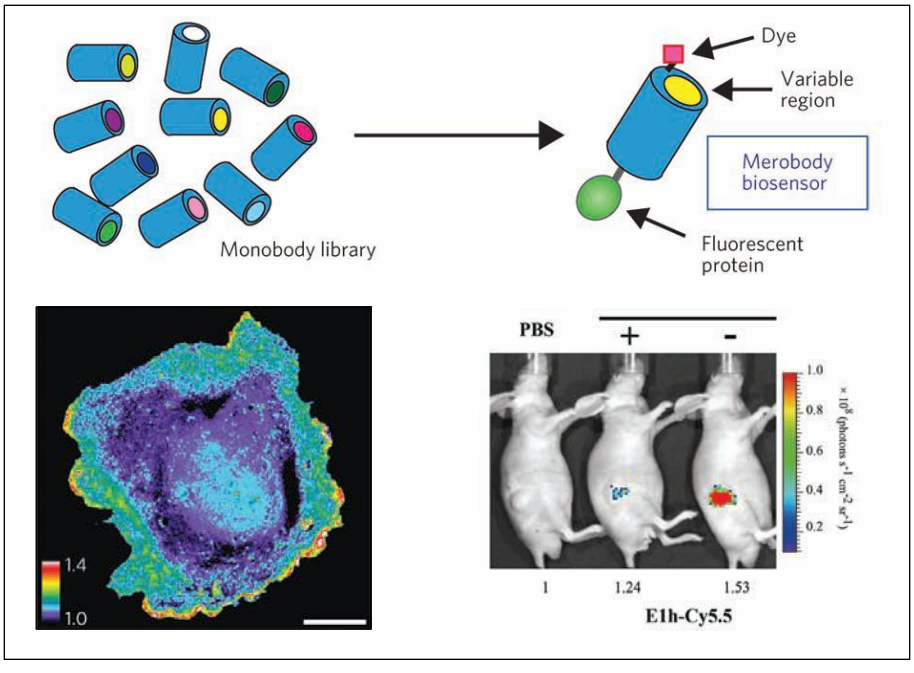
WHAT THEY ARE: In contrast to DARPins, Affimers, and monobodies, which are synthetic, nanobodies are actual antibodies that are produced in llamas, camels, and sharks. For some reason unknown to science, these animals make especially small antibodies—about 16 kDA—consisting only of heavy chains, which bind targets.

Unlike their synthetic cousins, nanobodies are obtained by immunizing an animal. From a llama's (or alpaca's) immune response, researchers can isolate total RNA from B cells to amplify nanobody cDNA and create a library. This large cDNA library encodes many different nanobody variants, of which scientists can select a few with high binding affinity, often in pm ranges, in about three months' time. Researchers can access nanobody libraries through one of several companies, such as Belgium-based Ablynx, or through labs that make them, such as that of Serge Muyldermans at the Vrije Universiteit Brussel in Belgium (*Annu Rev Biochem*, 82:775–97, 2013).

"Once you have the cDNA, you know the exact sequence of your nanobody," says Oleg Dmitriev, a biochemist at the University of Saskatchewan in Canada. "If you decided, you could tweak its specificity by going back to the cDNA library and doing new selection rounds. That's not very common with monoclonal antibodies."

To improve availability and to get around the development time needed to create nanobodies, researchers have recently developed a synthetic yeast platform, bypassing the need for animal immunization completely. Although there are some concerns about the specificity of synthetic nanobodies, this library is freely available to academic researchers upon request from Andrew Kruse's lab at Harvard Medical School in Boston or from Aashish Manglik's lab at the University of California, San Francisco (*Nat Struct Mol Biol*, 25:289-296, 2018).

USES: Aside from standard uses to track and perturb protein function, nanobodies are especially handy in coaxing temperamental proteins to crystallize. Over the past decade, protein crystallographers have included nanobodies in their bag of tricks because these affinity reagents can pin down highly dynamic proteins, stabilize their flexible regions, and shield hydrophobic surfaces on membrane proteins—all key steps in crystal formation. ■



MONO MODULATORS: Monobodies can be used for both in vitro and in vivo imaging. Top left: schematic of the monobody library binders against Src family kinases (SFK); top right: biosensing mechanism of the SFK-targeting monobody. Bottom left: ratio image of a cell microinjected with the monobody biosensor (scale bar: 20 μm). Bottom right: in vivo targeting of the monobody seen in a nude mouse model of prostrate cancer.

