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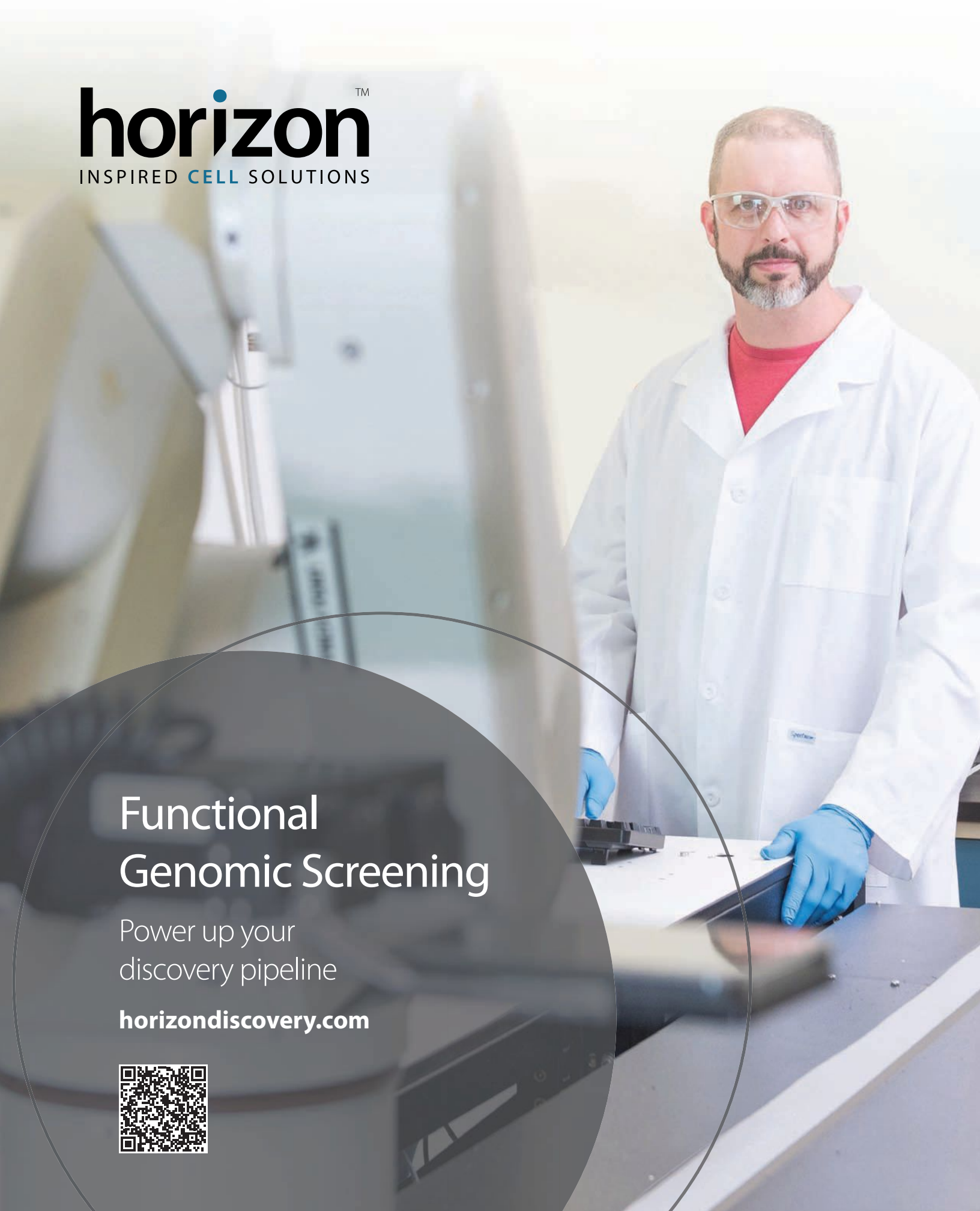
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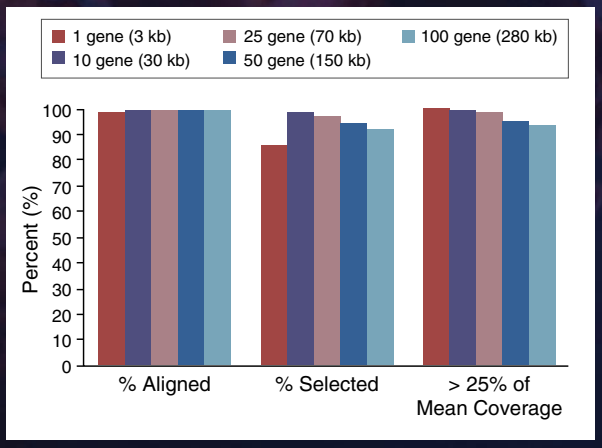


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# Contents

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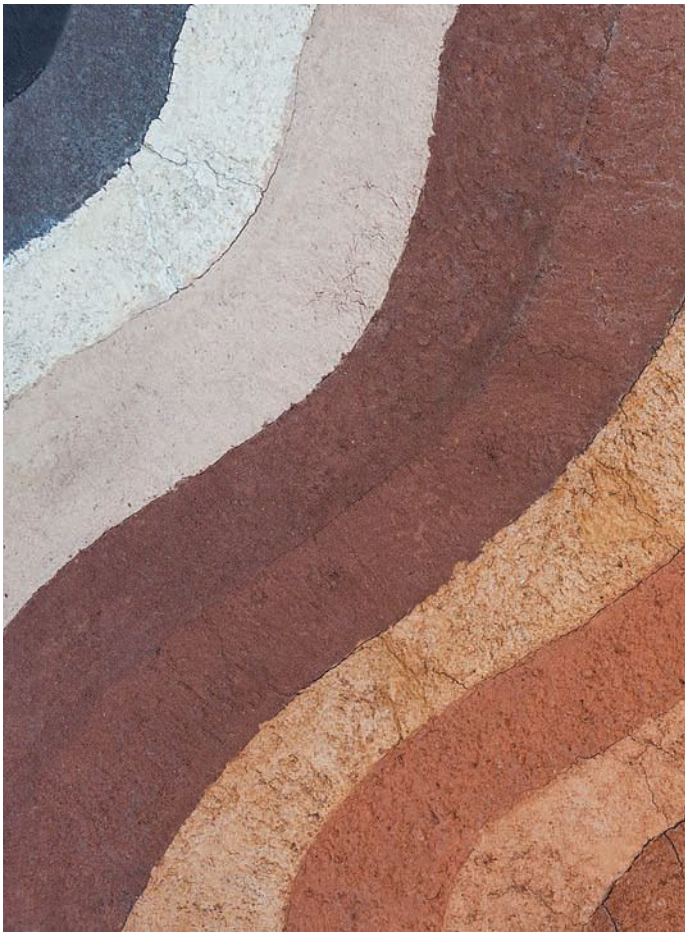

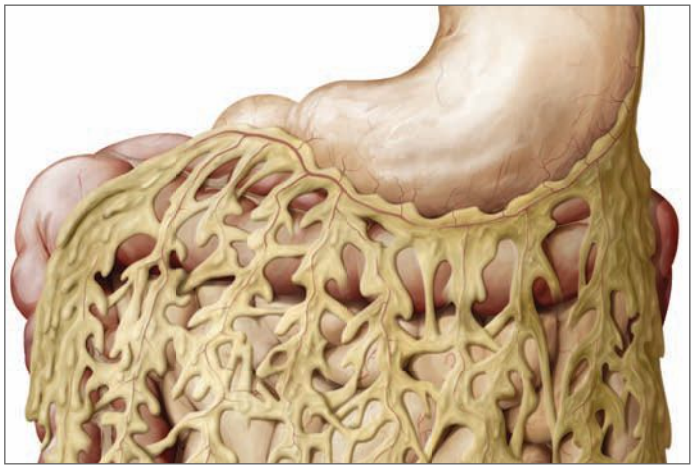

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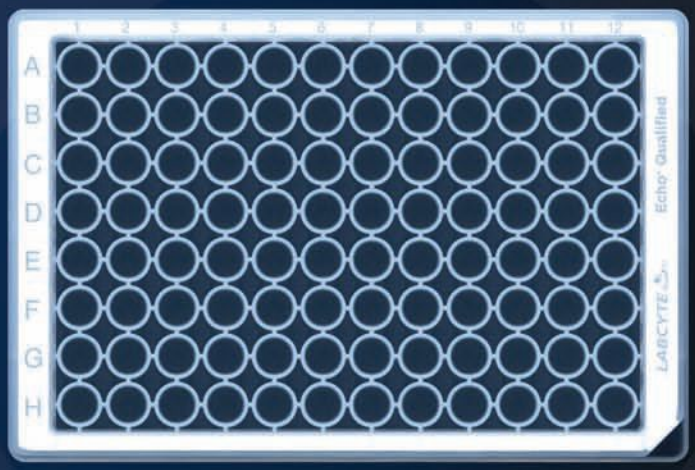


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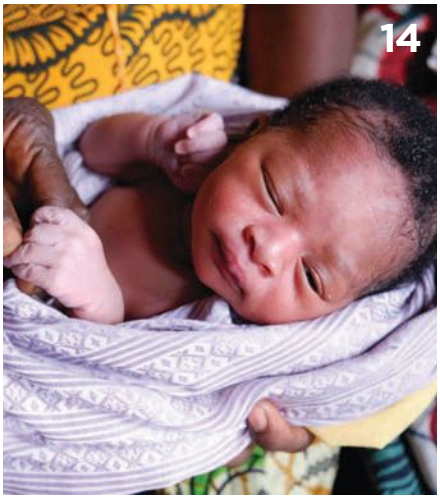


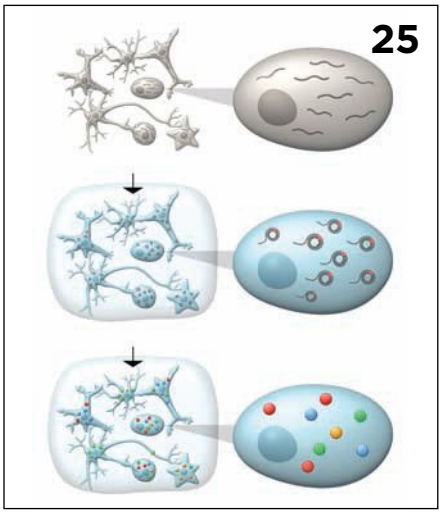


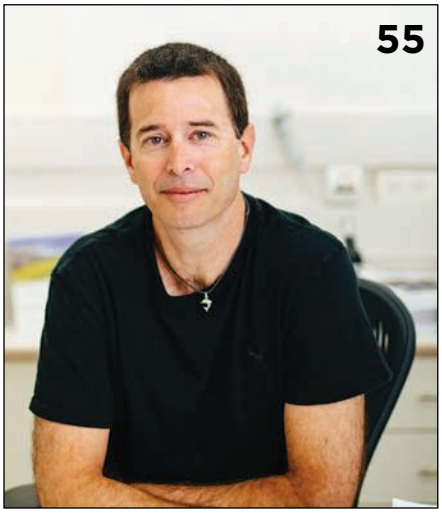


FELICIA WEBB; © GEORGE RETSECK; DAVID VAAKNIN



**CORRECTIONS:**
In "The Elderly Muscle" (September 2018), the story incorrectly stated that John Faulkner worked with Heather Carlson at the University of Michigan in the late 1980s. Rather, Bruce Carlson was Faulkner's collaborator. In addition, the muscle stem cells were depicted as appearing inside the sarcolemma, when in fact these cells exist outside of this membrane. Finally, a misleading sentence about the role of satellite cells in muscle aging has been removed. Both the number and function of satellite cells likely plays a role in muscle decline. *The Scientist* regrets these errors.

**PUZZLE ON PAGE 12**

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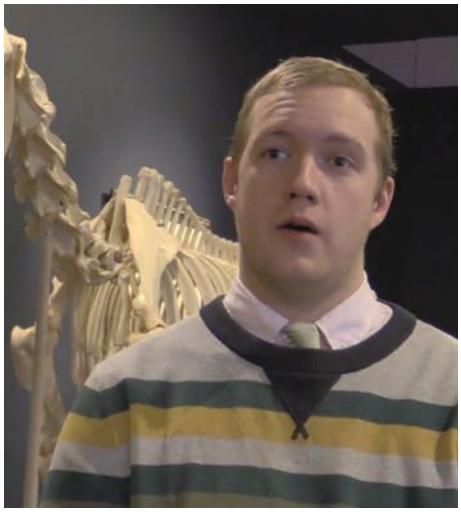


THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
**Rememberers**
Authors Hilde Østby and Ylva Østby introduce their book, *Adventures in Memory*, and explain why it was initially titled *Diving for Seahorses*.

VIDEO
**Sickle-Cell Roots**
Pediatrician Beverley Nelson relays the history of Walter Clement Noel, the first patient diagnosed with sickle-cell disease.

VIDEO
**Gift-Horse Mouths**
William Taylor, archaeologist at the Max Planck Institute for the Science of Human History, describes his PhD work studying equine skeletal remains.

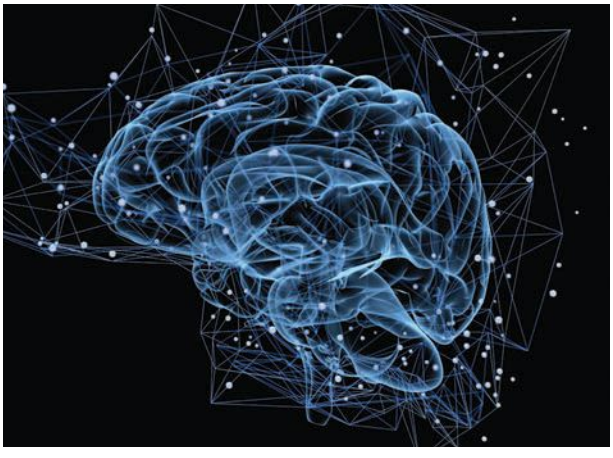
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

# Coming in November

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

- Can science define intelligence?
- The emerging field of neuroepigenetics
- Techniques that go beyond optogenetics
- Ancient brain surgery

AND MUCH MORE



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# Contributors

As a biology major undergrad at the University of Denver, **Troy Randall** took an immunology course that he found "incredibly interesting." Now, as an immunology researcher at the University of Alabama at Birmingham (UAB), Randall realizes that he "probably understood nothing about it." His interest in immunology drove him to study the subject as a doctoral student at Duke University, where he met his wife, Frances Lund, with whom he now runs his lab at UAB. One aspect of his current research involves understanding belly fat's role in generating an immune response, and how that function gets disrupted by ovarian cancer.

As a nine-year-old, **Selene Meza-Perez** grew interested in science while watching Carl Sagan's television show *Cosmos* with her father. "Instead of looking at the sky" Meza-Perez swapped her telescope for a microscope, she says, to study biology. She wound up completing her doctoral studies at the National Polytechnic Institute in Mexico, in immunology. She began working for Randall after a chance meeting led to the realization that fatty tissue's immune role was a topic of mutual interest. Meza-Perez says a "passion for knowledge" inspires her to produce work that eventually may contribute to improving human health. When not reading books about science or discussing the field with her husband (also an immunology researcher), Meza-Perez enjoys hiking and playing with their dog, Leika.

On pg. 44, Randall and Meza-Perez write about the evolving understanding of a fat deposit called the omentum.

In 2006, after training as a clinical neuropsychologist at the University of Oslo, **Ylva Østby** began working with epilepsy patients at Oslo University Hospital. Initially interested in psychology, she was drawn to basic neuroscience and took up doctoral work in a laboratory at the university, where she studied the development of brain structures and memory formation. As a postdoc at the same university, Østby currently studies "how memories are experienced in our consciousness," particularly in patients with memory dysfunction and epilepsy. When her older sister Hilde suggested that she write a book, Østby said, "If we could do it together, I would do it." The result is their newly published *Adventures in Memory*. The process of writing a book for the general public has been "eye-opening," says Østby, adding that it helped her get a fresh perspective on the neuroscience of memory. Further broadening her outlook, Østby is working with researchers from the University of Oslo's Department of Literature on a study on the interplay between cognition, emotion, and literature.

From the time she was 10 years old, **Hilde Østby** was a storyteller—at least to her sisters. Now, as a freelancer, she writes for *Aftenposten*, Norway's largest newspaper, and other outlets, while also exploring several book ideas. "In my professional life, I've never had so many stupid quarrels," Hilde says of writing a book with her sister. The elder Østby, who has a master's degree in the history of ideas from the University of Oslo, adds that the book's coauthors have really been working on parallel subjects their whole careers. Read an essay based on the Østby sisters' collaborative effort, *Adventures in Memory: The Science and Secrets of Remembering and Forgetting*, on pg. 63.

When **Sukanya Charuchandra** was in high school, she made the decision to follow in the footsteps of her parents, who were both involved in the scientific enterprise. Her father sold and serviced laboratory equipment, and her mother was a geneticist. "One of my teachers showed us a video of the heart and how the heart muscles look from the inside," Charuchandra recalls. Fascinated by the physiological insight, the young student rushed home to relay her excitement to one of her older sisters. "I remember describing it to her and telling her that it's all so exhausting. I just wished the poor thing could take a break."

She first earned a bachelor's degree in life science from St. Xavier's College in Mumbai, her home town. But after getting a master's degree in biotechnology from Maharaja Sayajirao University of Baroda in Gujarat, Charuchandra, disillusioned with the realities of laboratory work, decided to take her own break. She took a year off of school instead of forging on to do a PhD and discovered that science journalism might be a way for her to parlay her academic achievements into a nonresearch career.

After getting a master's degree in science journalism from Boston University in 2017, Charuchandra freelanced and did internships at Boston Children's Hospital and for the Johns Hopkins University Office of Communications. She started as an intern at *The Scientist* this May and has written numerous pieces for the website and the print magazine. "Just being able to put out so much . . . it taught me a lot," she says. Read her story, "Toothy Tales," on pg. 20, one of several articles she authored in this issue.

KATINKA HUSTAD; CAPPELEN DAMM

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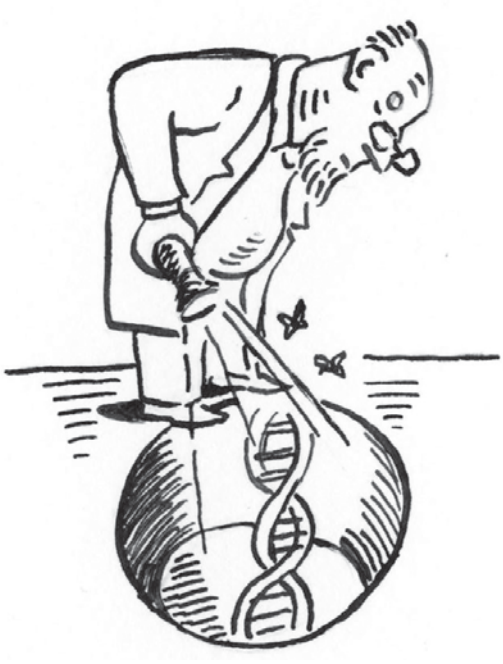
# Genes and Blues

Learning about your own genetic idiosyncrasies comes with complex emotions.