ANNU REV ANAL CHEM, 10:293–320, 2017

Life Science on Cloud 9

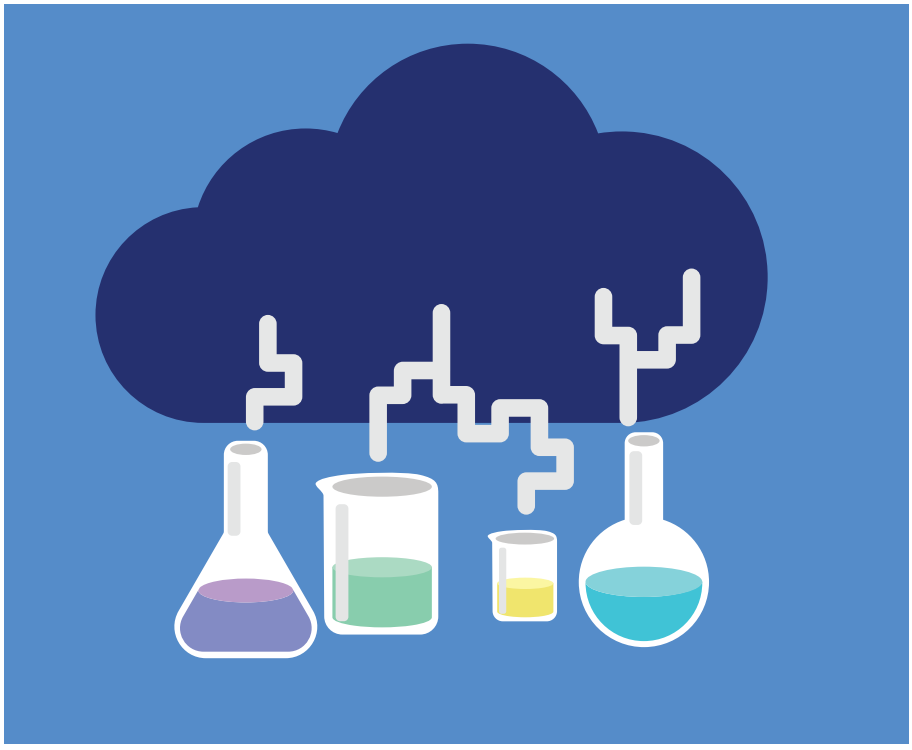
The Internet of Things has the potential to connect many facets of research—from laboratory equipment to ideas—but scientists must be ready for the questions its implementation could raise.

BY ABBY OLENA

One of the two high performance liquid chromatography (HPLC) machines in Lakshminarasimhan Pranatharthiharan's lab at Sunovion Pharmaceuticals in Marlborough, Massachusetts, wasn't working. The instrument's readouts—a series of peaks that indicate the components of the drugs that Pranatharthiharan's group analyzes—were jumping around all over the place.

Troubleshooting the machine and complaining to the supplier of the columns used in the instrument failed to turn up a solution. So Pranatharthiharan's team stuck a cloud-connected temperature sensor right next to the machine in question. After several days of using an electronic lab notebook (ELN) to record the temperature readings from the device, the team identified the problem: the building's climate control system was blowing hot and cold air at specific times every day in the vicinity of the instrument. "If you happen to be running the analysis at that time: boom" —the readouts wouldn't be accurate, Pranatharthiharan says. Once they figured out their building's temperature fluctuations, he and his group were able to get their analyses back on track.

It's a prime example of how the emerging Internet of Things (IoT)—a virtually connected network of physical devices, such as lab equipment and sensors—can help solve problems that researchers have traditionally addressed over hours or days of direct monitoring. In addition to collecting data about equipment performance and laboratory conditions, scientists such as Pranatharthiharan can use the IoT to deposit vast amounts of experimental data into the cloud directly from instruments and to control experiments remotely. "It's very clear that research in laboratories—particularly in biology and biomedicine—is becoming fully intertwined with online technologies," says Sabina Leonelli, a philosopher who studies data and life science at the University of Exeter in the U.K.



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The trend is aided by a growing number of tools to gather data and metadata into the cloud. Both IoT-specific startups and larger equipment manufacturers are moving to develop cloud-based initiatives in order to grab their piece of an overall IoT market projected to grow more than threefold, to $561 billion, by 2022. Proponents say that scientists can use network-ready tools to scrutinize lab data more closely and make connections that weren't obvious before, potentially facilitating more-efficient and more-reproducible research. But some observers emphasize the need for caution in adopting the IoT, and note that it remains to be seen how science will cope with the challenges and ethical implications of the transition to a fully connected lab.

Getting connected

Several startups have emerged in the last few years with the express aim of developing the IoT for laboratory use—an endeavor they say can help address the crisis of reproducibility that plagues scientific research. For instance, Massachusetts-based Elemental Machines, the company that made the temperature sensor that Pranatharthiharan used to solve his HPLC problems, offers several tools that help experimentalists stay on top of anomalies in how an instrument runs or help predict equipment failures. These include wifi-connected hardware for monitoring laboratory conditions, a device that can plug into any

instrument with a data port, and an online and mobile dashboard that collects all of a team's data and metadata in one place and can send alerts to researchers.

Sridhar Iyengar, Elemental Machines CEO and founder, says the company specifically focused on sensors that monitor temperature, humidity, carbon dioxide levels, light, and air pressure because those metrics are the most likely to perturb chemical reactions or biological processes. "Our mission, very broadly speaking, is to increase reproducibility in science and science-based activities," he says.

Researchers can use the sensors to monitor more than just room conditions. In the past, chemists in Pranatharthiharan's group recorded temperature readings to track the progress of chemical reactions by standing next to the hood with their tablets and manually inputting data into their ELNs every minute or so. Now, they plug a probe into one of Elemental Machines's sensors and the data stream straight into their ELNs. "I want to use tools that make the life of my folks a little bit easier," says Pranatharthiharan. "Now they can just plug the stuff in and then go and do something else."

Other companies are going further than just monitoring lab equipment; they're offering remote control. Boston-based TetraScience is one such company. "There are all these different pieces of instrumentation and devices that produce extremely valuable data," says Alok Tayi, TetraScience CEO and cofounder. "Yet the way that data is accessed and the way those instruments are operated right now is all manual." The firm collaborates with equipment manufacturers to integrate cloud connectivity, makes a wifi-based hardware link that can talk to sensors and simple instruments such as balances and stir plates, and provides an online dashboard that links it all together.

Chemist Jonathan Barnes, now at Washington University in St. Louis, used early versions of TetraScience hardware and software for free as a beta tester during his postdoc at MIT to monitor and control polymer synthesis reactions via an app on his phone. "I could turn the stir plate off, or I could change the temperature, or just completely cut power to the entire thing," he says. It was especially useful because the reactions were time sensitive; heated for too long, the materials would start to decompose. Without remote monitoring and control, "I couldn't set it up at 6 or 7 o'clock because then that meant I would have to come back anywhere between 1 and 3 AM, depending on the length of the experiment," Barnes says.

Connecting animal research to the cloud has helped minimize stress on lab organisms.

In some cases, the IoT can provide immediate feedback to researchers using connected equipment. University of California, San Francisco (UCSF), graduate student Valentina Garcia is one of the first users of Gilson's internet-connected pipettes, which she borrows from the local company representative. The pipettes send data about liquid quantities and number of pipetting steps to a tablet via Bluetooth that can then be shared with an ELN. Garcia says she plans to use the connected pipetting system in her studies of the malaria parasite *Plasmodium falciparum*, where making a mistake setting up an experiment could set her back three days or more. The system is "really great for high-throughput assays, when you're just going to be doing the same thing over and over again, and you want to make sure you're not getting lost in the pipetting."

The cloud menagerie

It's not just humans seeing the changes brought about by a connected lab. Moving animal research to the cloud has fostered the rise of international collaborations, given small companies the option of pursuing costly and time-consuming work in rodents, and minimized stress on lab organisms.

Veterinarian Steven Niemi, director of the Office of Animal Resources at Harvard University, has investigated the effect of bringing tools for animal research and the IoT together and found reasons to pursue the integration, especially where international collaborations are concerned. If you can share animal data directly, collaborators don't have to repeat experiments or use other animals for the same purpose, he says. "My hope is that some of this new technology can enhance" animal research, Niemi adds.

Ethan Perlstein, CEO and founder of biotech Perlara, has seen this enhancement firsthand. His company uses model organisms to study rare genetic diseases and then seeks drugs to treat those diseases. Perlara houses most of its model organisms—yeast, human cells, worms, fruit flies, and zebrafish—in labs at its headquarters in San Francisco. But working with mice presented the challenge and expense of building a vivarium, an undertaking that Perlstein says didn't make sense for the small company.

California-based Vium houses a cloud-connected vivarium and contracts with labs to do preclinical studies of drug candidates in rodents. Perlara used Vium's services to test potential therapeutics for Niemann-Pick Type C, a lysosomal storage disorder, and the project grew into a collaboration with pharmaceutical giant Novartis.

Vium's goal is to accelerate drug development while fundamentally changing the way animal research is done, says Joe Betts-Lacroix, the company's cofounder and chief technical officer. To that end, the firm's animal housing monitors temperature (which provides information about in-cage animal activity), controls airflow and lighting, and employs high-definition video cameras so sensitive that computer vision algorithms can detect the movement of an animal's chest walls and determine its breathing rate. Each cage has one or more computers that transmit data from sensors straight to the cloud, where animal care staff and researcher clients can access and analyze them online.

Not only does the setup constantly collect data from every animal, it does so without forcing the rodents to interact with people—an added benefit from a research perspective. "Mice perceive humans as

deadly predators, and by the time there is a human hand reaching into the cage . . . the animals are already freaking out," Betts-Lacroix says. Minimizing these interactions could thus help researchers separate the effects of handling the animals from experimental treatment variables.

"Right now there are [approximately] eight million analog cages out there, and it's our mission to digitize them all so that we can derive the maximum amount of information from each animal," says Betts-Lacroix. "In that sense it's an animal welfare contribution because we can use fewer animals and get the same amount of information."

Connectivity problems

The promise of the IoT comes with both long- and short-term issues that scientists must consider. Leonelli explains that one pressing question is how far labs should go toward automation and standardization, as a one-size-fits-all approach risks overlooking nuances in data gathering or experimental design particular to a certain discipline. "Very often it is the case that people in different parts of biology have very good reasons to do things in a specific way, which is not the same as somebody who is in a different subfield," she says. "So one has to be very careful to capture this kind of system-specific knowledge when implementing these higher-level technological solutions."