BY BOB GRANT



ANDRZEJ KRAUZE

Earlier this year, I ordered a genetic sampling kit from a website, rubbed a cotton swab vigorously on the inner surface of my cheek, sealed and returned my biological sample via the US Postal Service, and some weeks later, received information about the DNA that sits inside my cells. In doing so, I joined the ranks of millions of people—more than 12 million as of the end of 2017—who've explored their own genetic blueprints in similar fashion.

So what did I learn from dipping my toes into the bustling direct-to-consumer (DTC) genetics market? The cocktail party talking points are too numerous to list, but here's a taste: I'm 0.9 percent Ashkenazi Jewish, devoid of two of the genetic variants associated with celiac disease, likely to wake up around 8:02 AM (strike one, DTC genetics company), and unlikely to have a bald spot (strike two).

Even if the results are not 100 percent reflective of my biological reality, these insights are entertaining, and can inform decisions—whether accurate or not—concerning diet, exercise, sleep, procreation, and a suite of other choices for how to behave in light of my genetic backdrop. But in a clinical setting, via a different DNA test, I also recently learned that I harbor abnormalities in a gene whose dysfunction leads to a very serious condition.

At first, I greeted this newly acquired knowledge with something like relief. I am in fine fettle, and have been so for 40+ years of life. Therefore, my children, should they too have the variant, are likely to continue enjoying good health through childhood, adolescence, and adulthood—at least in the context of this one tiny bit of genetic makeup.

But as I thought about the molecular mechanics at play, fear and guilt started to creep into my thinking. What if I'm not as flush with good health as I believe I am? Is my ultimate demise encoded in my cells, lying in wait like a patient predator, biding its time before lurching to the surface and snapping me up? And more importantly, could my surreptitiously scripted denouement signal a less rosy outlook for my kids or my unborn decedents? I found myself looking up from my perch in my family tree, wondering about the genetic legacy being passed on from branch to branch.

I also found myself looking down the tree. How many generations of my ancestors carried this now-identifiable genetic change without knowing it? Such is the weight that comes with science's increasing ability to plumb the depths of life. Learning about the unexpected intricacies of one's own DNA, about previously undiscovered biological communities, or about unprecedented evolutionary dynamics resulting from recent anthropogenic forces is only half the battle. The other half is ethical and moral.

In this issue, you'll meet researchers working to characterize a biome that exists kilometers beneath our feet ("Underworld," pg. 28); realize that ecological damage and evolutionary changes are wrought by light pollution ("The Vanishing Night," pg. 36); and gain a new appreciation for a traditionally vilified tissue that serves vital cellular and molecular, immune and metabolic functions ("How Fat Fights Infection," pg. 44). These quests, much like the science that goes into illuminating human genetics, are monumentally important. But they should, and often do, lead to more questions, many involving human behavior.

Knowledge brings responsibility. The implications of all the new information science is acquiring extend beyond the laboratory, into the halls of government, the corporate boardroom, and even the family dining room. Here's to hoping that our species can progress forward, guided by an increasingly robust body of scientific knowledge to lead politicians, educators, businesspeople, and consumers toward more-informed decisions and a better future. ■

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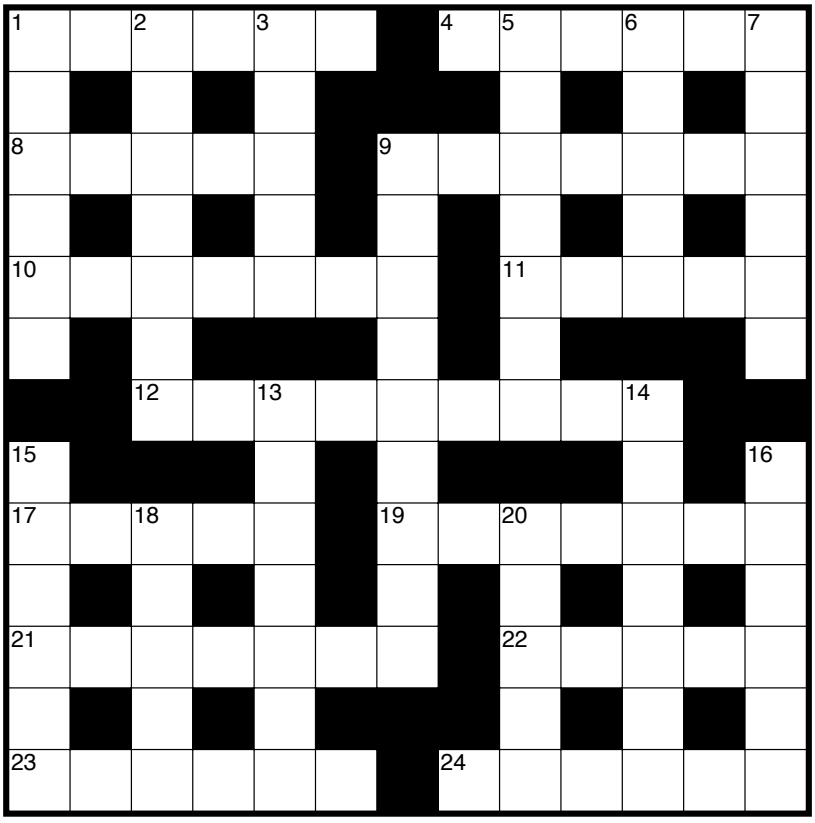
# Speaking of Science



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

BY EMILY COX AND HENRY RATHVON

## ACROSS

1. Sandbanks or gravel bars
4. Fluoxetine's trade name
8. Ellipsoidal yellow fruit
9. Like dodos and dinos
10. Relating to foxes
11. Inventor played by Bowie in *The Prestige*
12. Resembling a duck, pedally (hyph.)
17. Decapods with claws
19. Kin of an orange
21. Drupe whose husk yields coir
22. Coat defect caused by mite bites
23. Having a low pH
24. Protection for a pangolin

## DOWN

1. Secretion that starts the digestive process 2 I = V/R (2 wds.)
3. "Pineapple Island"
5. Sound of a woodpecker (hyph.)
6. Torrid and Frigid, for two
7. China, to Marco Polo
9. Orbital area (2 wds.)
13. Dog without a bark
14. Active by day, like the snowy owl
15. Tree that yields gum arabic
16. Mammals whose den is called a holt
18. Computer code for text
20. Myna or chimp, at times

*Answer key on page 5*

> The thing that is going to be tricky here is that it's going to be very tempting to use [artificial intelligence] as a weapon. In fact, it will be used as a weapon. The danger is going to be more humans using it against each other most likely, I think.

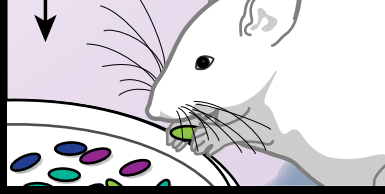
—Entrepreneur and engineer **Elon Musk** speaking with comedian and podcaster Joe Rogan about misgivings concerning the future of AI (September 6)

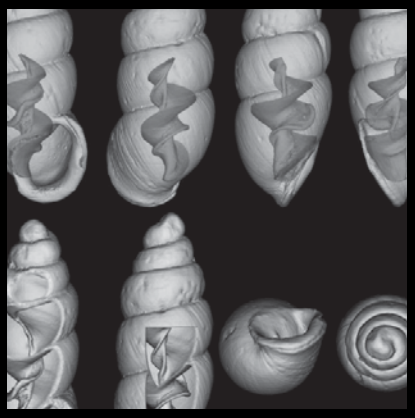
> In the life of the planet, destruction of night is as important an issue as the poisoning of water and air.

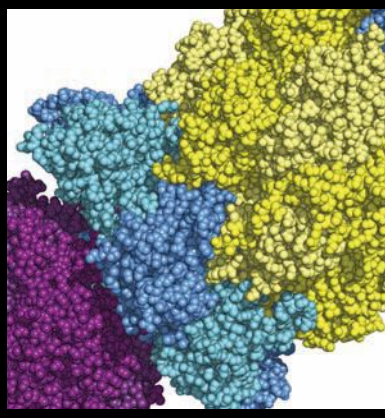
—**Richard "Bugs" Stevens**, a professor at the University of Connecticut School of Medicine, on the ecological and human health damage wrought by light pollution (*Aeon*, August 3)


FRANK COTHAM, CARTOON BANK



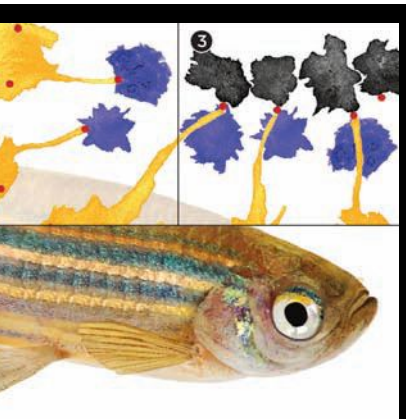
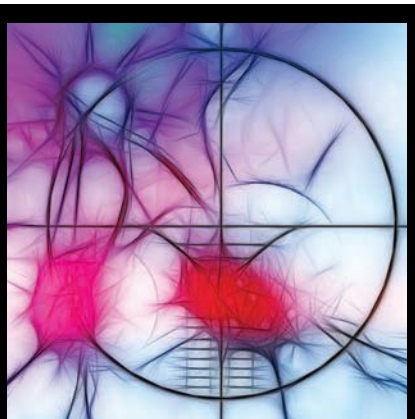
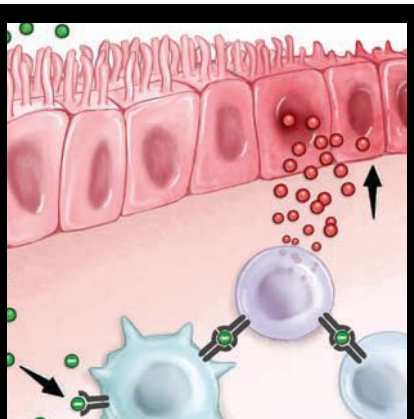














# Notebook

OCTOBER 2018

FELICIA WEBB

**FROM DAY ONE:** Children in Keneba, The Gambia, including this hours-old baby, are part of a long-term investigation into the epigenetics of nutrition and health.

## Time Stamps

Summertime in The Gambia, a tiny West African country of rivers, salt flats, and baobab trees, means the start of the rainy season. "Everything is turning green," says nutrition scientist Andrew Prentice over the phone from his home in the rural village of Keneba. "It's gorgeous." Prentice leads the UK's Medical Research Council International Nutrition Group station in Keneba, about three hours' drive from the main MRC unit in Fajara, near the coast.

For the subsistence farmers in Keneba, the rainy season is also the hungry season; stores of last year's harvest run low, and the next round of crops is just being planted. The cyclical nature of the villagers' nutrition has provided what Prentice calls an "experiment of nature" that he and his colleagues can track to explore how diet affects health during early fetal development.

For 80 years, researchers have studied the residents of Keneba, documenting everything from basic demographics such as birth dates to, in recent years, gene methylation patterns. Two decades ago, Prentice's team made a remarkable discovery in this community: the time of year when study subjects were born was a strong predictor of their risk of dying in young adulthood. Specifically, people born during the hungry season are much more likely to die during their 20s, 30s, or 40s than those born in the harvest months (*Nature*, 388:434, 1997). Ever since then, Prentice has been trying to figure out the biology underlying this phenomenon. What is this memory from early life that rears its head years later to make people more or less likely to die?

Fast forward a decade, zoom across the globe to a bar in Cleveland, pour a few beers, and a lead in this investigation emerges. Rob Waterland, a geneticist at Baylor College of Medicine and a friend of Prentice, is well-regarded for work he did as a postdoc in epigeneticist Randy Jirtle's lab, then at Duke University. Waterland showed that a

mother mouse's diet during pregnancy influences the colors of her pups' coats via the methylation of a particular gene (*Mol Cell Biol*, 23:5293–5300, 2003). "That was the first time that had ever been shown," says Waterland. "A transient nutritional stimulus during a critical period of development could form a permanent phenotypic change by an epigenetic mechanism."

## Conception at times of low food abundance was linked with higher methylation.

Loci that possess this type of individual-specific epigenetic marking that occurs early in development, is present in all tissues, and lasts for a lifetime are called metastable epialleles. (Epigenetic marks are more commonly thought of as specific to certain cell types.) Since his mouse work, Waterland has been on the hunt for metastable epialleles in humans to figure out their roles in phenotypic variation. He likens the search to an archeological dig, with these epigenetic marks acting as fossils representing the time before gastrulation.

So when Prentice and Waterland met at that bar in Ohio, they decided to blend their interests in metastable epialleles and early-life nutrition. "We said, 'Let's test this in humans,'" recalls Prentice. "And the experiment of nature we have here seemed the prime way of doing it."

Using blood samples from 25 Gambian children born in the rainy and dry seasons during the 1990s, Prentice, Waterland, and colleagues examined methylation patterns at five sites in the genome thought to be metastable epialleles based on a separate genomic screen they had done in Caucasian adults. They found that children conceived during the rainy season had greater methylation levels than those conceived during the dry season (*PLOS Genet*, 6:e1001252). In other words, conception at times of low food abundance was linked with higher methylation. Following up in 2014, the researchers provided evidence that it wasn't the abundance of food per se, but the mothers' nutritional status, as gleaned from blood biomarkers, that correlated with children's DNA methylation at metastable epialleles (*Nat Commun*, 5:3746). "Then comes the so-what question," says Prentice. "What do these genes do? Does it really matter at all?"

The researchers began probing the human genome to identify more metastable epialleles. In 2015, they pinned down a tumor suppressor, *VTRNA2-1*, the methylation of which is tied to season of conception (*Genome Biol*, 16:118); a year later, they showed the same for *POMC*, a gene whose methylation patterns are related to an individual's body mass index (*Cell Metab*, 24:P502–09, 2016).

Along the way, they've come across numerous other sites in the human genome that appear to be metastable epialleles. In the group's most recent study, published this past summer in *Science Advances* (4:eeat2624), the researchers identified 687 candidate metastable epialleles—and that's still likely an incomplete list. In unpublished work, they carried out an unbiased screen of methylomes from 10 individuals and found a "treasure trove" of metastable epialleles, says Waterland.

The study from this year used data from an analysis of embryos generated by IVF, confirming in humans that these sites indeed form a record of our earliest days. The same loci that have methylation variation between individuals and consistency across tissues in adults also have these characteristics in embryos. But still, the marks' functional significance remains unclear. "It's all speculation at this point," says Doug Ruden, who studies epigenetics in development at Wayne State University School of Medicine. One attractive idea, according to Ruden, is that metastable epialleles might record environmental conditions for some potential benefit later in life.

Waterland suggests that metastable epialleles might not have a specific function in response to a specific stimulus, but rather exist for the sake of species diversity in the face of unpredictable future conditions. "We don't see particular enrichments for genes associated with any types of disease, but we see genes that affect a wide range of phenotypes. . . . I really think there's a possibility that epigenetic variation at metastable epialleles has an important role to play in determining risk of disease."

FELICIA WEBB

**GROWING UP:** Some of these girls from The Gambia are part of a long-term study into nutrition and health by researchers at the MRC International Nutrition Group.

For Dana Dolinoy, who studies epigenetics and nutrition at the University of Michigan School of Public Health, the work of Waterland, Prentice, and their group helps scientists navigate to the places in the genome where it's most likely that in utero conditions influence development or later health. "Before, you didn't know where to look," she says. Now, investigators know where to dig.

—Kerry Grens

## Breaking In

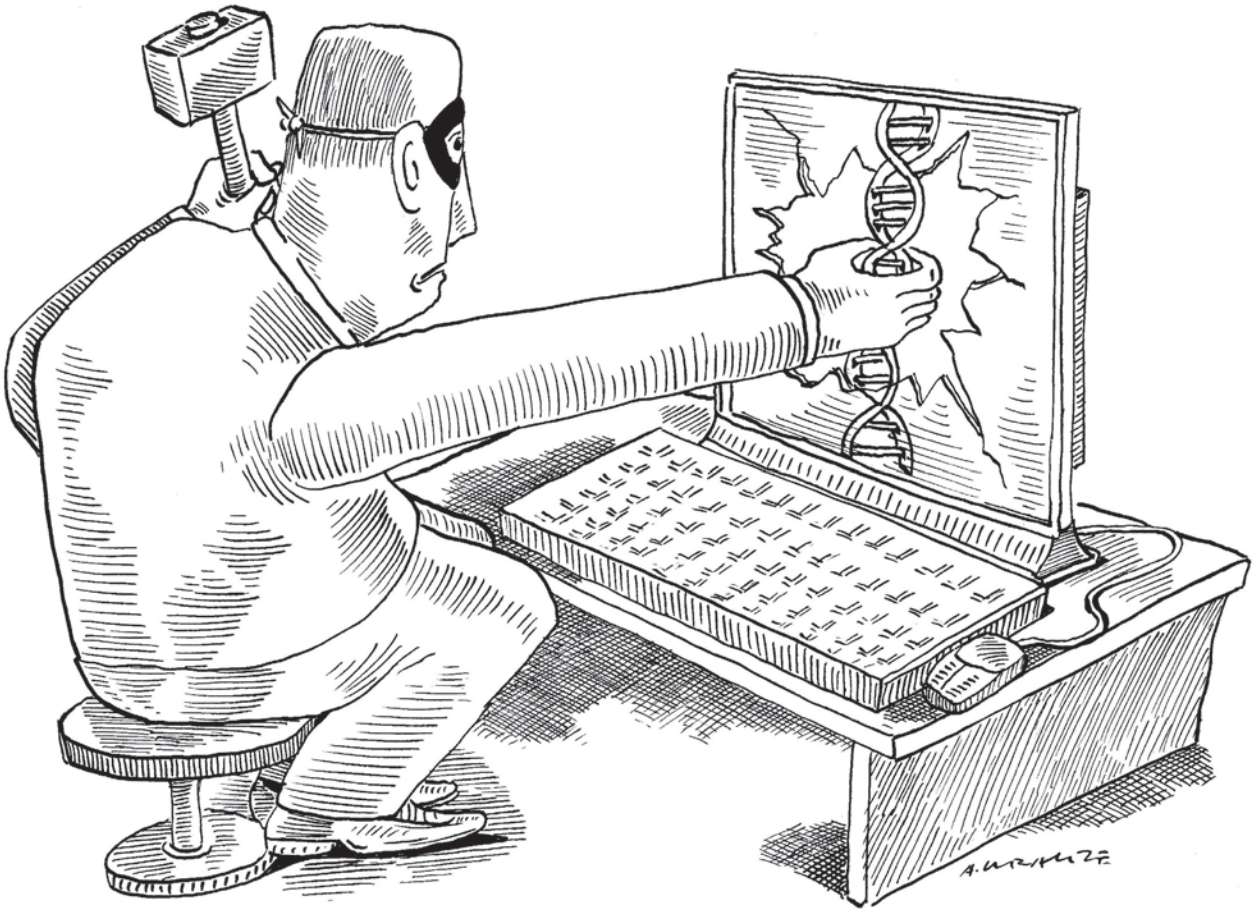
The first few times Ben Sadeghipour hacked into a computer, it was to access the video games on his older brother's desktop. "He would usually have a password on his computer, and I would try and guess his password," Sadeghipour tells *The Scientist*. Sometimes he'd guess right. Other times, he wouldn't. "So I got into learning about how to get into computers that were password protected," he says. "At the time, I had no clue that what I was doing was considered hacking."

The skills he picked up back then would become unexpectedly useful later in life. Sadeghipour now breaks into other people's computer systems as a profession. He is one of thousands of so-called ethical hackers working for HackerOne, a company that provides services to institutions and businesses looking to test the security of their systems and identify vulnerabilities before criminals do.

Although HackerOne has already landed contracts with hundreds of companies—its clients include Yahoo and Airbnb—since its founding in 2012, it attracted particular attention in the health industry earlier this year thanks to a deal with the National Institutes of Health (NIH). The agency's *All of Us* program, which aims to collect genetic and health data from 1 million people in the US to spur research in human biology and medicine, started enrolling volunteers in May. Although the database is still in development—researchers may be able to access the data as early as spring 2019—the program's launch immediately prompted questions about privacy risks.

By their nature, personal health records offer easy routes to identity theft, ransom demands, or the illegal sale of medical information—and attacks are on the rise. In the summer of 2014, 1.3 million patients with health data stored by Montana's Department of Public Health and Human Services were notified of a database breach. This past summer, Singapore announced that an attack on its central health system had compromised the data of 1.5 million people.

HackerOne's approach to minimizing this risk is to deploy experts such as Sadeghipour to find vulnerabilities in the system before anyone else does. From a client's perspective, "working



ANDRZEJ KRAUZE

**You pound your head on the table and say, "My goodness, we should have caught that."**

—Kermit Littlefield, NIH

with ethical hackers basically helps you improve your security capabilities by augmenting your existing processes with a wider pool of talent," explains Adam Bacchus, director of program operations at HackerOne. "It acts almost like a neighborhood watch that helps you find and fix bugs before criminals can actually exploit them." HackerOne won't disclose which hackers are taking part in the *All of Us* project and does not allow its hackers to talk openly about projects they are working on.

The NIH offers bounties—monetary rewards ranging from a few hundred to a few thousand dollars—for each vulnerability that any hacker in a group approved by HackerOne can find in the initial version of its *All of Us* database. Those discoveries complement the constant monitoring and security testing that the NIH is already running on a daily basis, explains Kermit Littlefield, information systems security officer for the *All of Us* program. "We take very seriously the trust our participants place in us," he says. "This was just another way to ensure we're safeguarding the data."

Sadeghipour notes that there are some typical approaches to looking for holes. "It all comes down to understanding the application," he says. Working out how different parts of a site share data, manage logins, and let users navigate around offers insights into how to trick a server into granting access to someone it shouldn't, he explains. Many hackers speed through such tests with the help of customized software that can run scans and automate attacks on websites. During the projects he's worked on, "there have been times where I have found people's addresses, phone numbers, emails," Sadeghipour says. "There've been times where I've got complete access to a company's server or website."

On finding such a vulnerability, a hacker submits a report explaining what he did via HackerOne, and the client's security team works on fixing the problem. The NIH's Littlefield tells *The Scientist* that, "in general, 95 percent [of cases] you pound your head on the table and say, 'My goodness, we should have caught that.' They're straightforward fixes." Low-level issues of this sort could include server misconfigurations that lead to a leak of information that isn't particularly sensitive, but that a company wouldn't necessarily want to be available

on the internet, Bacchus explains. Littlefield would not discuss specific weaknesses regarding the *All of Us* database.

Occasionally, though, bugs can be more serious, offering a hacker the ability to download private data with a few tricks. A find like that could fetch a bounty of around $2,000, Littlefield says. It's enough for a hacker to make a living—some reportedly make more than $200,000 a year chasing bounties—and the industry is experiencing a boost in popularity, with hackers such as Sadeghipour even creating programs to train new recruits and expand the community. But, as everyone working in data security emphasizes, ethical hacking is ultimately just an extra layer of protection. "No database is perfectly secure," says Michael Szego, a clinical ethicist at the University of Toronto. "You can do the best that you can, but there will always be people trying to get in these things."

For some companies and organizations, the data privacy solution consequently lies elsewhere. Some startups are now seeking to drastically reduce health databases' hackability by incorporating blockchain—a technology originally implemented to keep track of the cryptocurrency bitcoin—to produce a supposedly fraud-proof record of every time a database is accessed (see "Data Rush," page 60). Other efforts, such as the Personal Genome Project, are doing away with privacy altogether. "We actually make the data publicly available," explains Szego: the Canadian branch of the project, with which he is affiliated, recently published an analysis of its first 56 genomes. Participants provide informed consent to share their data online after reviewing their health and genetic records with a specialist, he explains. "We don't make any claims that our data will be secure."

—Catherine Offord

# Climate Cycles

Pieter De Frenne, an ecologist and cycling fan at the University of Ghent, was watching old footage of the Tour of Flanders—a popular Belgian 260-kilometer race held every April—on the web when something in the background caught his eye. The trees lining the racecourse in the clips from the 1980s were bare. But, he recalled, in footage of the most recent races, they were covered in leaves.

That got him thinking: perhaps archived footage from sporting events such as the Tour of Flanders could provide data on the effects of climate change on trees' phenology—the timing of leafing and flowering each season. "Video archives can be a very useful tool but have been largely unused until now to study the connection between climate change and phenology," says Pieter Vangansbeke, an environmental scientist at Ghent who also watched the cycling footage and recounted De Frenne's story to *The Scientist*.

Sunlight, temperature, and other environmental signals spur plants to leaf and flower. With global temperatures increasing in recent decades, plants have started to grow their leaves and flowers earlier each spring. Scientists have wanted to track this climate-driven creep in plant phenology for decades, and have traditionally used herbarium specimens to do so. However, "few botanists collect specimens these days, especially in Europe and North America, and there are now large gaps in the herbarium records," Claude Lavoie, a biologist at Laval University in Quebec City who was not involved in the research, tells *The Scientist* by email. "It is becoming extremely difficult to use herbarium data for phenological studies, because there are very few modern specimens covering the 21st century. We have records for the past, but no data for the present."


CLIMATE CAM: Researchers use footage of the Tour of Flanders cycling race to study the effects of climate change on plant phenology.

**Few botanists collect specimens these days, especially in Europe and North America.**

—Claude Lavoie, Laval University

To get around the problem, some researchers have made use of descriptions of natural environments in famous written works, such as Henry David Thoreau's *Walden*, and pored over photographs of outdoor landscapes to track plant communities' response to climate change. But with the advent of mobile technology, such an approach is easier these days. "People now take thousands of photographs with their smartphone every day of the year, and you can then reconstruct

FLEMISH RADIO AND TELEVISION BROADCASTING ORGANIZATION


FLEMISH RADIO AND TELEVISION BROADCASTING ORGANIZATION

**HOT RIDE:** Sports videos reveal that rising winter temperatures are associated with trees growing leaves and flowers earlier in the year.

phenological events, like leafing, with these photographs—especially in botanical gardens where couples go to take their marriage pictures, often always in front of the same pond or tree," Lavoie says. "The challenge here is to find a way to collect those pictures. And this is where the idea of footage is brilliant, since all those images, taken at the same places and the same periods of the year, are stored in a unique collection."

That consistency was key, Vangansbeke says: the archival video footage of the Tour of Flanders is standardized. It shows the same trees and shrubs from various angles year to year, so it has an advantage over ad hoc images or herbarium specimens. He and De Frenne tracked down old tapes—more than 200 hours of footage—of the Tour in the archives at the Flemish Radio and Television Broadcasting Organization and sent PhD student Lisa Van Langenhove to watch it.

Over the course of five weeks, Van Langenhove identified 46 individual trees and shrubs that had been caught on film from multiple angles, giving the

team 523 images to use to track when the plants leafed and flowered each year, and to measure the size of the leaves. When analyzing the data, the team found that during races that took place in the 1980s, almost no trees or shrubs on the course had begun to flower, and only 26 percent showed any leaves. But from 2006 onward, 45 percent of the same woody plants had started to leaf and 67 percent had started flowering by the time the cyclists hit the road in early April. And when the team correlated the plant data with local climate data—which have logged a temperature increase of 1.5 °C since 1980—the researchers found a solid link between warmer winter temperatures and earlier leafing and flowering (*MEE*, 9:1874–82, 2018).

"When we started the study, we expected to see some sort of change. Still, we were quite surprised to see how well we could see the change in the data, demonstrating significant connections between temperature and phenology," Vangansbeke says.

"This is a fun paper," Elizabeth Wolkovich, an ecologist at the University of British Columbia who was not involved in the study, tells *The Scientist*. "What's novel here is the idea of using footage from sporting events such as cycling races." A lot of phenology data already exist from Western Europe, she notes. Applying this method to footage from winter sports in the Arctic and other less studied regions could provide even more-valuable insights about the effects of climate change.

Such studies will also be "extremely important," when it comes to providing evidence of climate change for the public, notes Lavoie. While "there is overwhelming evidence of climate warming, there are still a lot of people, especially politicians, who do not believe this," he says. Displaying temperature trends on a chart is clear to a scientist, but often remains an abstraction for people with only limited scientific knowledge, he adds. "Showing images, especially sports images—that strikes the imagination."

—Ashley Yeager

# Toothy Tales

Mongolian horse herders extract certain premolars, called wolf teeth, from the mouths of horses before the animals turn two years old, usually with the aid of a screwdriver. This form of equine dentistry makes wearing metal mouthpieces, or bits, a tad more comfortable for creatures whose long service to humans galvanized the spread of civilizations throughout history, aiding in trade, warfare, transport, and communication.

William Taylor, an archaeologist at the Max Planck Institute for the Science of Human History in Jena, Germany, was studying horse bones at the National Museum of Mongolia and carrying out his own excavations in the East Asian country when local researchers told him about this form of oral health care that modern-day Mongolian herders administer to their horses. Taylor wondered "how deep the history of this practice is," he tells *The Scientist*, and whether more-ancient versions of equine dentistry might help explain some of the "funky specimens that we're finding in the museum."

With his Mongolian colleagues, Taylor began systematically looking at ancient horse teeth from the museum's collections and from his own expeditions. In all, the group studied horse specimens from 29 different sites across Mongolia, including horse remains that were ritualistically entombed alongside stone monuments featuring carvings of deer—important symbols in early Mongolian art. Some of the sites contain hundreds or thousands of horse remains—often just the heads, given "that the rest of the horse would have been eaten" by its owners, Taylor tells *The Scientist*.

Studying these samples in detail, the team identified two different dental procedures carried out by early, pastoral Mongolians. One set of specimens, dated to 1150 BCE, included milk teeth that had been sawed down, probably with a stone instrument. In modern horse dentistry, these baby teeth are often extracted to prevent interference with the development of permanent teeth or the wearing of a bridle and bit.

This attempted taming and removal of milk teeth is the earliest-known instance of a veterinary dental procedure (*PNAS*, 115:E6707–15, 2018). "People were really investing in the health care of horses in a way that had never happened before," says Taylor. The research-

**OPEN WIDE:** A Mongolian herder removes this horse's first premolars, or wolf teeth, using a screwdriver.

DIMITRI STASZEWSKI, TAYLOR ET AL. 2018, *PNAS*

**ANCIENT PRACTICE:** Researchers detected evidence of dentistry in horse skulls dating back more than 3,000 years.

WILLIAM TAYLOR

ers suggest in their paper that the practice dates back to a period when horses were increasingly being used for transportation, meat, and milk—ultimately, when the animals became "the foundation of the economy here in Mongolia," Taylor says.

He and his colleagues also noticed that horse skulls from around 750 BCE showed signs of having had the first premolar tooth on either side of the upper jaw extracted—the same teeth modern herders now remove with a screwdriver. These so-called wolf teeth are vestigial and usually fall out within the first three years of a horse's life, but their presence can interfere with the positioning of a bit in younger animals, leading to oral trauma.

Taylor and his colleagues propose that the emergence of this more recent practice ties in with the appearance of metal bits, which largely replaced bits made from leather around the late second or early first millennium BCE. With the move toward horse riding, early Mongolians—whose livelihood and nomadic lifestyle was greatly facilitated by the animals—grew more concerned with the health care of their horses, the researchers propose.

"I think it's very interesting that it is so early that people are starting to make adjustments to different teeth in the horse's mouth," says Alan Outram, an archaeologist at the University of Exeter in the UK, who was not involved in this research. Outram suggests broadening the study sites to include the Central Steppe of Kazakhstan and its surrounding areas—where some researchers think horses were first domesticated—to shed more light on the evolution of horse health care as people's use of metals increased. "I think [the current study] is showing that as technology improves . . . dentistry is coming along with it fairly quickly," he says.

The findings are also a reminder of the importance of the relationship between horses and humans, not just for Mongols—whose civilization once used horse-borne armies to rule over a 33-million-square-kilometer territory—but across Eurasia and beyond.

"We wouldn't have been able to have moved around the world, we wouldn't have been able to populate much of the world in the way we have, we wouldn't have been able to build the structures that we have without animal power," says Krish Seetah, a zooarchaeologist at Stanford University who did not participate in Taylor's research. "Human society would not look the way it looks today if it weren't for the relationships we developed with animals."

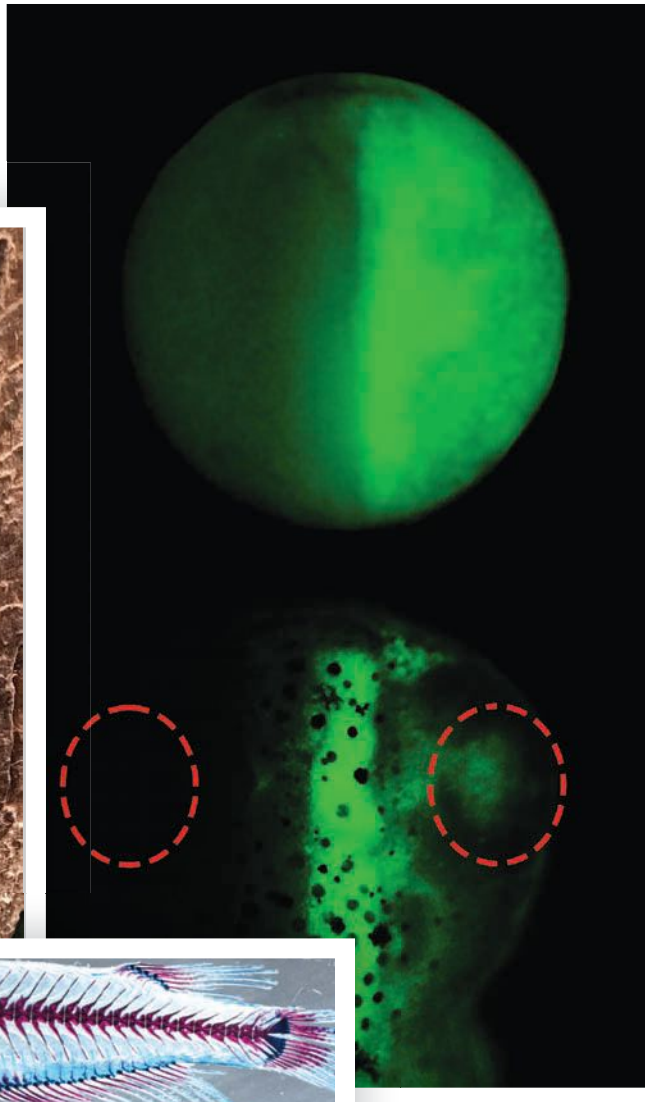
—Sukanya Charuchandra

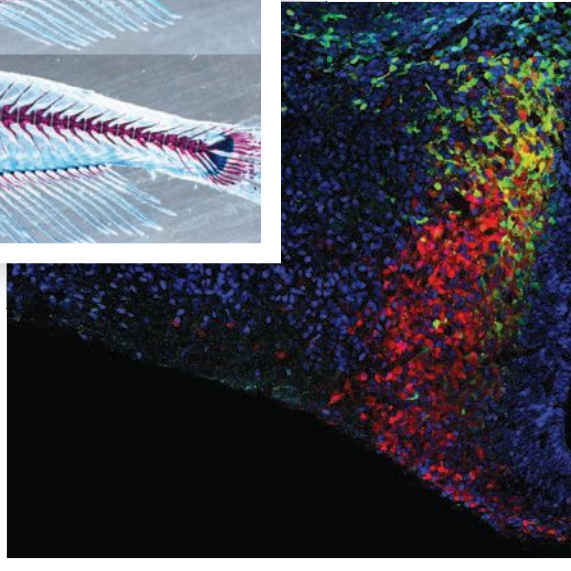
# Caught on Camera

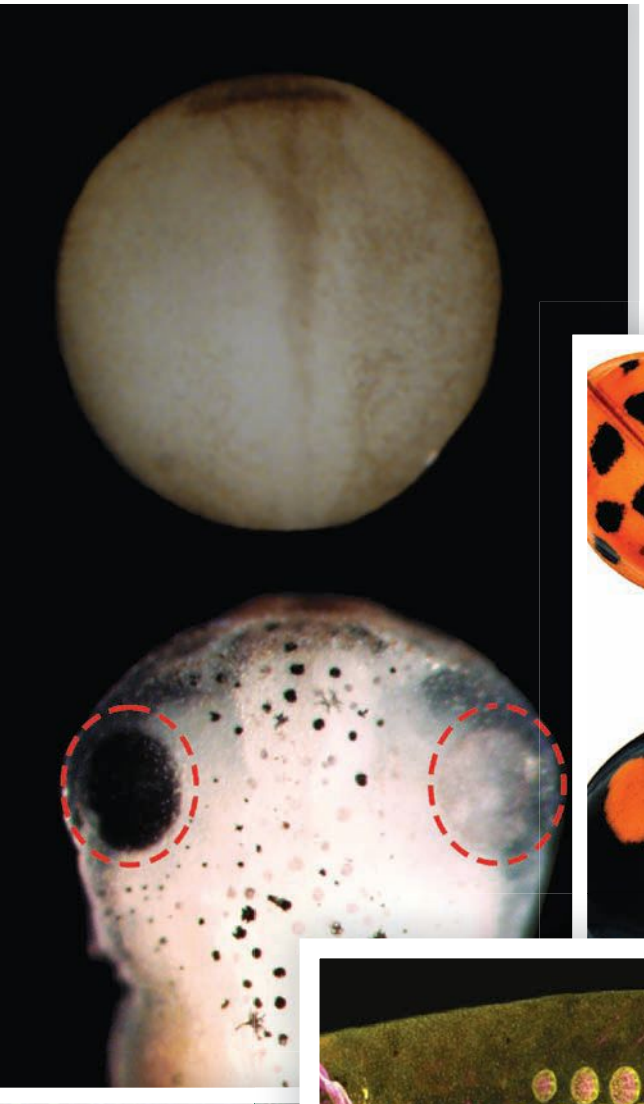
Selected Images of the Day from the-scientist.com