The IoT's sustainability and security, particularly when it comes to data storage, are also important considerations. Leonelli says that most people and companies that are storing vast amounts of data gathered from instruments and sensors do so in clouds provided by either Google or Amazon. "What does it mean when Google and Amazon end up [with access to] most of the research data that has been produced in the public sector?" she asks. "That's a very big question that has really not been resolved."

Niemi highlights related concerns about data privacy, particularly when integrating the IoT into animal research—a perennially controversial area. "As your data become even more shared or more distributed, could you be compromised or could you be compromising your own program through this kind of connectivity?" Niemi asks. Among researchers working on animals, "there's just as strong a cultural resistance to openness and sharing, even if the technology was rock solid secure," he adds.

There are also more-practical considerations for scientists hoping to reap the benefits of introducing the IoT to their labs, not least its associated costs. For instance, although chemist Barnes found increased connectivity useful during his postdoc, he has not yet brought the IoT into his own lab at Washington University. While he is not opposed to it, connecting the lab to the cloud is not an expense that he wants to prioritize over buying chemicals or equipment, Barnes says (see table below).

And then there's the need for scientists to get used to the protocol changes. UCSF student Garcia currently faces the practical challenge of figuring out how to integrate the new digital tool with her years of training in analog methods, such as using a paper lab notebook to track her research. "Even if I have the tablet there, if something goes wrong, I'm still writing in a notebook what happened," she says.

All the same, to many researchers the prospect of bringing their labs online, and connecting equipment and ideas, is attractive. "The technology's new, so it's going to be the most buggy and the hardest to work with at this moment," Garcia says. "But I bet ten, fifteen years from now, if I'm running my own lab, it's going to be entirely different. It's going to be a lot faster, a lot easier, and I think people are just going to be able to keep track of what they're doing so much better." ■

Abby Olena is a freelance science journalist based in Carrboro, North Carolina.

GOING DIGITAL: Several companies offer cloud-enabled lab equipment and services to help bring labs online. Below is a selection of products and services currently on the market.

Company	Product or Service	Approximate Retail Cost
Consolidated Sterilizer Systems	Retrofitting an existing autoclave for network connectivity	$2,000
Elemental Machines	Subscription for monitoring one connected device via Elemental Insights, a web-based dashboard	Starts at $249/device/year
Gilson, Inc.	PIPETMAN M Connected: Bluetooth-enabled pipette that accumulates data on pipetting volumes and protocol steps with TRACKMAN Connected	$750 to $1,500, depending on whether single- or multichannel
Gilson, Inc.	TRACKMAN Connected: Kit including a tablet with a pre-installed microplate tracker app, PipettePilot, to complement the PIPETMAN M Connected	$1,400
TetraScience, Inc.	Subscription for monitoring one connected device through TetraScience's web-based dashboard	Starts at $50/device/month

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Glorious Varieties

Using race to categorize genetic differences is suspect at best.

BY ROB DESALLE AND IAN TATTERSALL

In two opinion pieces that ran earlier this year in *The New York Times*, David Reich, professor of human genetics at Harvard University, discussed the genetics of race and racial differences. In the first piece, Reich addressed race and the genetics of complex behavioral and anatomical differences among humans. In the second piece, Reich responded to the "hundreds" of letters to the editors provoked by his first essay. Here, we hope to tease apart this highly visible exchange on race between a preeminent scientist and the public.

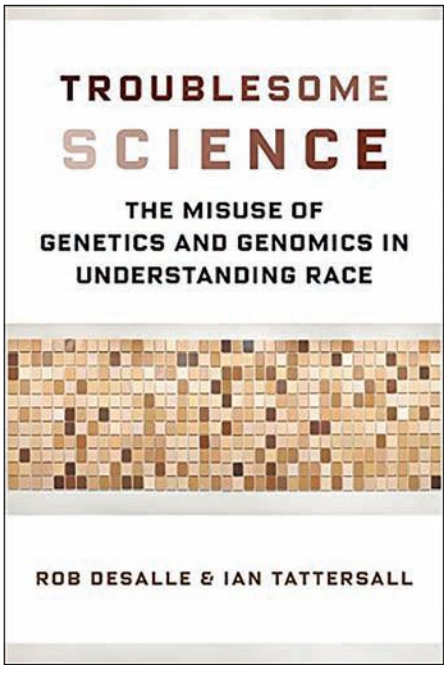
We find Reich's two editorials controversial because they seem to further the concept that race is a genetic, biological construct. His underlying claim is embodied in the following quote: "[M]any traits are influenced by genetic variations, and . . . these traits will differ on average across human populations." It is impossible to argue that this statement is false, so Reich asserts that scientists who deny the genetic basis of race are "anti-scientific, foolish, and absurd." But do these genetically controlled traits that differ across populations characterize the people that carry them?