**KOALA CODE**
This summer, researchers published the entire genome sequence of the koala (*Phascolarctos cinereus*).
Posted: *July 3, 2018*

**ORIGINAL FIN**
A Japanese rice fish (*Oryzias latipes*) with normal dorsal and paired pectoral/pelvic fins (top). When researchers knocked out a single genetic enhancer, named ZRS, the fins did not develop normally (bottom).
Posted: *April 2, 2018*



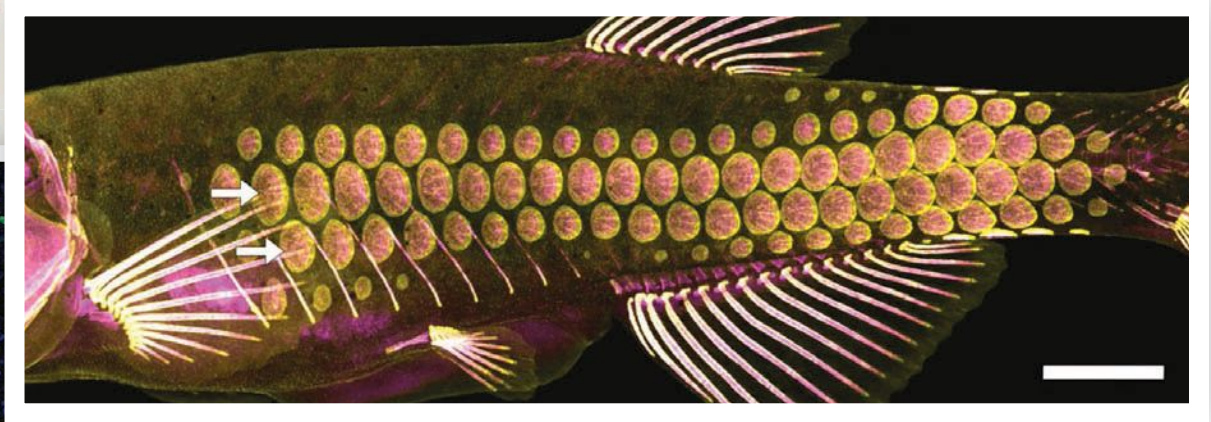


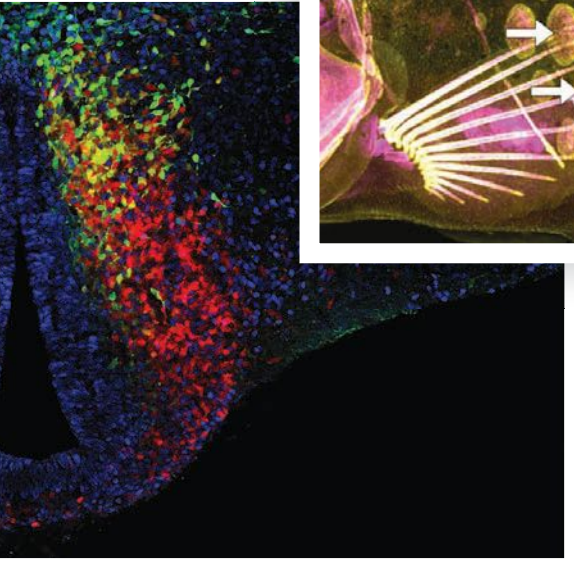


« *XENOPUS* PIGMENT
Earlier this year, researchers used single-guide RNAs with a fluorescent tracer to alter pigment genes in embryos of the African clawed frog (*Xenopus laevis*). The treatment resulted in the right half of embryos lacking pigment, as shown in the neurula stage (top) and at a later developmental period (bottom).
Posted: *May 18, 2018*

« KALEIDOSCOPE
Scientists recently determined that different alleles of one transcription factor, called Pannier, lead to the four main color morphs of the harlequin ladybird (*Harmonia axyridis*).
Posted: *August 27, 2018*

» SINGLE ORIGIN
The same genes direct the development of scales in zebrafish (*Danio rerio*) and skin appendages in terrestrial animals, according to recently published research.
Posted: *August 10, 2018*





« NEW NEURONS
This spring, scientists reported a unique "genetic fingerprint" that allowed them to follow a type of neuron in mouse eyes, from birth to maturity.
Posted: *March 28, 2018*

**Koala Code**: R.N. Johnson et al., *Nat Genet*, doi:10.1038/s41588-018-0153-5, 2018. Courtesy of Rebecca Johnson; ***Xenopus* Pigment:** B. Delay et al., *Genetics*, doi:10.1534/genetics.117.300468, 2018. Courtesy of Vanja Krneta-Stankic; **New Neurons:** M. Niquille et al., *eLife*, doi:10.7554/eLife.32017, 2018. Courtesy of Alexandre Dayer; **Kaleidoscope:** M. Gautier et al., *Curr Biol*, doi:10.1016/j.cub.2018.08.023, 2018. Courtesy of Benjamin Prud'homme and Junichi Yamaguchi; **Original Fin**: J. Letelier et al., *Nat Genet*, doi:10.1038/s41588-018-0080-5, 2018; **Single Origin:** A.J. Aman et al., *eLife*, doi:10.7554/eLife.37001, 2018.

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TheScientist TOP 10 INNOVATIONS

# Gene Expression in 3-D

STARmap enables expression analysis of multiple genes in multiple cells within thick tissue sections.

BY RUTH WILLIAMS

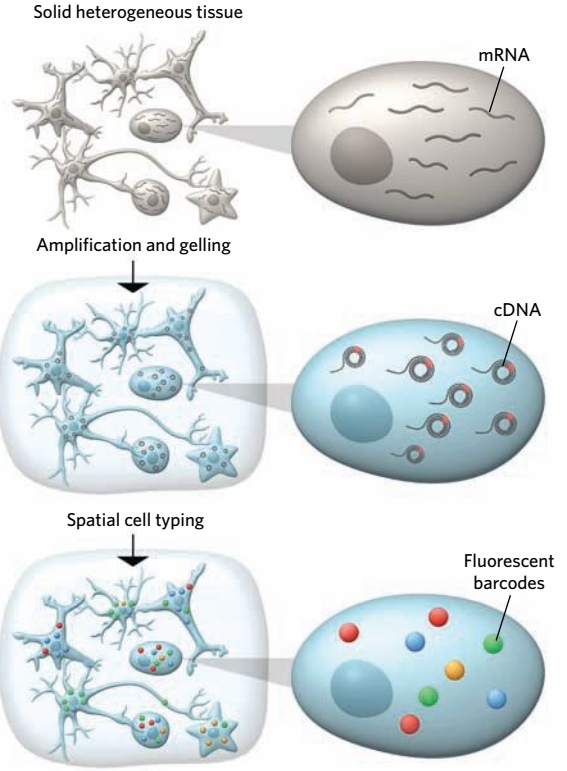
Cells of a given type or tissue may appear similar and yet behave differently. In the brain, for example, neurons of the same subtype may play very different roles depending on their locations and connections.

In short, when it comes to specific cell functions, "spatial information is absolutely critical," says gene-expression researcher Je Lee of Cold Spring Harbor Laboratory. Researchers are therefore developing tools to examine the expression of multiple genes in tissue sections, and a technique called STARmap, developed by Xiao Wang, a postdoc in the lab of Karl Deisseroth of Stanford University, and colleagues, is the latest approach.

STARmap (Spatially Resolved Transcript Amplicon Readout Mapping) is "a significant step toward true 3-D gene expression analysis," says molecular systems biologist Sten Linnarsson of Sweden's Karolinska Institute who was not involved in the work. In the past, a researcher wanting to examine the expression of multiple genes at once in a tissue was "pretty much limited to working with thin sections" because of imaging difficulties, Linnarsson explains. For example, a principal technique used for such analyses—RNA fluorescence in situ hybridization (FISH)—can suffer from background fluorescence due to probes' binding to nonspecific sequences and glomming on to tissue proteins.

To maximize the signal-to-noise ratio with STARmap, Wang and her colleagues first amplify their RNA targets within the tissue section using a technique that produces hundreds of tandem cDNA copies, each with a unique DNA barcode. They then add acrylamide to the sample, to which the amplified cDNAs crosslink, forming a tissue hydrogel. Fats and proteins are then stripped away from the gel to leave a transparent yet structurally preserved specimen. Lastly, the team sequences the amplified barcodes in situ, using confocal microscopy to image the patterns of fluorescent nucleotides at individual spots.

"The [image] quality they demonstrate here is very impressive," Linnarsson says.




**SEE-THROUGH:** To visualize gene expression in a thick section of brain tissue with various cell types, RNA targets (top) are amplified to create multiple cDNA copies, each containing a barcode specific to the particular gene. Acrylamide is then added to the tissue and cross-linked to the cDNA amplicons (middle). From the resulting hydrogel, fats and proteins are removed to leave a clear tissue within which the barcodes can be sequenced (bottom).

The team has used the technique to study the expression of up to 28 genes simultaneously in 150-micron slices of mouse brain tissue, and more than 1,000 genes simultaneously in 8-micron slices. These analyses revealed previously unappreciated differences in the distribution of excitatory and inhibitory neuronal subtypes (identified by their expressed genes) over the brain's cortical layers.

Next, says Wang, "We are moving up to 1-mm sections, and ultimately aiming for the whole mouse brain." (*Science*, doi:10.1126/science.aat5691, 2018) ■

## AT A GLANCE

| IN-TISSUE MULTIPLEX GENE EXPRESSION DETECTION | TARGET RNA DETECTION | MAXIMUM TISSUE THICKNESS | NUMBER OF EXPRESSED GENES POSSIBLE TO VIEW AT ONCE | TISSUE |
|---|---|---|---|---|
| Multiplex single-molecule RNA FISH | In situ hybridization of target mRNAs with fluorescently labeled probes | 15 microns | 249 in 15-μm slices (Shah et al., *Neuron*, 92:342–57, 2016) | Brain |
| STARmap | In situ amplification of mRNA targets followed by their in-situ sequencing with ligation of fluorescent probes | 8 microns for hundreds of genes | 1,020 in 8-μm slices | Brain |
| | | 150 microns for tens of genes | 28 in 150-μm slices | |

TS WEBINARS

# COMING**SOON** | Cell Signaling and Metabolism: Bridging a Gap in Cancer Therapies

Researchers have known that cancer cells possess an altered metabolism for almost a century. But the cell-signaling mechanisms responsible for this phenomenon are just now coming to light, with some drugs targeting key regulators of both cellular metabolism and tumor progression. The repurposing of old drugs for the activation and/or inhibition of proteins at the center of the cell-signaling and metabolism web has particularly received much attention. Drugs such as metformin, chloroquine, and various statins are just a few examples of those in new, cancer-focused clinical trials. To further explore cell metabolism as a novel avenue of drug discovery based on already established cell-signaling mechanisms essential for tumor progression, *The Scientist* is bringing together a panel of experts to discuss their research into this exciting and recently acknowledged aspect of cancer biology. Register today!

**THALES PAPAGIANNAKOPOULOS**, PhD
Assistant Professor
Department of Pathology
NYU School of Medicine

**M. CELESTE SIMON**, PhD
Scientific Director and Investigator
Abramson Family Cancer Research Institute
Associate Director-Shared Resources
Abramson Cancer Center
Arthur H. Rubenstein, MBBCh Professor
Department of Cell and Developmental Biology
University of Pennsylvania Perelman
School of Medicine

## WEDNESDAY, OCTOBER 24
## 2:30–4:00 PM EASTERN TIME

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

**TOPICS TO BE COVERED:**

- Metabolic symbioses in tumor microenvironments
- Uncovering metabolic bottlenecks in genetic subtypes of lung cancer

**WEBINAR SPONSORED BY:**

LABCYTE nanoString MilliporeSigma ROCKLAND antibodies & assays

TS WEBINARS

# ON**DEMAND** | CAR T-cell Therapy and Bispecific Antibodies: Frontiers in Cancer Immunotherapy

Cancers are the most heterogenous group of diseases affecting humans. Multiple compensating pathways work to promote disease progression. Various immunotherapies have shown anticancer promise, but their targeted nature limits the scope of their action and applicability. CAR T-cell therapy and bispecific antibodies are two approaches that have addressed the targeting of more than one epitope, the redirecting of T lymphocytes to kill tumor cells, and the improvement of efficacy in solid tumor types. For a closer look at the design, development, and success of CAR T-cell therapy and bispecific antibodies, *The Scientist* brings together a panel of experts to discuss their research and share insights into the potential these therapeutics hold in the fight against various tumor types.

**BRUCE LEVINE**, PhD
Netter Professor, Dept. of Cancer Gene Therapy
Founding Director, Clinical Cell and Vaccine Production Facility
Deputy Director, Center for Cellular Immunotherapies -
Technology Innovation & Assessment
Department of Pathology and Laboratory Medicine
Perelman School of Medicine
Abramson Cancer Center
University of Pennsylvania

**JOHN H. SAMPSON**, MD, PhD, MBA, MHSc
Robert H. and Gloria Wilkins Professor
of Neurosurgery
Chair, Department of Neurosurgery
Duke University Medical Center

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**www.the-scientist.com/immunooncfrontiers**

**TOPICS TO BE COVERED:**

- Where cancer immunotherapies are living up to their promise, and where they're falling short
- The art and science of developing novel immunotherapies

**WEBINAR SPONSORED BY:**

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LAB TOOLS
THE SCIENTIST WEBINAR SERIES

# COMING**SOON** | Phenotypic CRISPR Screening: Looking Beyond Cell Viability to Study Complex Genetic Interactions

CRISPR screening, whether using knockout, activation, or inhibition approaches, has become an essential tool for drug discovery. A powerful adaptation of CRISPR screening involves coupling pooled NGS-linked or arrayed screening strategies with phenotypic measurements. By using an advanced combination of techniques, this screening strategy can detect genes that drive complex biological responses via biomarker signal readout or a panel of high-content parameters. Scientists at Horizon, the sponsor of this LabTools webinar, have developed both FACS-linked and HCA-based strategies for analysis where cellular pathophysiology is uncoupled from cell health, providing much richer and biologically relevant outcomes. In this webinar, we will discuss data obtained and strategies learned from several successful screens. In addition, a representative of Horizon will present ongoing research that uses single-cell RNAseq-based screening approaches and work that employs increasingly relevant cell models, such as T cells.

**BEN CROSS**, PhD
R&D Manager
Horizon Discovery

## TUESDAY, OCTOBER 30
## 2:30–4:00 PM EASTERN TIME

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

**TOPICS TO BE COVERED:**

- The strengths and considerations associated with complex phenotypic screens
- Examples of successful applications of pooled and arrayed phenotypic screens

**WEBINAR SPONSORED BY:**

horizon
INSPIRED CELL SOLUTIONS

LAB TOOLS
THE SCIENTIST WEBINAR SERIES

# ON**DEMAND** | Evaluating New 3-D Systems for Disease Modeling, Drug Discovery, and Toxicology

Cell- and tissue-based models are a necessary step in the workflow for toxicology testing, drug discovery, and disease modeling. In recent years, the research and pharmaceutical industries have adopted more-complex ex vivo and in vitro 3-D tissue models because of their better predictive power. In this webinar, representatives from Bio-Techne, the sponsor of this LabTools webinar, will discuss the history of cell- and tissue-based model systems for toxicology, drug screening, and disease modeling. They'll also introduce MimEX™ Tissue Model Systems, a culturing platform that builds upon the physiological and structural advancements of current organoid models by producing 3-D tissue with better accessibility and lower variability. Tune in to learn more about MimEX 3-D Tissue.

**SCOTT SCHACHTELE**, PhD
Product Manager
Stem Cells and Cell Culture
Bio-Techne

**KEVIN FLYNN**, PhD
Scientist
Stem Cell Research and Development
Bio-Techne

**WATCH NOW!**
**www.the-scientist.com/mimex**

**TOPICS TO BE COVERED:**

- History of cell and tissue model systems for toxicology and disease modeling
- Troubleshooting variability in iPSC-based models
- Advantages and disadvantages of current 3-D culture systems

**WEBINAR SPONSORED BY:**

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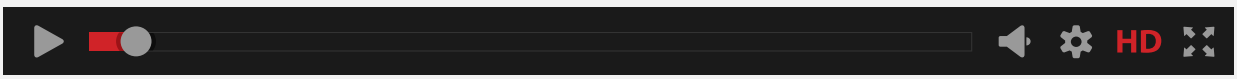


# Underworld

The Earth's crust is home to an extraordinary array of microbial life, thriving kilometers beneath our feet.

BY CATHERINE OFFORD

About a 20-minute drive north of the industrial town of Timmins, Ontario, the ground gives way to a gaping pit stretching more than 100 meters across. This pit is the most recognizable feature of Kidd Creek Mine, the deepest copper and zinc mine in the world. Below the Earth's surface, a maze of underground tunnels and shafts pierces 3 kilometers of ancient volcanic rock. Were it not for a huge ventilation system keeping the passages cool, the air temperature at this depth would be 34 °C (93 °F).

It's here that Barbara Sherwood Lollar, a hydrogeologist at the University of Toronto, journeys into the planet's crust to hunt for signs of life. "You get into a small truck or vehicle and go down a long, winding roadway that corkscrews down into the Earth," she tells *The Scientist*. By the time she and her fellow passengers clamber out into the corridors at the end of the roadway, "we are literally walking along what was the ocean floor 2.7 billion years ago," she says. "It's an utterly fascinating and magical place to visit."

Unlike miners, who navigate these tunnels in search of metal ores, Sherwood Lollar and her colleagues are on the lookout for pools of salty water. "These aren't waters you'd pump into your cottage and drink or spread on your crops," Sherwood Lollar says. "These are waters that have been in contact with the rock for long geochemical timescales—they're full

of dissolved cations and anions that they've leached out of the minerals." So full, in fact, that they give off a distinctive, musty odor. "As we're walking along these tunnels, if I get a whiff of that stenchy smell, then we head in that direction."