At the heart of Reich's argument is not a fact of nature, but the human mind's interpretation of what it perceives. Statistician John Gower was quoted as saying in a 1972 symposium: "The human mind distinguishes between different groups because there are correlated characters within the postulated groups." Gower pointed to this "underlying correlation structure" as a way for humans to find regularity in the "blooming, buzzing confusion" of our world. The first and most obvious way we do this is by visual observation. In New York City, we easily recognize the ethnic background of large numbers of people every day simply by looking. But there is an even larger number of people for whom we cannot infer ethnicity by using our eyes. Genes, even more than facial features, are traces of our ancestral origins and interrelationships. And so genomicists have developed powerful computational and statistical tools that identify underlying correlation structures for the purposes of medicine and ancestry studies.

While genomics is a much more precise way of discovering underlying correlation structure than eyeballing, both modes of observation suffer from the same problems when applied to understanding race. Their most severe deficiency is that defining any population falls prey to a "moving target" syndrome, making the scientific validity of tests for the existence of race fuzzy at best. For example, on the subway we can routinely recognize people who likely have Asian ancestry. We can also visually infer that a subset of those people is from Cambodia. If we look even closer at this group of people, we might see a man and woman with two kids, and recognize familial similarities. This shifting perceptual goalpost dictates the underlying correlation structure we process. And so it goes for genomics.

Both eyeballing and genomics can detect the underlying correlation structure of populations. But are underlying correlation structures a basis for identifying races? From the perspective of systematics—the reigning methodology for recognizing functional groups of organisms in nature—they are not. When organisms of a species or genus broadly interbreed, clear boundaries and hierarchies within and between subgroups are destroyed even though ancestry is not, and the underlying correlation structure remains. Still, while that structure is useless for delineating "races," it can be used to trace our individual geographic roots, and the legacy of our forebears' marriages and misadventures.

When we pivot away from trying to round up genetic differences into a basis for races, and turn instead toward a better understanding of their use in interpreting ancestry, we eliminate the very shaky assumption that predetermined, biological races exist. And as we emphasize in our latest book, *Troublesome Science*, "race is a totally inadequate way of characterizing diverse humankind or even of helping understand humanity's glorious variety." All argument, speculation, and prediction made about group traits and the existence of races is therefore suspect. ■



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Rob DeSalle is Curator in the Comparative Genomics Institute and Ian Tattersall is Curator Emeritus in the Division of Anthropology, both at the American Museum of Natural History. Read an excerpt of their book, Troublesome Science: The Misuse of Genetics and Genomics in Understanding Race, *at www.the-scientist.com.*

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COMING**SOON** | Growing Pains: Cell Culture Challenges and Best Practices

Cell culture is an essential technique in modern biological laboratories and is employed in a wide range of research fields, including oncology, genetics, pharmacology, and bioproduction. Cell-line contamination and misidentification is a significant threat facing cell culture, with the potential to invalidate years, if not decades, of data. Other common obstacles to research reproducibility involving culture systems include environmental variability, media inefficiency, and inappropriate scaling up or down of operations. Addressing these challenges will ensure the continued utility and reliability of cell culture across the biological sciences. Join *The Scientist* for an webinar on this topic of growing importance.

SHARON BAHIA, PhD
Product & Distributor Manager, Culture Collections
National Infection Service
Public Health England (PHE)

JIM COOPER
Cell Biology Applications Scientist,
Culture Collections
Public Health England (PHE)

TUESDAY, JUNE 12
2:30 – 4:00PM EASTERN TIME

REGISTER NOW!
www.the-scientist.com/culturechallenges
The webinar video will also be available at this link.

TOPICS TO BE COVERED:

- Standardization of training, reagents, protocols, and analysis methods
- Steps for improving reproducibility of culture setup and maintenance

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TS WEBINARS

ON**DEMAND** | Trends in Synthetic Biology: Antibody Engineering

As the body's circulating army of responders, antibodies recognize and destroy foreign and potentially disease-causing pathogens. The engineering of synthetic antibody forms and conjugates has produced a whole new arsenal of tools for applications in research, diagnostics, and therapeutics. Forward-engineering approaches create new recognition sites and molecular functions for antibody products to accurately and efficiently detect previously undetectable molecular targets with an increased response lifetime. For a closer look at the fastest growing class of synthetic therapeutics, *The Scientist* brings together a panel of experts to share their research, to discuss current design and optimization approaches, and to offer insight on the future outlook of antibody engineering.

ANNE MESSER, PhD
Senior Scientist
Neural Stem Cell Institute
Regenerative Research Foundation

YASMINA ABDICHE, PhD
Chief Scientific Officer
Carterra, Inc.

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TOPICS COVERED:

- Producing more and better antibodies
- Designing your antibodies for optimal effect

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LAB TOOLS
THE SCIENTIST WEBINAR SERIES

COMING**SOON** | LabTools: Beyond 3-D: Recapitulating Nature for Optimal Bioproduction

When cells are your factory, it's imperative to ensure that they are kept under the right conditions throughout the life of the culture. Standard 2-D and 3-D culture systems, whether dish-, bag-, roller bottle-, or bioreactor-based, all share the same limitations; these cultures fail to keep the cells in tissue-like contact with neighboring cells, depriving them of important signaling cues. Hollow fiber bioreactors (HFBR) are able to promote a physiologically relevant interaction between cells while enabling product retrieval without perturbation. Learn more about HFBRs from FiberCell Systems, the sponsor of this webinar event, and learn how your standard methods are letting you down by design. Bring your questions and comments; FiberCell has the answers you seek.