Where there's water, there's the potential for life. In 2006, Sherwood Lollar was part of a team led by Tullis Onstott at Princeton University that discovered an anaerobic, sulfate-reducing bacterium thriving in the sulfate-rich fracture waters of Mponeng gold mine in South Africa, 2.8 kilometers underground.[1] A few years later, a different group described a diverse microbial community living at a similar depth in the Earth's crust, accessed via a borehole drilled into the ground in Finland.[2] With the recent discovery of 2-billion-year-old, hydrogen- and sulfate-rich water seeping out of the rock in Kidd Mine, Sherwood Lollar and her colleagues are hoping they might again find life.[3]

**Before the rise of the land plants, deep biomass could have outweighed life on the surface by an order of magnitude.**

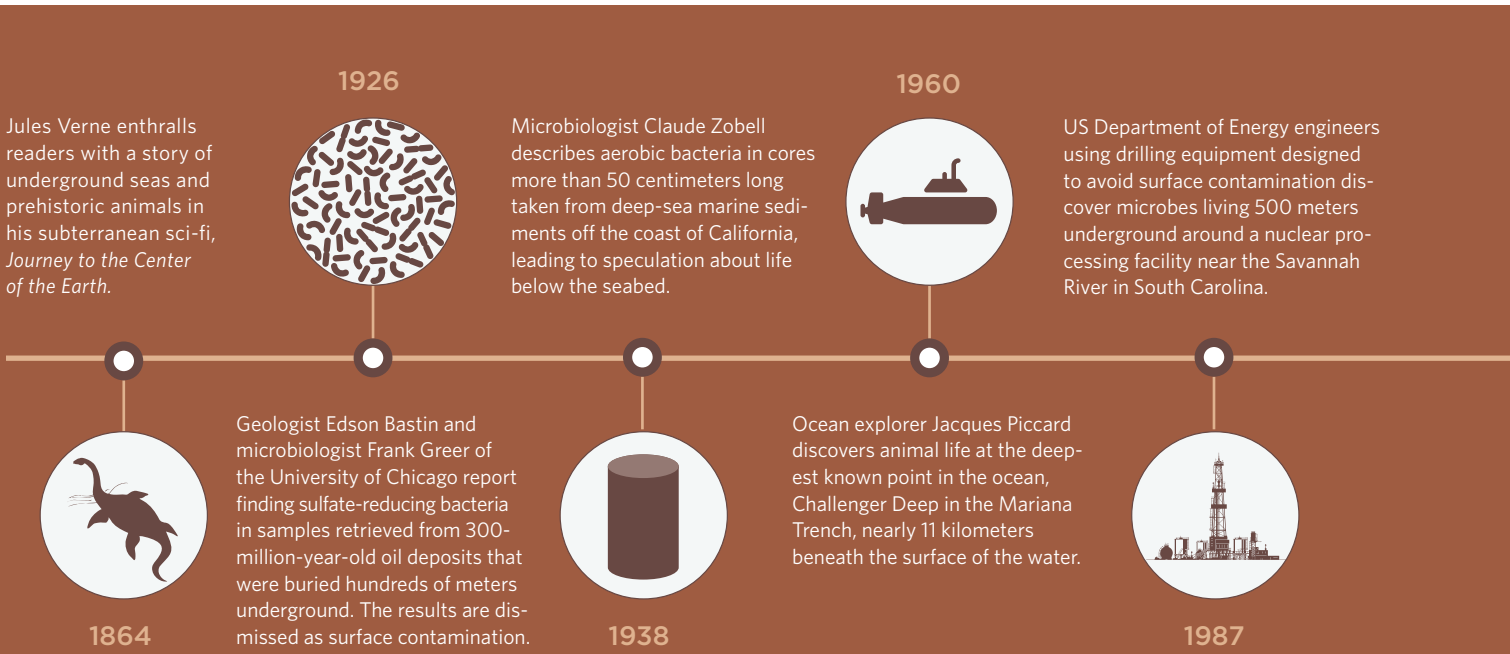
These expeditions are just one part of a rapidly expanding field of research focused on documenting microbial and even eukaryotic life dwelling hundreds of meters deep in the Earth's crust—the vast sheath of rock encasing the planet's mantle. Researchers are now exploring this living underworld, or deep biosphere, not only in the ancient, slow-changing continental crust beneath our feet, but in the thinner, more dynamic oceanic crust under the seafloor. (See illustration on page 32.) Such habitats have become more accessible thanks to the last two decades' expansion of scientific drilling projects—whereby researchers haul up cores of rock to study on the surface—as well as a growing number of expeditions into the Earth via mines or cracks in the ocean floor.

Studies of these dark—and often anoxic and hot—environments are challenging scientists to rethink the limits of life, at the same time highlighting how little we know about the world beneath our feet. "It's a really good field if you don't mind not knowing all the answers," says Jason Sylvan, a geomicrobiologist at Texas A&M University. "For some people, that freaks them out. For me, a field is more exciting when you can ask really big questions."

## Holes in the ground

A desire to explore the deep biosphere has led Julie Huber, a microbial oceanographer at Woods Hole Oceanographic Institution in Massachusetts, to some of the remotest places on Earth. Huber is interested in the huge volumes of water swilling around between rock particles in the oceanic crust, and the extent and diversity of microbial life within them. One way to access that water is via expensive drilling projects, many organized by the International Ocean Discovery Program (IODP), that bore through marine sediments to the crust. In 2013, this approach revealed bacteria living in 3.5-million-year-old basalt rock underneath the Pacific Ocean.[4]

The other way, Huber explains, "is to find where that water is naturally leaking out through the seafloor, and then try to capture it just as it's coming out." For that purpose, Huber has not only worked with teams of engineers to guide remotely operated vehicles down to the bottom of the ocean, she's also joined the ranks of scientists who have taken the plunge with *Alvin*,

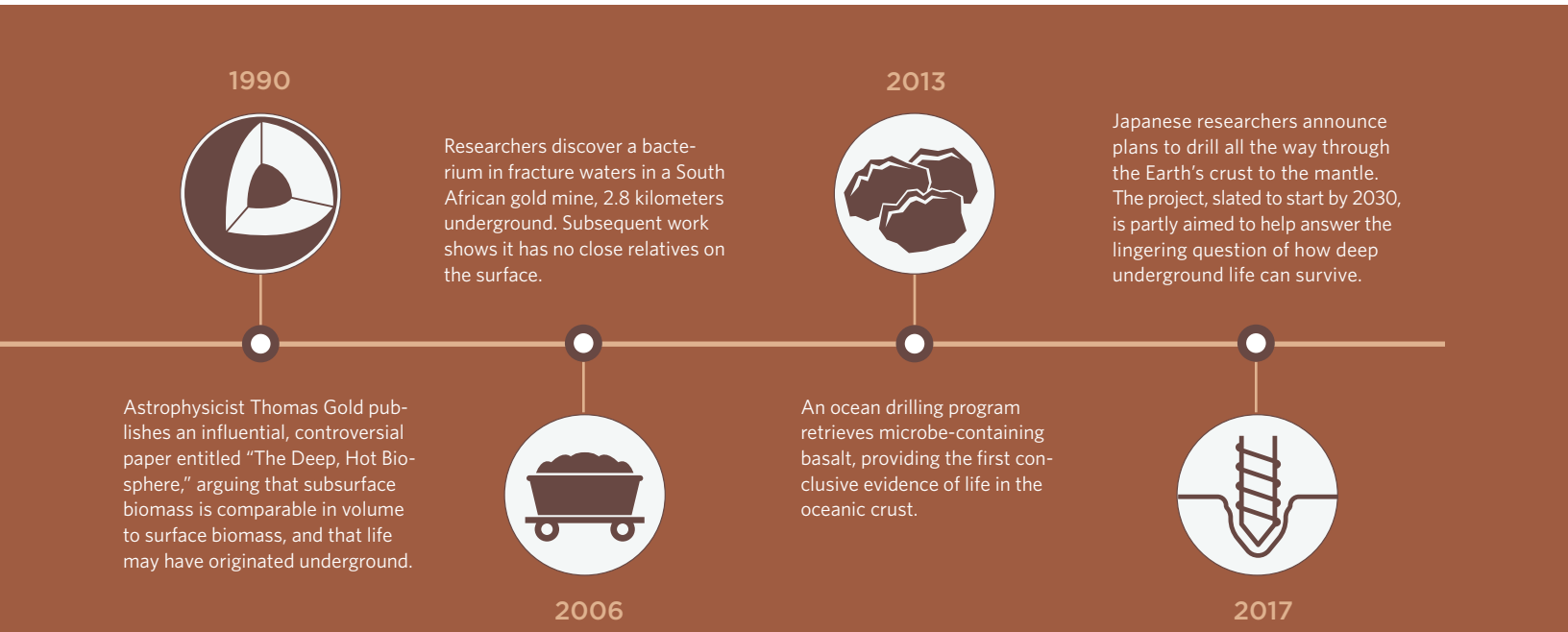


K. VOGLESONGER, UNIVERSITY OF TORONTO; G. BORGONIE

**LIFE IN A LAND DOWN UNDER:** Mines offer researchers direct access to the deep biosphere, kilometers into the Earth's continental crust. Scientists have now used several of these sites, from Kidd Creek Mine in Ontario (left) to gold mines in South Africa (right), to search for underground life.

a three-person submersible research vehicle owned by the US Navy that can dive down as far as 4,500 meters. "Claustrophobic people don't do well in there," Huber acknowledges—adding that anyone planning to dive is invited to try sitting in the sub before it leaves the boat deck to avoid "a full-on panic being launched into the ocean."

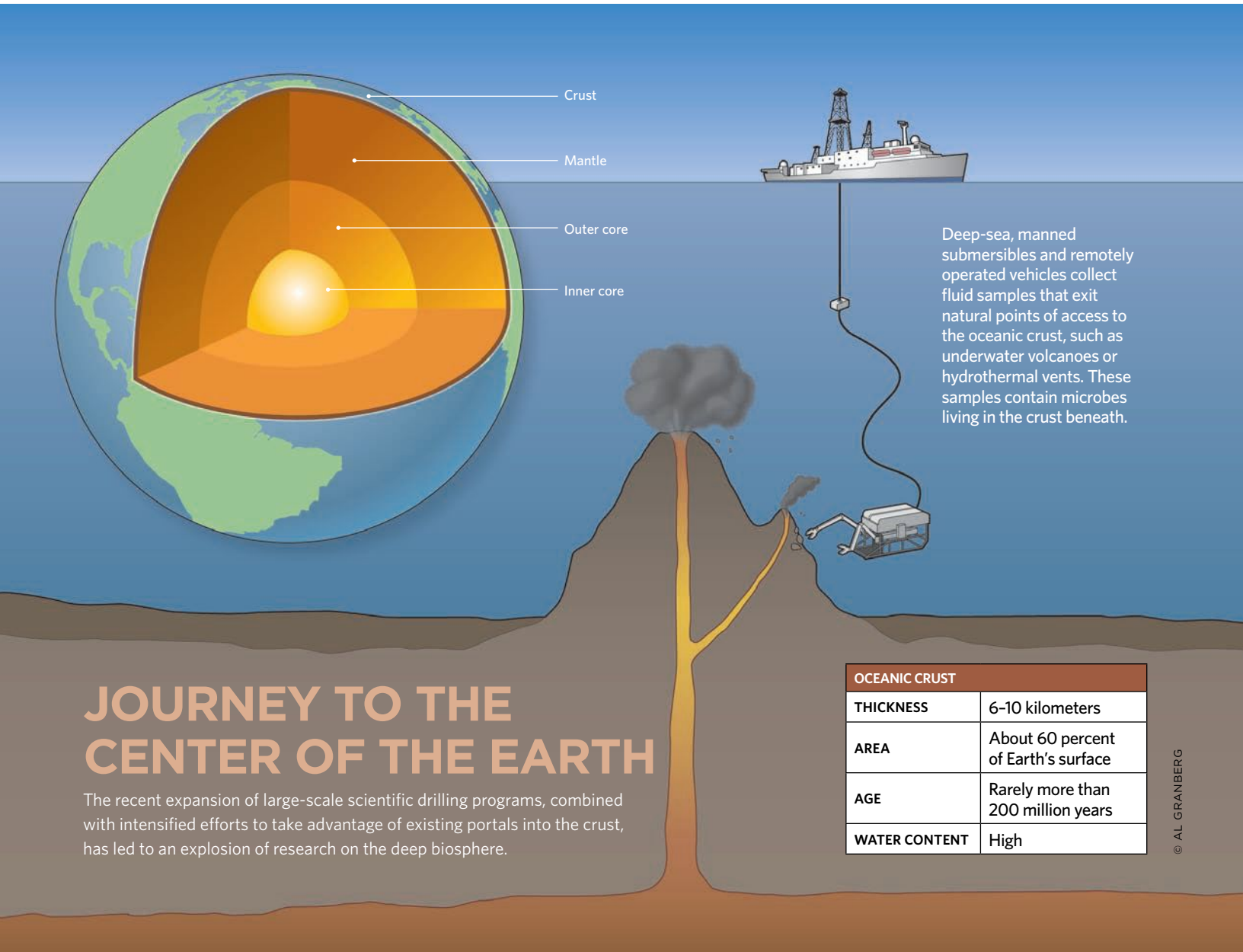
These technologies allow Huber to collect samples of the fluids seeping, or sometimes exploding, out of the oceanic crust from underwater volcanoes and hydrothermal vents. In the early 2000s, she and her colleagues used 16S rRNA gene sequencing to analyze subseafloor microbial diversity following multiple eruptions of Axial Seamount, an underwater volcano about 480 kilometers west of Oregon and nearly 1.5 kilometers under the water's surface. Compared to background seawater, samples collected at the vent site revealed multiple unique bacterial[5] and archaeal[6] taxa that appeared to have been blasted out of the crust, pointing to a diverse microbial community thriving below the seafloor. More recently, Huber's group carried out a detailed survey at the world's deepest hydrothermal vent field—a site known as Piccard, after Swiss deep-sea adventurer Jacques Piccard—and turned up thousands of vent-specific microbial taxa in fluids exiting the crust at temperatures of up to 108 °C (226 °F).[7]



Such findings are becoming typical of this young research field. To date, studies of crustal sites all over the world—both oceanic and continental—have documented all sorts of organisms getting by in environments that, until recently, were deemed inhospitable, with some theoretical estimates now suggesting life might survive at least 10 kilometers into the crust. And the deep biosphere doesn't just comprise bacteria and archaea, as once thought; researchers now know that the subsurface contains various fungal species,[8] and even the occasional animal. Following the 2011 discovery of nematode worms in a South African gold mine, an intensive two-year survey turned up members of four invertebrate phyla—flatworms, rotifers, segmented worms, and arthropods—living 1.4 kilometers below the Earth's surface.[9]

Unsurprisingly, as researchers explore these unusual habitats, they're finding a number of organisms that were until recently unknown to science. The discovery of "extremophile" archaea species in the last decade has led scientists to rethink the entire domain's phylogeny. (See "The Old Ones," *The Scientist*, June 2018.) And while many of the bacteria and archaea discovered in the deep biosphere have analogs or close relatives on the surface, some are unlike anything found anywhere else.

One example is *Candidatus Desulforudis audaxviator*, first found by Onstott's team in Mponeng gold mine in 2006. ("Audax viator," which translates from Latin to "bold traveler," is a reference to a line in Jules Verne's *Journey to the Center of the Earth*.) Researchers have since identified bacteria resembling this species in other sites a kilometer or more into the crust, but haven't yet found any close relatives in surface communities. Another bacterial species, unearthed more than 1,000 meters down in the Henderson molybdenum mine in Colorado, shows faint phylogenetic links to members of the phylum *Nitrospirae*, but is otherwise unlike anything on the surface.[10]

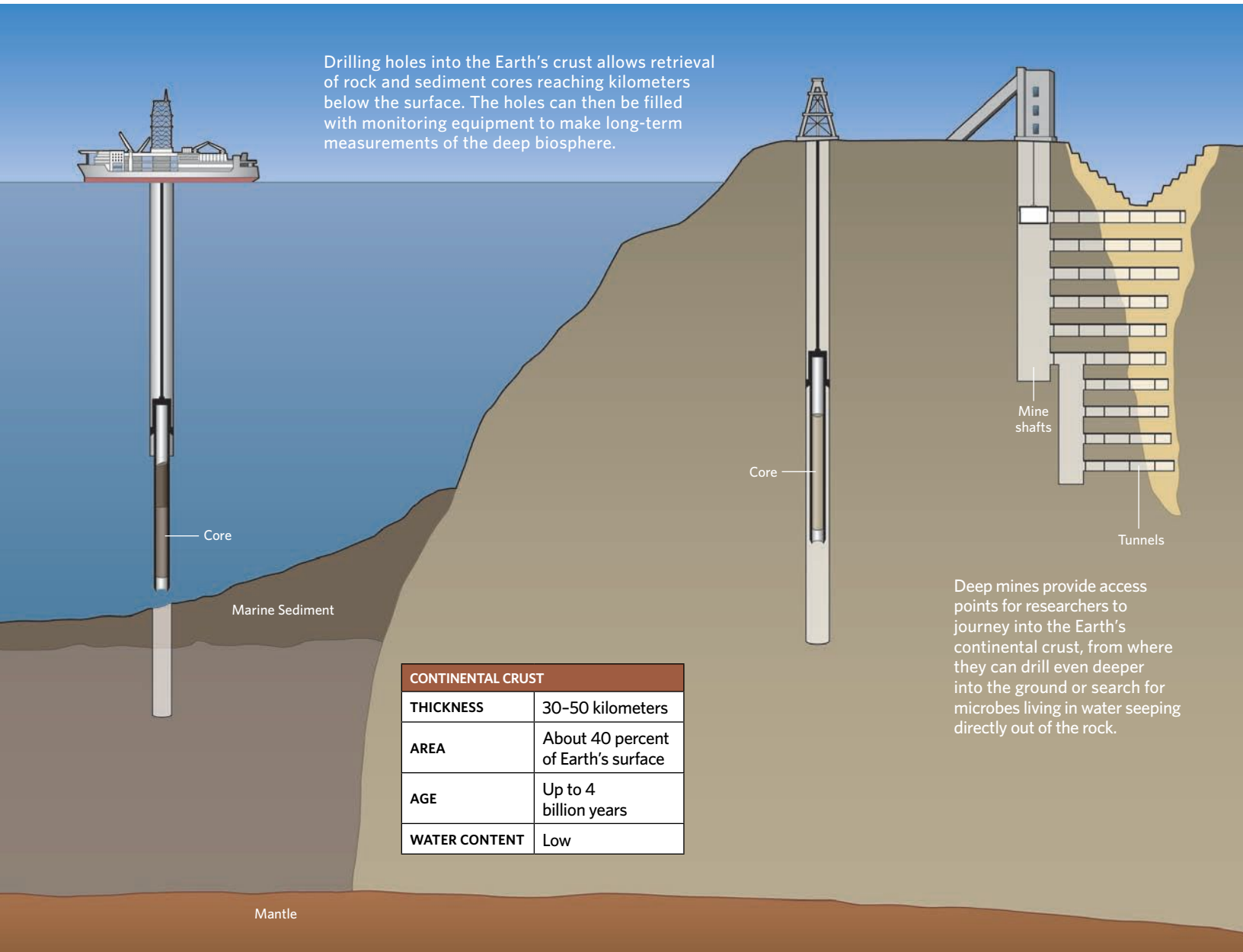


A key area of research now is understanding how such life survives. Devoid of sunlight, "these systems are typically energy-poor," says Sherwood Lollar. Compared to surface communities, microbes in the deep biosphere are thought to be relatively slow-growing and sparsely distributed, she adds. While surface soil may contain in excess of 10 billion microbes per gram, oceanic crust usually contains around 10,000 cells per gram, and continental crust—where water is unsurprisingly in shorter supply—holds fewer than 1,000 cells per gram.