JOHN J.S. CADWELL, MS
President and CEO
FiberCell Systems, Inc.

WEDNESDAY, JUNE 20
2:30 – 4:00 PM EASTERN TIME

REGISTER NOW!
www.the-scientist.com/hollowfiber
The webinar video will also be available at this link.

TOPICS TO BE COVERED:

- The spatial and nutritional constraints faced by standard cultures, and how HFBRs address them
- Using HFBRs to generate monoclonal antibodies, exosomes, difficult-to-express proteins, and more

WEBINAR SPONSORED BY:

FiberCell Systems
A BETTER WAY TO GROW CELLS

LAB TOOLS
THE SCIENTIST WEBINAR SERIES

ON**DEMAND** | LabTools: Genetic Variant Detection in Cancer: Using ISH to Track Tumor Evolution

Intratumor heterogeneity (ITH) is a major underlying cause of therapy resistance and disease recurrence and constitutes a history of a specific tumor's growth. Current methods to analyze genetic ITH rely on the sequencing of "bulk" or flow-sorted populations, in which the spatial context of tumor subclones is not preserved, and rare subclones may not be detected. These shortfalls can be addressed with the BaseScope™ ISH assay—a unique mutation-specific RNA in situ hybridization assay made by ACD, the sponsor of this webinar. The BaseScope assay represents a significant technical advance for in situ mutation detection and provides new insight into the mechanisms of tumor evolution with potential ramifications for selecting patients for treatment. Join us to learn more about this new approach to ITH analysis.

ANN MARIE BAKER, PhD
Centre for Tumour Biology
Barts Cancer Institute
Queen Mary University of London

COURTNEY ANDERSON, PhD
Group Leader Applications
Advanced Cell Diagnostic

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TOPICS COVERED:

- How ITH influences treatment successes and failures
- How the BaseScope ISH assay enables reliable detection of ITH

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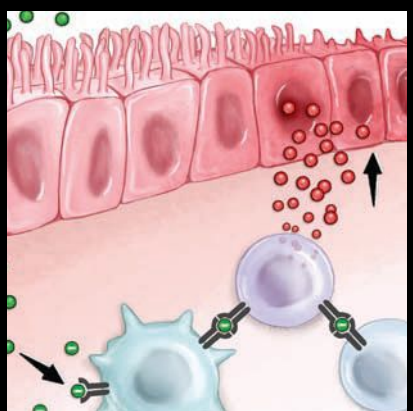

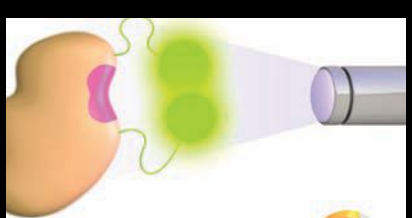

the 2018-2019 Keystone Symposia Series

October	7–11	**Drivers of Type 2 Diabetes: From Genes to Environment** (S1) Seoul | South Korea
	14–18	**Framing the Response to Emerging Virus Infections** (S2) Pok Fu Lam | Hong Kong
	17–20	**21st-Century Drug Discovery and Development For Global Health** (S3) ♦ Berlin | Germany
November	11–14	**From Rare to Care: Discovery, Modeling and Translation of Rare Diseases** (S4) Vienna | Austria
	25–29	**Leveraging Genomic Diversity to Promote Animal and Human Health** (S5) ♦ Kampala | Uganda
December	11–15	**Role of the Genital Tract Microbiome in Sexual and Reproductive Health** (S6) ♦ Cape Town, Western Cape | South Africa
January	13–17	**DNA Replication and Genome Instability: From Mechanism to Disease** (A1) Snowbird, Utah | USA
	13–17	**Host and the Environment in IBD: Scientific Advances Leading to New Therapeutics** (A2) Taos, New Mexico | USA
	13–17	**Mitochondrial Biology in Heart and Skeletal Muscle** (J1) *joint with* **Mitochondria in Aging and Age-Related Disease** (J2) Keystone, Colorado | USA
	13–17	**Single Cell Biology** (L1) Breckenridge, Colorado | USA
	17–21	**Tuberculosis: Mechanisms, Pathogenesis and Treatment** (A3) Banff, Alberta | Canada
	20–24	**Integrated Pathways of Disease in NASH and NAFLD** (A4) Santa Fe, New Mexico | USA
	20–24	**Cancer Vaccines** (L2) Vancouver, British Columbia | Canada
	21–25	**Digital Health: From Science to Application** (A5) Keystone, Colorado | USA
	21–25	**Windows on the Brain: Formation and Function of Synapses and Circuits and Disruption in Disease** (A6) Taos, New Mexico | USA
	27–31	**Cellular Plasticity: Reprogramming, Regeneration and Metaplasia** (J3) *joint with* **Signal Dynamics and Signal Integration in Development and Disease** (J4) Keystone, Colorado | USA
February	2–5	**Transcription and RNA Regulation in Inflammation and Immunity** (B1) Tahoe City, California | USA
	10–14	**Molecular Approaches to Vaccines and Immune Monitoring** (J5) *joint with* **B Cell–T Cell Interactions** (J6) Keystone, Colorado | USA
	10–14	**Obesity and Adipose Tissue Biology** (J7) *joint with* **Functional Neurocircuitry of Feeding and Feeding Behavior** (J8) Banff, Alberta | Canada
	17–21	**Autophagy: From Model Systems to Therapeutic Opportunities** (B2) Santa Fe, New Mexico | USA
	18–22	**Uncovering Mechanisms of Immune-Based Therapy in Cancer and Autoimmunity** (B3) Breckenridge, Colorado | USA
	19–23	**Genome Engineering: From Mechanisms to Therapies** (B4) Victoria, British Columbia | Canada
	24–28	**Tumor Metabolism** (B5) Keystone, Colorado | USA
	24–28	**Cell Competition in Development and Disease** (B6) Tahoe City, California | USA
	24–28	**Myeloid Cells** (B7) Santa Fe, New Mexico | USA
	24–28	**RNA-Protein Interactions** (X1) *joint with* **Long Noncoding RNAs: From Molecular Mechanism to Functional Genetics** (X2) Whistler, British Columbia | Canada
March	3–7	**Phenotypic Drug Discovery: Recent Advances and Insights from Chemical and Systems Biology** (C1) Breckenridge, Colorado | USA
	3–7	**Diabetes: Innovations, Outcomes and Personalized Therapies** (X3) *joint with* **Unraveling the Secrets of Kidney Disease** (X4) Whistler, British Columbia | Canada
	10–14	**Cancer Immunotherapy: Mechanistic Insights to Improve Clinical Benefit** (C2) Whistler, British Columbia | Canada
	10–14	**Microbiome: Chemical Mechanisms and Biological Consequences** (C3) Montréal, Québec | Canada
	10–14	**Innate Immune Receptors: Roles in Immunology and Beyond** (M1) Taipei | Taiwan
	15–19	**Mammalian Sensory Systems** (C4) Seattle, Washington | USA
	15–19	**Cancer Metastasis: The Role of Metabolism, Immunity and the Microenvironment** (M2) Florence | Italy
	17–21	**Epigenetics and Human Disease** (X5) *joint with* **3D Genome: Gene Regulation and Disease** (X6) Banff, Alberta | Canada
	24–27	**Origins of Allergic Disease: Microbial, Epithelial and Immune Interactions** (M3) Tahoe City, California | USA
	24–28	**Innate and Non-Classical Immune Cells in Cancer Immunotherapy** (C5) Keystone Resort | Keystone, Colorado | USA
	24–28	**HIV Vaccines** (X7) ♦ *joint with* **Functional Cures and the Eradication of HIV** (X8) ♦ Whistler, British Columbia | Canada
	31–4	**Lipidomics and Functional Metabolic Pathways in Disease** (C6) Steamboat Grand | Steamboat Springs, Colorado | USA
April	7–10	**Imaging Across Scales: Leveraging the Revolution in Resolution** (D1) Snowbird, Utah | USA
	7–10	**Protein Replacement through Nucleic Acid Therapies** (R5) Steamboat Springs, Colorado | USA
	7–11	**Antibodies as Drugs: New Horizons in the Therapeutic Use of Engineered Antibodies** (D2) Breckenridge, Colorado | USA
	7–11	**Proteomics and its Application to Translational and Precision Medicine** (D3) Stockholm | Sweden
	8–11	**Skin Health and Disease: Immune, Epithelial and Microbiome Crosstalk** (D4) Hannover | Germany
	10–13	**Biomolecular Condensates: Phase-Separated Organizers of Cellular Biochemistry** (D5) Snowbird, Utah | USA
	14–18	**Immunometabolism, Metaflammation and Metabolic Disorders** (D6) Vancouver, British Columbia | Canada
	14–18	**Small Regulatory RNAs** (D7) Daejeon | South Korea
May	6–9	**Delivering Therapeutics Across Biological Barriers** (E1) Dublin | Ireland
	13–16	**Climate Change-Linked Stress Tolerance in Plants** (M4) Hannover | Germany
June	9–13	**Positive-Strand RNA Viruses** (E2) ♦ Killarney, County Kerry | Ireland
	16–20	**Neural Environment in Disease: Glial Responses and Neuroinflammation** (Z1) *joint with* **Neurodegenerative Diseases: New Insights and Therapeutic Opportunities** (Z2) Keystone, Colorado | USA

Scholarship deadlines precede meetings by four months, abstract deadlines by three months and discounted registration deadlines by two months. View details for each conference at **www.keystonesymposia.org** followed by **/19** and the alpha-numeric **program code** (e.g., **www.keystonesymposia/19A1**). 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. ♦ Global Health Series conference.