Working with such low-biomass samples presents a challenge of its own, but researchers are using a combination of techniques, including metagenomic analyses and incubation of subsurface rocks or fluids with different potential food sources in the lab, to probe the function of subsurface microbes. Such studies are revealing genes for metabolic enzymes that suggest these organisms can gain energy from a suite of sources—particularly hydrogen and other molecules that are released by chemical reactions between water and rock. When geomicrobiologist Lotta Purkamo of the University of St Andrews and her colleagues characterized the ecosystem of a 600-meter-deep borehole in northern Finland, for example, they found evidence of metabolic pathways based on reducing or oxidizing sulfate, nitrate, methane, ammonia, and iron, as well as fixation reactions involving carbon.[11]

Additionally, thanks to metatranscriptomic analyses, "we're learning that these organisms have a lot of potential metabolisms that they could be expressing," says Huber, who recently carried out this sort of assay on the Axial Seamount community.[12] "But depending on the conditions and the geological setting, just a small subset of those genes are being used." Such results hint at flexible and opportunistic lifestyles, she adds, where microbes make use of whatever they can, whenever they can.

These findings are chipping away at some of the big questions about the diversity and uniqueness of life in the deep bio-




| CONTINENTAL CRUST | |
|---|---|
| THICKNESS | 30–50 kilometers |
| AREA | About 40 percent of Earth's surface |
| AGE | Up to 4 billion years |
| WATER CONTENT | Low |

sphere. But the insights afforded by a single drill core or fluid sample can be frustratingly fleeting, says University of Bergen geobiologist Steffen Jørgensen. One sample "doesn't give us any understanding of the dynamics of the system and how it evolves over time," he says. For a longer-term view of life deep in the Earth, researchers are taking their experiments underground.

## The fourth dimension

Last summer, Jørgensen stepped out of a helicopter onto a tiny basalt island about 30 kilometers from the south coast of Iceland. Too rocky to access by boat, the island of Surtsey is the tip of a huge mound of magma blown out of the seafloor by an underwater volcanic eruption that went on for nearly four years in the mid-1960s. This newly formed oceanic crust "gives us a huge advantage," Jørgensen says. "We can actually drill into what is a marine system, but from land."

Using equipment flown to Surtsey by helicopter, Jørgensen and a large team of engineers drilled down into the basalt. They didn't just remove cores from the island; rather, the researchers set up a mini observatory to take in situ measurements of the deep biosphere. Into a 190-meter-deep hole in the rock, the team installed a series of 10-meter-long aluminum tubes, several with a number of small slits to allow fluids to trickle through from the surrounding rock. Then, into the tubes the team lowered a cable with various bits of equipment—temperature and pressure loggers, and microbial incubators—attached at specific intervals, until the equipment lined up with the slits. Since then, the instruments in the observatory have been collecting data from the oceanic crust, and next summer, Jørgensen and his colleagues will go back to see what they've found.

The Surtsey installation is now one of a handful of deep observatories around the world and part of a larger effort to establish long-term studies in both oceanic and continental crust. Such sites offer a window into the activity of the deep biosphere, as well as an opportunity to collect time-series data that are critical to understand how that biosphere changes over time. "It's the only way that we can . . . make observations that are more than 'I went to this place, one time in the history of the world, and I grabbed a bunch of rocks, and here's what I saw,'" says Sylvan.

Data coming out of long-term studies of the deep biosphere paint a dynamic picture. This July, a team that included Onstott and Sherwood Lollar published metagenomic, metatranscriptomic, and metaproteomic analyses of data collected over a period of two and a half years at a depth of 1,339 meters from a borehole drilled into South Africa's Beatrix gold mine.[13] Over the course of the study, the microbial community structure shifted in concert with natural fluctuations in the groundwater's geochemistry—in particular, the availability of electron-accepting compounds such as nitrates and sulfates.

Meanwhile, Huber's group published an analysis of data gathered over two years from two so-called CORK (circulation obviation retrofit kits) observatories installed in the oceanic crust below North Pond, a site on the Mid-Atlantic Ridge, through which circulates well-oxygenated and—at less than 15 °C (59 °F)—relatively cold water.[14] Metagenomics showed that the microbial communities, which were substantially different from those of warmer and anoxic environments, went through substantial shifts over time—with one phylum dominating one month, and another taking over the next—despite only minor fluctuations in the water's geochemistry.

Such underground observatories can also act as in situ laboratories. By incubating rocks inside these sites for years at a time, researchers can study how microbial communities colonize new material in their natural environments rather than in the lab, and how the mineralogical composition of the crust influences who grows where.[15] The sites might even reveal subsurface dynamics on much longer timescales, by helping scientists identify signs of ancient life. To date, many of the clues about deep microbial communities throughout geological history come from what look like fossilized or mineralized remains of bacteria and archaea on rocks retrieved from the crust. But given how little researchers know about the processes

LAVA LAND: The Icelandic island of Surtsey (left) was created by a four-year volcanic eruption in the 1960s. Researchers have now installed a deep observatory into a hole they drilled (right) to monitor life in the deep marine biosphere.

SOLVEIG LIE ONSTAD; PAULINE BERGSTEN

of mineralization in the deep subsurface, the authenticity of at least some of these remains is in question.

"It's quite difficult to tell whether you're actually looking at a fossil of an organism that lived in the deep biosphere billions of years ago," explains University of Edinburgh geobiologist Sean McMahon. "Not only is it difficult in general to recognize fossil bacteria, which look very much like minerals at that size scale, it's difficult to show, if it really is a fossil bacterium, that the organism lived below the surface at the time it was living billions of years ago."

**It's a really good field if you don't mind not knowing all the answers.**

—Jason Sylvan, Texas A&M University

To get a better grip on the long-term dynamics of the deep biosphere, groups such as McMahon's are trying to recreate deep mineralization in the lab. They do this by inoculating rocks with bacteria, McMahon explains, then tweaking physical and chemical conditions to trigger fossilization. "The idea is to try and find the sweet spot where the microbes are able to live happily, but you only have to change a small thing for them to become entombed in minerals and fossilized," he says.

Underground observation stations such as the one at Surtsey will soon be able to complement this research, says Jørgensen. "By having the observatory, we can hopefully clarify whether these [fossil-like] structures can be produced abiotically, or if we only see them where there's microbes present," he says. "It is a very difficult question to get to the bottom of."

## Missing pieces

Despite the infancy of research into the deep biosphere, it's clear to many in the field that science has long held a warped view of what constitutes life in our universe. Researchers are far from agreeing on the extent of this underworld—one 1990s paper controversially suggested that deep life constituted 50 percent of the Earth's current biomass,[16] though most estimates are now below 15 percent. Before the rise of land plants around 400 million years ago, though, deep biomass could have outweighed life on the surface by an order of magnitude, according to calculations published this summer by McMahon and the University of Aberdeen's John Parnell.[17]

However much life exists below the Earth's surface, its mere presence is forcing a reevaluation of biological normalcy, not only on Earth but deep within other planets such as Mars. After all, in the Earth's crust, "we had made an assumption that there was no life," notes Purkamo, who has also been affiliated with St Andrews's Centre for Exoplanet Science. "And then, tada!"

Findings from the underground frontier are also pushing scientists to consider how subsurface microbes—and the reactions they carry out—influence global processes occurring above the surface. "I'm quite sure that people don't really think about that," notes Jørgensen. "That they're walking on this enormous biosphere that could have a really significant impact on how the system works." The same goes for attempts to understand physical and biological evolution throughout the planet's history. "When we think about how life on Earth has changed over time, and how it's interacted with the chemistry of rocks, sediments, groundwater, oceans, atmosphere, we shouldn't be thinking just about charismatic animals and plants," says McMahon. "We should be thinking about this huge quantity of microorganisms, most of which are living on the surfaces of mineral grains and interacting with them."

That's exactly the view today's deep biosphere researchers are trying to expand, and to most in the field, it's an exciting journey. "It's like: Damn, there's so much we do not know about what is happening down there," says Huber, whose team is currently exploring the deep biosphere at an active underwater volcano known as Loihi, about 35 kilometers off the coast of Hawaii's Big Island. "And what a privilege to be able to ask these questions and to do this type of science and try to figure it out." ■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# The Vanishing Night

The loss of darkness can harm individual organisms and perturb interspecies interactions, potentially causing lasting damage to life on our planet.

BY DIANA KWON

FIDEL FERNANDO, UNSPLASH

As darkness fell over Manhattan on the ninth anniversary of the September 11 attack, two beams were shot into the sky at the site where the Twin Towers once stood. The commemorative lights had appeared annually since the towers fell, but in 2010 onlookers noticed something unusual: countless white sparkles glittering within the white beams.

The mysterious white objects turned out to be thousands of migrating birds. Although the public had just taken notice of this spectacle, conservationists had been aware of the phenomenon for several years. Shortly after the tribute first appeared, New York City Audubon, a conservation group, helped initiate a program to monitor the installation and temporarily shut off the lights whenever too many birds got caught in the beams. In a later analysis of the bird populations on memorial nights between 2008 and 2016, researchers found that, although the short-term shutdowns were effective, approximately 1 million animals had been attracted to the glowing memorial and had become distracted from their normal migratory routes.[1]

This annual demonstration of how artificial illumination can influence animal behavior is but one instance of a much bigger problem. Around 80 percent of all humans—and more than 99 percent of people in the US and Europe—now live under light-polluted skies. In addition to direct lighting from urban infrastructure, light reflected from clouds and aerosols, known as skyglow, is brightening nights even in unlit habitats. As electric lights become more energy- and cost-efficient, the proportion of lit surfaces keeps rising. Meanwhile, the list of organisms that researchers document to be affected by Earth's unnatural glow is growing right along with it.

In 2002, the University of Southern California geographer Travis Longcore, also science director of the Los Angeles–based nonprofit The Urban Wildlands Group, and colleagues organized the first North American conference on the ecological consequences of light pollution. This inspired a growing interest in the scientific community that eventually led to a handful of large-scale projects that launched in Europe around 2010, says Thomas Davies, a postdoctoral ecologist at Bangor University in the UK. "That's when we started to see this exponential growth in the research output in this field."

Over the last 16 years, researchers have uncovered the many nuanced ways that light can affect individual species and have started to build a bigger picture of the effects on ecosystems. "It's become clear that light pollution is a major anthropogenic pressure on the environment," says Kevin Gaston, an ecologist at the University of Exeter in the UK.

> WE HAVE NOTHING IN OUR GENETIC MAKE-UP THAT HAS BEEN EXPOSED TO THIS TYPE OF CHALLENGE. IT'S COMPLETELY UNPRECEDENTED IN THE HISTORY OF THE EARTH.
>
> —Therésa Jones, University of Melbourne

And it's a uniquely disruptive pressure in that life on Earth evolved to the beat of the circadian cycle, and bright, constant light at night is a very recent phenomenon in evolutionary time, adds Therésa Jones, a behavioral ecologist at the University of Melbourne in Australia. "We have nothing in our genetic make-up that has been exposed to this type of challenge. It's completely unprecedented in the history of the Earth."

## Fatal attraction

In the 1880s, Swedish-American ornithologist Ludwig Kumlien noted that a 200-foot-tall, illuminated observation tower in Milwaukee, Wisconsin, was attracting migrating birds in the evening—and that many perished after colliding with the lights or the surrounding electric wires.[2] This was one of the earliest reports of what is now a well-known effect of artificial nighttime lighting: its ability to draw in wildlife.

Since then, researchers have identified several other animals that succumb to light's fatal allure. Insects are perhaps the most obvious example—many of these critters are nocturnal, and a wide variety of species, including beetles, mayflies, and moths, will cluster around streetlamps, floodlights, and other sources of nighttime illumination. Although the factors underlying this so-called "flight-to-light" behavior remain unclear, the consequences are well documented: increased rates of injury, exhaustion, and predation.

Evening lighting can also fragment animals' habitats, as strings of lamps limit the movement of organisms from one place to another. In one field experiment, Franz Hölker, a freshwater ecologist at the Berlin-based Leibniz-Institute of Freshwater Ecology and Inland Fisheries, and his colleagues discovered that street lights could draw in moths passing within a radius of approximately 23 meters.[3] Given that lampposts—at least in Europe—are typically around 20 to 45 meters apart, Hölker explains, the area from which they draw in insects often overlaps, creating a magnet that traps the animals and reduces their ability to disperse through the environment.

Certain organisms may adapt to light over time, potentially limiting the negative effects of the exposure. In 2016, a pair of Swiss researchers discovered that adult ermine moths (*Yponomeuta cagnagella*) from bright urban areas were less likely to be attracted to lights than their counterparts from dark, rural regions.[4] This is likely a "genuine adaptation," Gaston says. "The selection pressure to not fly to light is quite high if you are constantly exposed to it, and you're suffering high mortality or energetic costs."

But so far, the ermine moths are the only documented example of such an adaptation to avoid artificial light. Whether the behavior of other animal populations will change in this way remains an open question, says Gaston. In addition, as light levels are one

of many characteristics that differ between urban and rural areas, it is difficult for scientists to rule out the contributions of noise, air pollution, and other environmental stressors present in developed regions of the world.

## Out of sync

Most organisms, from bacteria to people, have biological rhythms that help keep them aligned with the day-night cycles that occur as the planet rotates about its axis. These cadences are entrained by a variety of external signals, with light as the most important cue. As darkness disappears, that regulation can go awry.

Artificially extended days can modify the timing of tightly controlled daily activities, such as foraging and sleep.[5] Some diurnal species, such as the great tit (*Parus major*), may continue searching for food later in the day, while nocturnal organisms—certain mice or bats, for example—spend less time out hunting or foraging.

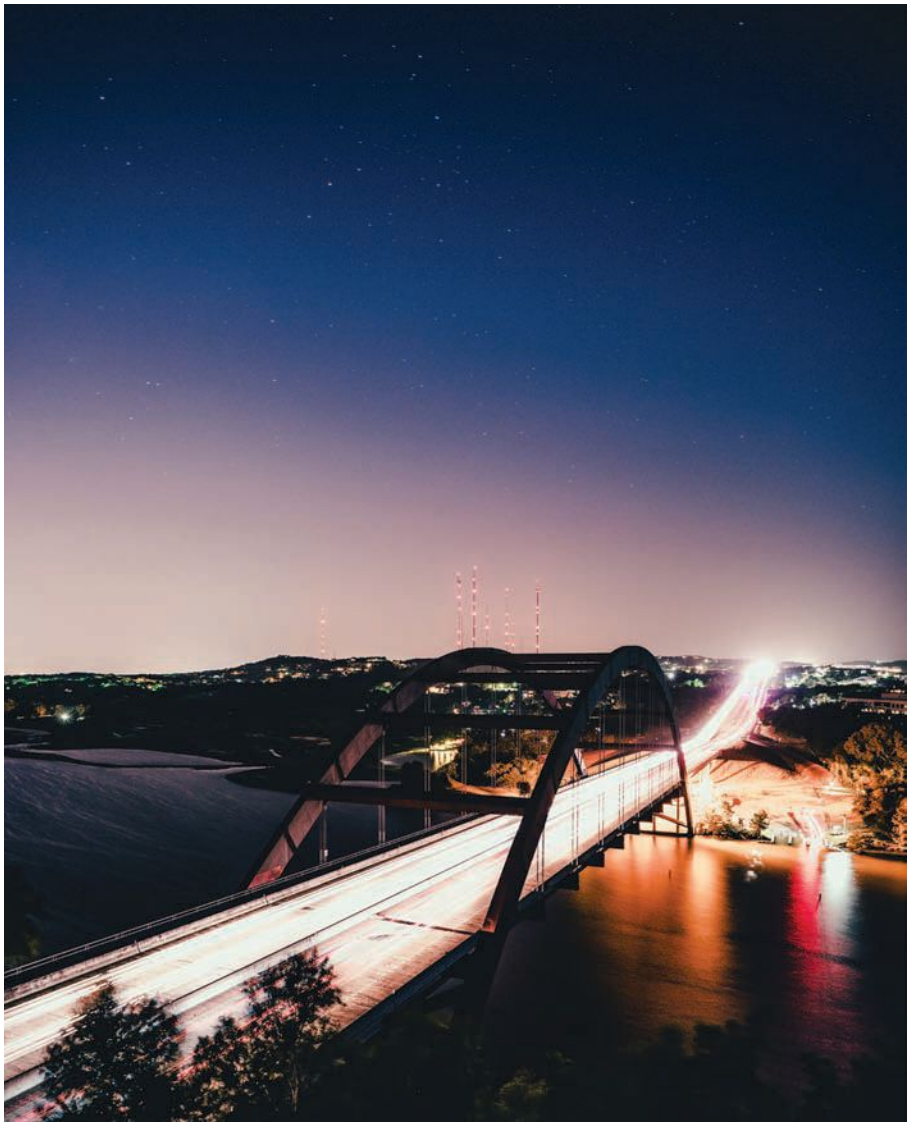
Light pollution can also distort seasonal and lunar rhythms, which are responsible for biological events such as reproduction and migration. Davide Dominoni, a postdoctoral ecologist at the Netherlands Institute of Ecology in Wageningen, and colleagues have found that constant, low levels of illumination at night (0.3 lux, 20 times lower than the intensity of the average street lamp in Munich, Germany, where the study took place) caused European blackbirds, *Turdus merula*, to develop their reproductive systems a month earlier than counterparts reared with dark nights.[6] Other researchers have found that light pollution can delay birth in wallabies,[7] advance egg laying in songbirds,[8] and alter the migration patterns of salmon.[9]

Such perturbations may be mediated by changes in levels of melatonin, a hormone that is produced primarily at night and plays a key role in light's effect on circadian cycles. The secretion of this chemical is known to be suppressed by blue light, which is present in high amounts in electronic devices and light-emitting diodes (LEDs), a type of lighting gaining popularity for use in street lamps thanks to its low cost and high energy efficiency. Reduced levels of melatonin have been measured in humans exposed to blue light.[10] Last year, a group led by researchers at the University of Haifa in Israel found that people exposed to computer screens at night experienced modified circadian oscillations—specifically, lower nighttime melatonin production and a smaller nocturnal drop in body temperature—as well as altered sleep patterns.[11]

Experiments in the lab have shown that exposure to light at night can also dampen melatonin secretion in a variety of other animal species, including birds, fish, and insects. Jones and her team, for example, found that crickets reared under constant light had lower melatonin levels and impairments in immune function compared with those exposed to 12 hours of illumination per day.[12]

Despite the growing evidence that nighttime glow can alter daily and seasonal cycles, scientists currently have "very little evidence for strong effects, at least in vertebrates, on the fitness of animals," Dominoni says. "I'm interested in trying to figure out whether the effects of light pollution on circadian and seasonal rhythms . . . have long-term consequences on the health of these animals."