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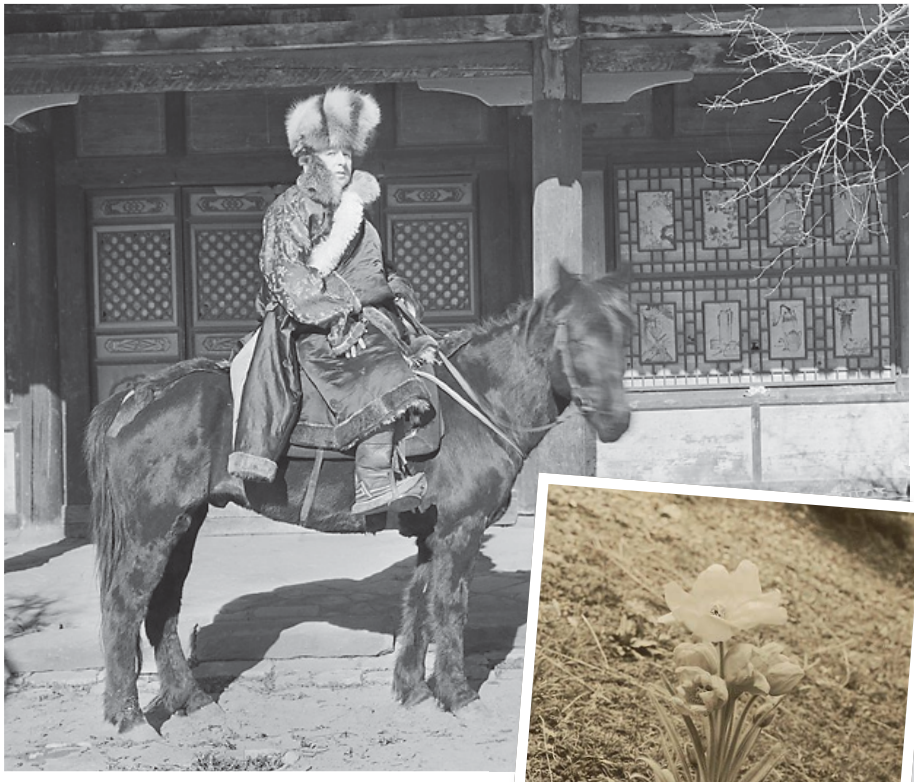
China's Flowers, 1922-1949

BY ASHLEY YEAGER

On a botanical expedition to China's Yunnan Province in 1925, explorer Joseph Rock and his entourage were unexpectedly ambushed by a group of bandits. The caravan, which included servants, porters, military guards, and research assistants, retreated to a nearby hill and waited, weapons ready. Another, less-well-armed caravan traveled the same path, and the bandits chose to prey on it instead. Rock watched through his field glasses, and witnessed native soldiers on horseback appearing out of nowhere to disperse the bandits.

Rock's adventures in China's far west weren't always so precarious. The Austrian-American also enjoyed fine dinner service and hot baths in a collapsible tub, even in remote desert regions. According to a contemporaneous *National Geographic* article, he once said, "You've got to make people think you're someone of importance if you want to live in these wilds."



FLOWER POWER: Joseph Rock, dressed in Tibetan clothing, prepares to explore. (Right) Rock's photo of *Meconopsis integrifolia* taken in 1925 in Gansu Province, China

The US Department of Agriculture, Harvard's Arnold Arboretum and its Museum of Comparative Biology, and other institutions paid Rock's way to Asia to collect plant and bird specimens and other treasures, and he spent three decades on the task. He first went to Assam in northeast India and the countries then known as Burma and Siam in 1920 to collect seeds of the chaulmoogra tree (*Taraktogenos kurzii*) and related species, which produced a substance used to treat leprosy. Rock was then sent to China, where he gathered tens of thousands of herbarium specimens.

Many of Rock's herbarium sheets—pieces of plants pressed and labeled on paper—represented the first documentation of the enclosed species, Michael Dosmann, the keeper of the living collections at Arnold Arboretum, tells *The Scientist*. These sheets are now used to identify species that have gone extinct in the regions of western China that Rock explored. "The land and the world has changed since he was in Asia almost 100 years ago," Dosmann says. "We're using his specimens to determine what you could return and restore."

Rock "wasn't solely a botanist," Dosmann says. "His ability to document language and understand culture was unprecedented." The explorer wrote two histories of the Naxi people of northwestern Yunnan Province, along with a 1,094-page Naxi–English dictionary; documented conflicts between Hui Chinese forces and Ngolok Tibetans; and photographed people of various ethnic groups. *National Geographic* published many of his travel diaries between 1922 and 1935.

Rock employed considerable visual skills not only in identifying plants and deciphering Naxi pictographs, but also in compiling maps, writes Tibetan and Himalayan studies scholar Michael Aris of Oxford University in his 1992 book, *Lamas, Princes, and Brigands: Joseph Rock's Photographs of the Tibetan Borderlands of China*. "His photographs, too, often taken under very difficult circumstances, provide eloquent testimony to his drive for classifiable visual evidence."

"Rock was a bit of a polymath," Dosmann says. "He was incredible." ■

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