For some species, the potential harm of light pollution may be offset by benefits. In recent work published as a preprint earlier this year, Jones and colleagues found



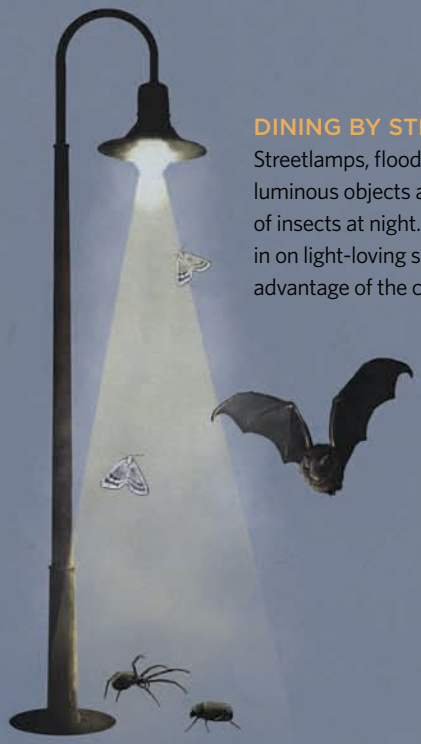
COSMIC TIMETRAVELER, UNSPLASH

# BRIGHT NIGHTS

As artificial light increases in volume and geographical coverage around the world, a variety of animal species are suffering ill effects. Some of these consequences are immediate and obvious—for example, a moth that flies into a streetlamp may die on impact. But there are also less visible, possibly more damaging effects, such as changes to predator-prey and plant-pollinator relationships that can reverberate through ecosystems.

## FATAL ATTRACTION

Illuminated skyscrapers and spotlights can lure migrating birds. Animals may become disoriented and end up in deadly collisions or perish from exhaustion.

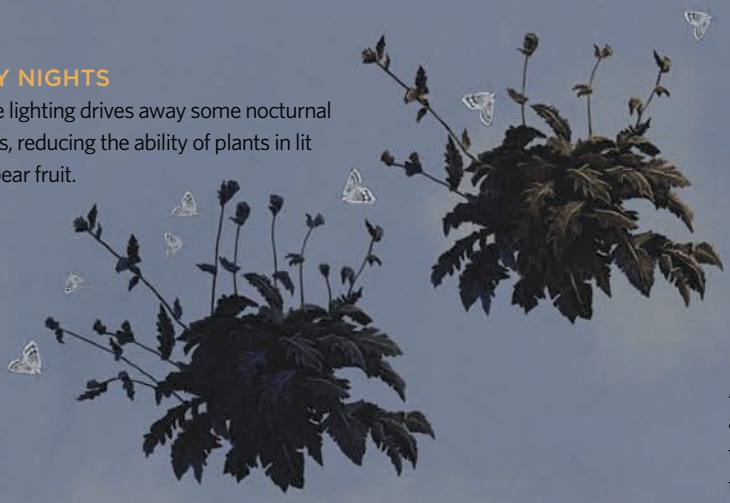


## DINING BY STREETLIGHT

Streetlamps, floodlights, and other luminous objects attract a wide range of insects at night. Predators home in on light-loving swarms to take advantage of the congregated prey.

## LONELY NIGHTS

Nighttime lighting drives away some nocturnal pollinators, reducing the ability of plants in lit areas to bear fruit.



## DESYNCHRONIZED

Artificially lit nights can perturb an animal's circadian rhythms, altering the timing of activities, such as sleep, foraging, mating, and migration, that are tightly controlled by the body's internal clocks.

## SHIFTING COMMUNITIES

Artificial illumination at night can increase the proportion of microorganisms in freshwater sediments that are able to photosynthesize under low light levels.

that Australian garden orb-weaving spiders (*Eriophora biapicata*) exposed to artificial light at night end up maturing faster and with fewer molts, being smaller as adults, and laying fewer eggs.[13] Outside the lab, however, the researchers observed a compensating advantage: spiders living near streetlights ate more than individuals living in darker locales, due to the abundance of potential prey congregating around the lights (unpublished). "Physiologically they were being affected, but ultimately they were doing okay because they gained this benefit from the change in the predator-prey relationship," Jones says.

## Chain reactions

During the summers of 2014 and 2015, Eva Knop, a community ecologist at the University of Bern in Switzerland, and her team spent several nights wandering through meadows wearing night-vision goggles, in search of nocturnal pollinators. The researchers had positioned street lamps over seven cabbage thistle (*Cirsium oleraceum*) patches in remote meadows in the foothills of the Swiss Alps previously unexposed to nighttime illumination. They then compared insects on the plants in the lit areas to those on plants in nearby, unlit control regions.

This investigation revealed a 62 percent reduction in nocturnal visits to the cabbage thistles in light-polluted areas, which led to a corresponding 13 percent drop in fruit production among the illuminated plants.[14] This decreased output could cause a decline in diurnal pollinator populations, which rely on the plants as a key source of food, the study authors suggest. "We need further experimental work to prove these indirect effects, but this shows that the negative effect at night could indirectly propagate into the day," Knop says.

A number of recent experiments in Europe have started to reveal how light pollution influences species interactions. At the University of Exeter, Gaston and his colleagues have set up grassland mesocosms, mini ecosystems within wooden cubes, each containing various plant, herbivore, and carnivore species. By exposing the enclosures to LEDs of varying intensities—low levels to mimic skyglow, medium levels corresponding to streetlamps, and high levels akin to stadium lighting—Gaston's team has found that light pollution can have profound effects on predator-prey interactions.[15]

> IT'S BECOME CLEAR THAT LIGHT POLLUTION IS A MAJOR ANTHROPOGENIC PRESSURE ON THE ENVIRONMENT.
>
> —Kevin Gaston, University of Exeter

In one experiment, for example, the team found that in 48 mesocosms that were exposed to low-intensity light for a few months, aphid populations shrank by approximately 50 percent due to increased predation by parasitoid wasps. Conversely, predators spent less time on the prey's host plants lit by more intense lights, leading to fewer aphid deaths. "Because of the interactions [between species], you might actually see quite severe effects even at quite low light levels," Gaston says. "It's not just how you respond, it's what your natural enemies and competitors are doing."

Such ecosystem-level effects of light pollution can influence population dynamics and even community productivity. At the Westhavelland Nature Park, one of the darkest regions in Germany, Hölker and his colleagues compared microbial communities in freshwater sediments from two sites in a shallow agricultural drainage ditch—one site lit by artificial light at night and the other left dark. After five months, they found a significant increase in photosynthesizing microbes, suggesting that these organisms were using nighttime lighting as an energy source.[16] Subsequent laboratory experiments revealed that exposing sediments to artificial light also perturbed the seasonal changes that typically occur in the microbial population. Without exposure to light pollution, there were clear winter and summer communities of bacteria and algae, Hölker says. "[But] after one year of illumination, this difference was no longer significant—the temporal structure was lost."

This change in the composition of microbial communities corresponded with a shift in the ecosystem's productivity. Under artificial lights, the microorganisms produced less carbon dioxide than those unexposed to evening illumination, likely a consequence of nighttime photosynthesis. In the long term, the researchers suggest, this could reduce the amount of carbon released from these freshwater systems into the biosphere over time.

"We naturally tend to think about the impacts [of light pollution] on individual species in isolation," says Gaston. "But I think what's becoming apparent now is that those networks of interactions are really vital to understanding the consequences of light at night."

## Shrinking numbers

Last year, researchers reported that flying-insect populations in Germany had dropped by more than 75 percent over the past three decades.[17] This dramatic loss in invertebrate life made headlines, and a coauthor of the study warned that such declines have set the Earth on course for an "ecological Armageddon." Of course, the question on everyone's mind was: What's the cause? "When this study came out, they were thinking about land-use change, climate change, and pesticides," Hölker says. But these factors alone could not explain the population plunge. Light pollution might be the missing piece of the puzzle, adds Hölker, whose team recently discovered that the decimated regions also had high levels of evening illumination.[18]

Light pollution could dramatically alter populations of vertebrates as well.

Field experiments by Kamiel Spoelstra, a biologist at the Netherlands Institute of Ecology, and colleagues have revealed that fast-flying *Pipistrellus* bats accumulate under certain colors of light that slow-flying *Myotis* and *Plecotus* bats avoid.[19] The slow bats may be light-shy because exposing themselves under light could make them more vulnerable to predators, whereas agile bats may be able to enjoy the feast of insects that accumulate under the lights, Spoelstra explains.

Over time, it is possible that "if you have many lights outside, these light-shy bats simply lose habitats," Spoelstra says. "It may be that the more lighting we have, the more common the common species and the rarer the less-common species become."

But examining the effects of artificial light on animal populations is difficult, and strong evidence of light pollution's long-term repercussions remains scarce. To appreciate the true scale of light pollution's effects, the best place to look would be in regions that have only recently been exposed to nighttime lights. A recent analysis found that between 2012 and 2016, the artificially lit outdoor surface area of the earth increased at an estimated rate of 2.2 percent per year, with much of the growth occurring in South America, Africa, and Asia.[20] This expansion has largely been facilitated by energy-efficient LEDs, Davies says. "So you get remote lights that are being put up in parts of the world that have previously been dark for the whole of evolutionary time."

As scientists uncover more and more evidence of the harms of nighttime light, they are beginning to work with designers, architects, and government officials to protect the planet's wildlife. Spoelstra, for example, has worked with Dutch policy makers to illuminate some areas with red light instead of white to prevent disruption to bat populations. However, this problem "can't be solved by changing the spectrum alone," Spoelstra says. Other modifications, such as limiting the times when roads are illuminated, putting motion sensors on lights, and shielding lamps so light does not spill into the sky or adjacent forests, are also necessary, he adds.

By themselves, these solutions are not enough to fight the effects of the increasingly luminous nights across the globe. Animals will not be able to evolve fast enough to adapt to the changes humans make on the planet, Longcore says. "We need to make either individual or collective decisions to not make the world even more light polluted than it already is." ■

NASA EARTH OBSERVATORY, IMAGE BY JOSHUA STEVENS

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ADAPTED FROM A FIGURE BY TRAVIS LONGCORE

## EVENING HUES

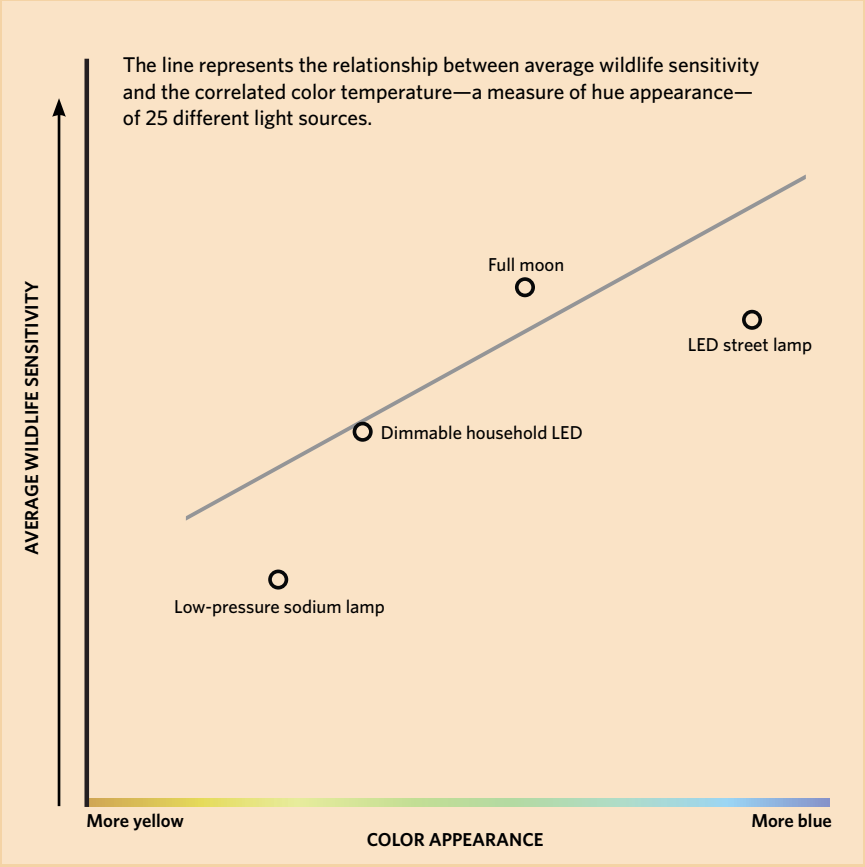
Artificial lights come in a range of colors. Low-pressure sodium lamps, which are typically used to brighten streets at night, have a distinct yellow hue. Light-emitting diodes (LEDs), on the other hand, offer illumination that is more energy-efficient, but the commonly used white LEDs typically produce large amounts of blue light.

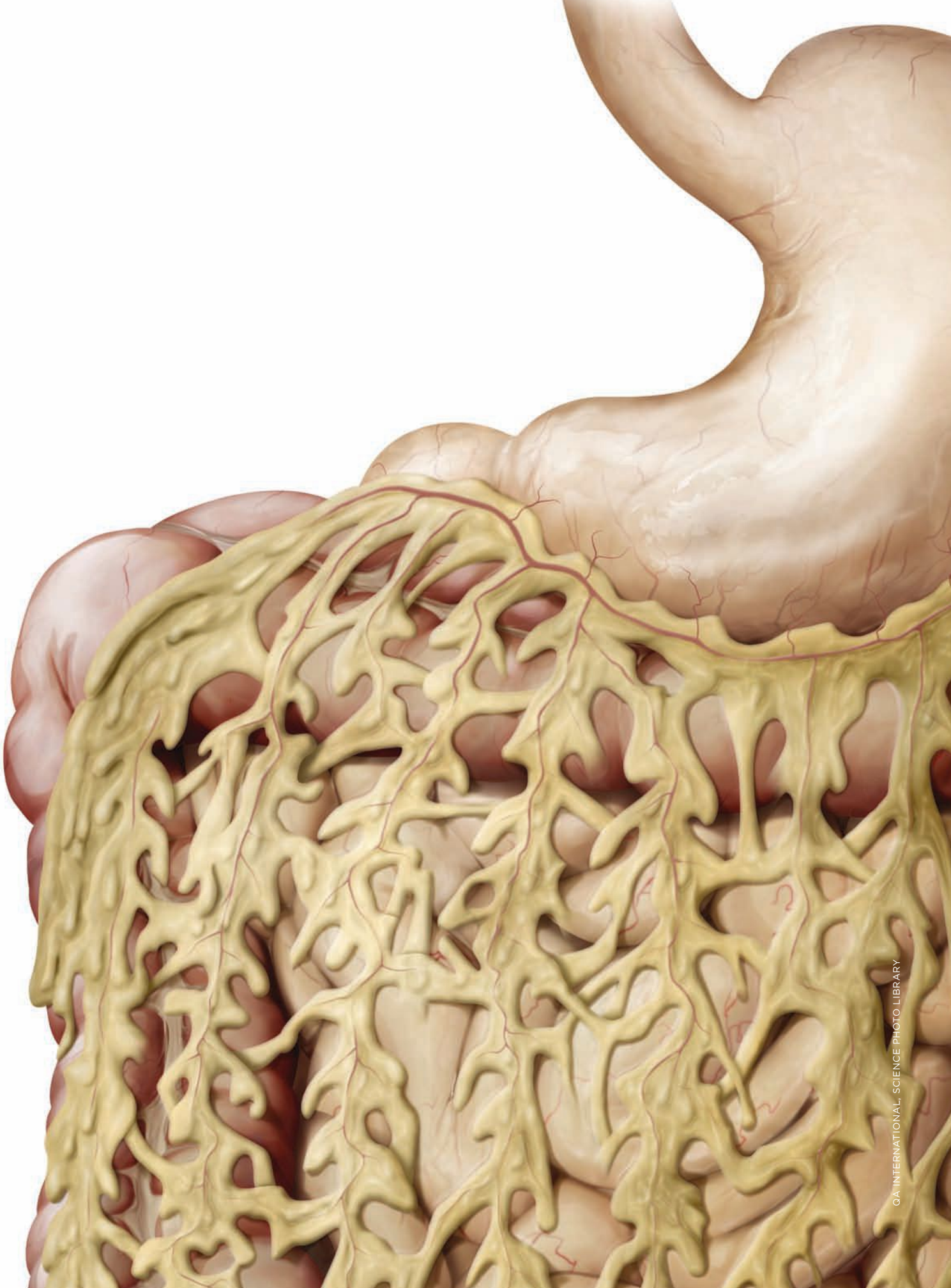
Scientists have long suspected that blue-rich lighting is the most harmful to wildlife. Decades of research have revealed that the cool hue is attractive to many animals—particularly insects—and can suppress the production of melatonin, a crucial hormone for regulating circadian rhythms.

In a recent analysis, University of Southern California geographer Travis Longcore and colleagues compiled previously published data on organisms' responses to light across the spectrum to calculate the predicted effects of different lighting types. This work demonstrated that blue-rich lights indeed pose the greatest risk for the well-being of a wide variety of species, including insects, birds, and fish (*J Exp Zool*, doi:10.1002/jez.2184, 2018).

Now, conservationists are looking to capitalize on this information to protect wildlife. In Florida, for instance, the Fish and Wildlife Conservation Commission now recommends the use of red or amber LEDs to avoid attracting hatchling sea turtles. Similarly, some areas in the Netherlands have installed red lights to make their evening skies safer for bats.

But even these colors can have adverse effects. For example, red lights tend to attract migrating birds—a problem recently recognized by the US Federal Aviation Administration, which announced in 2015 that it would require communication tower operators to replace steady red lights with flashing ones to reduce their allure.





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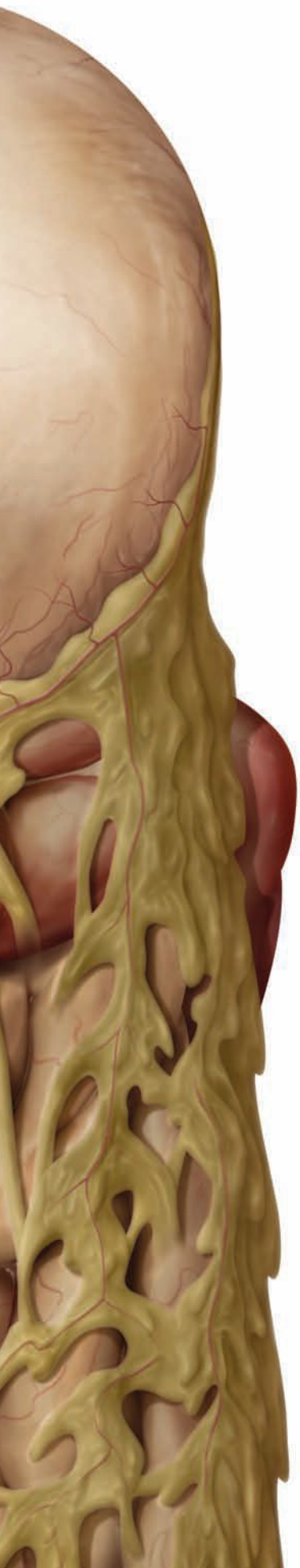


# How Fat Fights Infection

Hanging in front of the abdomen like an apron, the deposit of visceral fat known as the omentum helps regulate immune responses.

BY SELENE MEZA-PEREZ AND TROY D. RANDALL

Fat is a loaded tissue. Not only is it considered unsightly, the excess flab that plagues more than two-thirds of adults in America is associated with many well-documented health problems. In fact, obesity (defined as having a body mass index of 30 or more) is a comorbidity for almost every other type of disease. But, demonized as all body fat is, deep belly fat known as visceral adipose tissue (VAT) also has a good side: it's a critical component of the body's immune system.

VAT is home to many cells of both the innate and adaptive immune systems. These cells influence adipocyte biology and metabolism, and in turn, adipocytes regulate the functions of the immune cells and provide energy for their activities. Moreover, the adipocytes themselves produce antimicrobial peptides, proinflammatory cytokines, and adipokines that together act to combat infection, modify the function of immune cells, and maintain metabolic homeostasis.

Unfortunately, obesity disrupts both the endocrine and immune functions of VAT, thereby promoting inflammation and tissue damage that can lead to diabetes or inflammatory bowel disease. As researchers continue to piece together

Researchers continue to piece together the complex connections between immunity, gut microbes, and adipose tissues.

the complex connections between immunity, gut microbes, and adipose tissues, including the large deposit of fat in the abdomen known as the omentum, they hope not only to gain an understanding of how fat and immunity are linked, but to also develop fat-targeted therapeutics that can moderate the consequences of infectious and inflammatory diseases.

### The omentum's role in immunity

The omentum, a term derived from the Latin word for apron or cover, is a fold of fat that hangs below the stomach and covers the intestines. (See illustration on right.) Found in all people, even the most lean, the omentum is actually an independent organ. Hints about its functions (other than fat storage) exist in the early scientific literature. For example, after observing numerous cases in which the omentum adhered to ulcers of the gall bladder, stomach, and intestine; surrounded inflamed ovaries or ruptured appendices; and successfully plugged a hole in the diaphragm, a pioneering British surgeon at the turn of the 20th century referred to it as the "abdominal policeman."[1]

Consistent with this conclusion, we now know that the omentum supports the generation of blood vessels and fibrous connective tissue that can help repair damaged organs, and also promotes immune responses to fight infection. Many of the immune cells that reside within the omentum are found in aggregates termed fat-associated lymphoid clusters, or "milky spots" for their whitish appearance amidst the yellow fat. (See image on page 48.) In many ways, milky spots are analogous to lymph nodes—the small bean-shape organs that filter excess fluid from peripheral organs such as the skin, muscle, liver, and lungs. Milky spots, similarly, filter the fluid that flows from the abdominal cavity. The clusters of immune cells in both milky spots and lymph nodes sense microbes, damaged cells, and inflammatory mediators and initiate appropriate immune responses.

#### KNOW YOUR FAT

Adipose tissue is broadly divided into brown and white varieties. Brown fat cells express high levels of thermogenic genes and help maintain body heat by burning calories. Beige fat cells function similarly, but they are not of the brown fat cell lineage. Rather, they develop in white fat, the tissue that we typically think of as "fat." White fat cells are involved in whole-body energy homeostasis and lipid storage and are found both under the skin and in the abdomen, where they are known as visceral adipose tissue (VAT). It is this type of fat that can help detect and eliminate pathogens as well as maintain immune homeostasis in the gut.

Despite their analogous functions, milky spots and lymph nodes have very distinct populations of leukocytes. For example, the antibody-producing B cells in milky spots and lymph nodes develop from different progenitors and have unique repertoires of antigen receptors. In particular, B cells in milky spots are associated with T cell–independent responses to bacterial and carbohydrate antigens, and often differentiate into IgA-producing cells that react with commensal bacteria in the intestine.[2] Given that the omentum frequently adheres to ruptures in the intestine or appendix, it is likely that it helps defend against commensal organisms that might spill into the abdominal cavity after injury.

Like B cells, macrophages in the omentum also appear to tailor their activities to protect the host from bacteria that might escape the intestine. Studies from Ruslan Medzhitov's lab at Yale University show that these macrophages produce the transcription factor GATA6, which is regulated by retinoic acid, a metabolite generated from vitamin A by enzymes that are abundant in the omentum.[3] Peritoneal macrophages from mice fed a vitamin A–deficient diet do not produce GATA6

and consequently fail to make transforming growth factor–β (TGF–β), a cytokine that stimulates B cells to produce IgA in response to gut microbiota. Similarly, mice with a genetic deletion of *GATA6* in macrophages also fail to make TGF–β and IgA.

Meanwhile, T cells in the omentum produce a greater variety and higher amounts of infection-fighting cytokines than their counterparts in other parts of the body. Last year, Yasmine Belkaid of the National Institute of Allergy and Infectious Diseases and colleagues showed that VAT-resident memory $CD8^+$ T cells in mice respond more quickly to an infection than memory cells at other sites.[4] The rapid response, the researchers found, was due to an altered cellular metabolism in these T cells, characterized by the increased uptake of fatty acids and elevated mitochondrial function. Given that fatty acids and glycerol are major products of adipocytes, fat-resident memory $CD8^+$ T cells are likely taking advantage of the metabolic resources in adipose tissues for both their maintenance and their ability to defend against pathogens.

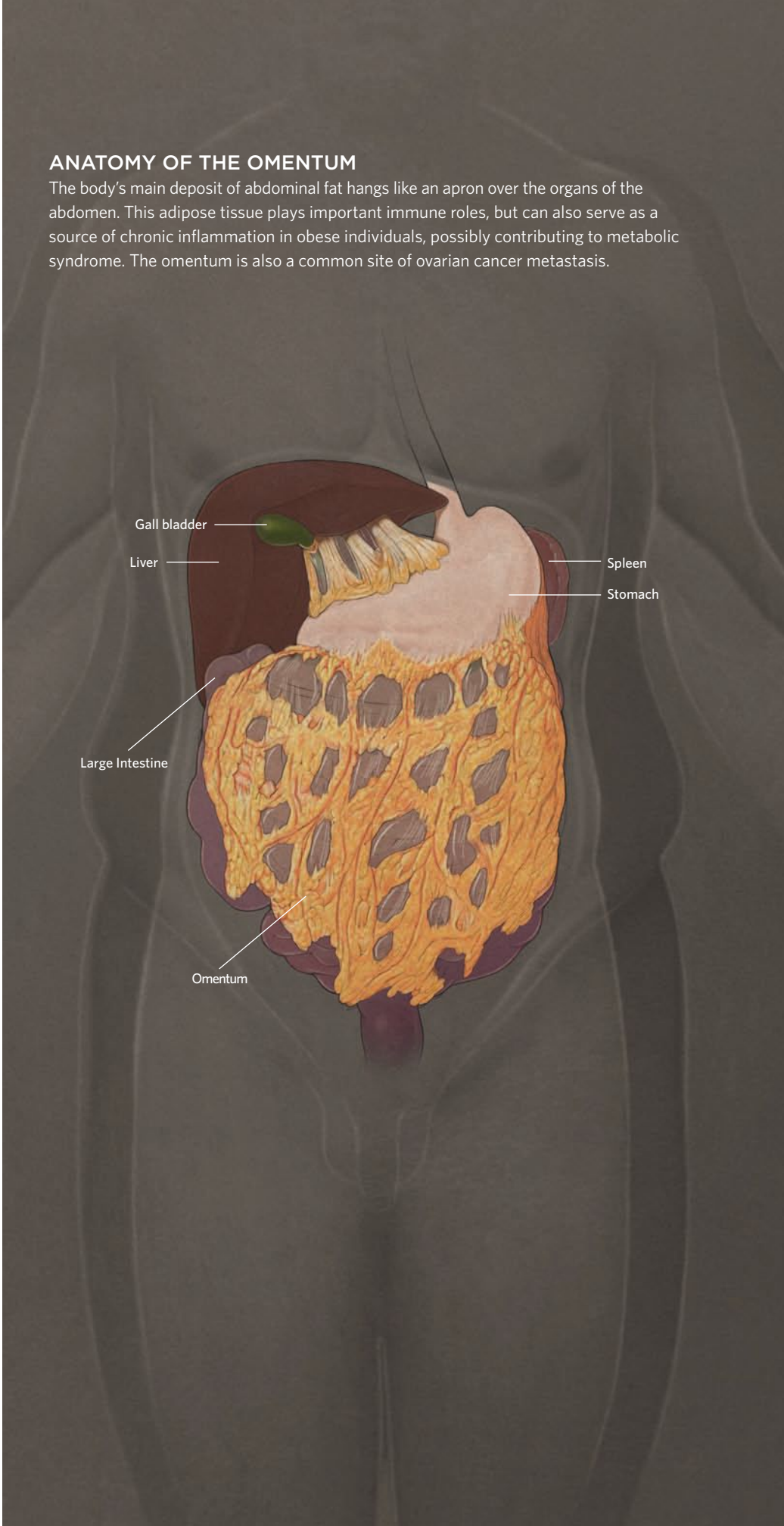
Importantly, the interactions between T cells and adipocytes go in both directions: recently activated T cells trigger adipocytes to reduce the expression of genes involved in lipid biosynthesis and instead produce antimicrobial peptides. This transient shutdown of lipid metabolism in favor of antimicrobial function is likely a two-pronged mechanism for limiting pathogen growth and facilitating antimicrobial immunity.

## The downside of fat's T cells

Unfortunately, T cell activation in adipose tissue isn't always so helpful. In a series of studies examining the link between immune-driven inflammation and insulin resistance in diet-induced obesity, Satoshi Nishimura and colleagues at the University of Tokyo reported that $CD8^+$ memory T cells in the VAT of mice fed a high-fat diet became chronically activated and produced inflammatory chemokines and cytokines, which recruited macrophages that further aggravate inflammation and tissue damage.[5]

### ANATOMY OF THE OMENTUM

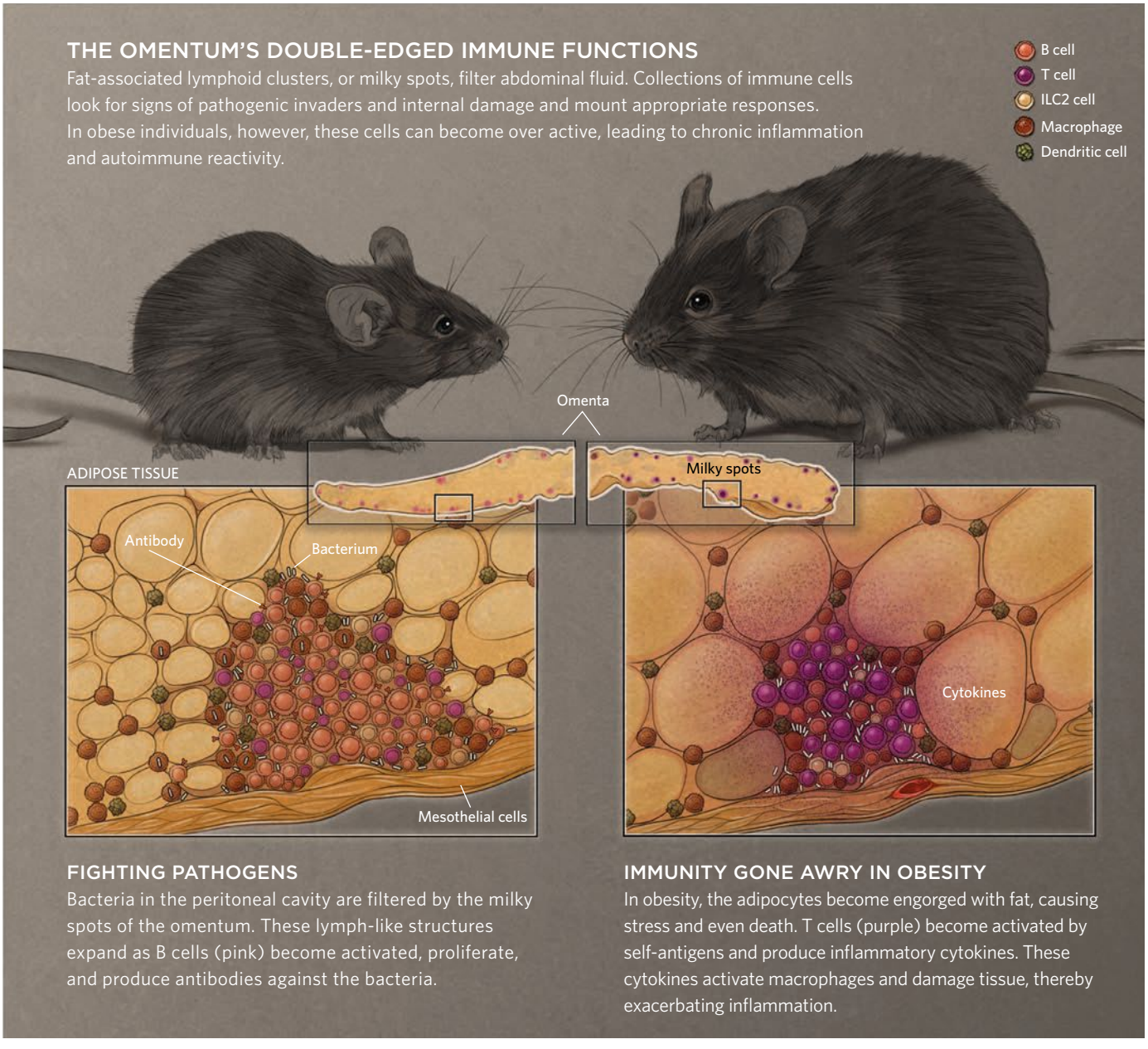
The body's main deposit of abdominal fat hangs like an apron over the organs of the abdomen. This adipose tissue plays important immune roles, but can also serve as a source of chronic inflammation in obese individuals, possibly contributing to metabolic syndrome. The omentum is also a common site of ovarian cancer metastasis.



Inflammatory T helper (Th1) cells and antibody-producing B cells also accumulate in the VAT of obese mice and contribute to metabolic dysfunction.[6,7] Like $CD8^+$ T cells, Th1 cells make inflammatory cytokines that activate local macrophages. Moreover, they promote the differentiation of B cells into antibody-secreting cells. In the case of diet-induced obesity, VAT-associated B cells often produce antibodies that, rather than binding to viruses or bacteria, latch on to adipocytes and other cells, including the insulin-producing β cells of the pancreas. This causes tissue damage that can ultimately lead to diabetes or other inflammatory diseases.

At the same time that inflammatory pathways are overly exuberant, immunoinhibitory mechanisms may also be dysfunctional. A recent series of papers from the lab of Diane Mathis at Harvard University defined a unique population of regulatory T cells (Tregs) that reside in the VAT.[8,9] Obese mice lacking VAT-associated Tregs became insulin resistant more easily and exhibited increased adipose inflammation, the researchers showed. In contrast, obese mice given the diabetes drug pioglitazone had improved insulin sensitivity and lowered blood sugar, due in part to increasing numbers and suppressive activity of VAT-associated Tregs.

Similarly, type 2 innate lymphoid (ILC2) cells normally dampen inflammatory responses in adipose tissue and, as an added benefit, promote the differentiation of adipo-



cyte precursors into beige fat, which increases caloric expenditure and reduces adiposity. Obese humans have fewer ILC2 cells in their white adipose tissue than lean individuals, suggesting they burn less fat, thereby promoting obesity and adipose inflammation.[10,11]

## Another tradeoff: tumor metastasis

The omentum's immune functions can also be subverted by malignancy, particularly ovarian carcinoma, which often metastasizes to the omentum. The same filtering activity that allows milky spots to detect pathogens also facilitates the collection and colonization of detached tumor cells floating in the abdominal fluid.[12] This activity is easily observed in mice following an injection of tumor cell suspensions: within hours, milky spots take up tumor cells, which grow into visible colonies within days. Because of this risk, surgeons often resect most or all of the omentum in the context of human ovarian cancer to eliminate as much metastatic disease as possible.

Researchers have long thought the high rate of metastasis in the omentum was due solely to the passive collection of tumor cells, but recent data from the laboratory of Anil Sood at MD Anderson Cancer Center are starting to upend this assumption. Sood and colleagues showed that the omentum is uniquely supportive of ovarian tumor growth, regardless of how the metastasizing cells get there.[13] Using a technique called parabiosis, in which mice are surgically paired side by side so that they have a common circulation, the researchers found that ovarian tumor cells can metastasize through the bloodstream from one mouse to the other. Despite initially lodging in the lungs, liver, and spleen, however, the cells only formed metastatic tumors in the omentum.

Tumor cells' ability to thrive in the omentum reflects a critical interaction with adipocytes, which transfer lipids to the cancerous cells. Visualizing fluorescently labeled lipids using microscopy, researchers observed the depletion of lipids from adipocytes and the accumulation of lipids in tumor cells, suggesting a direct transfer.[14] Tumor cells in the omentum also alter their cellular metabolism in favor of oxidizing fatty acids, as these molecules are abundant in adipose tissues such as the omentum. This metabolic switch is similar to that observed in VAT-resident T cells and is likely a common theme among cells that reside in belly fat.

> Cells residing in the omentum likely take advantage of the metabolic resources in adipose tissues.

In addition to altering their metabolic program, tumor cells that metastasize to the omentum upregulate (or are selected for) the production of HER3 (human epidermal growth factor receptor 3). The omentum is a rich source of neuroregulin, the ligand for HER3, which facilitates metastasis and colonization.[13] In fact, therapeutic disruption of this pathway in mice reduces metastasis to the omentum and impairs tumor growth.

Despite the omentum's role in promoting the growth of ovarian tumors, it also has the cell types and lymphoid architecture to initiate antitumor immune responses. For instance, the presence of tumor infiltrating lymphocytes (TILs), particularly $CD8^+$ T cells, positively correlates with overall survival in women with ovarian cancer, whereas the presence of immunosuppressive Tregs is associated with rapid tumor progression and accelerated mortality.[15] A better understanding of how immunity is regulated in the omentum should allow us to tap into these processes to eliminate malignant cells and improve the notoriously poor outcomes for women with metastatic ovarian cancer. ■

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# The Literature

CELL & MOLECULAR BIOLOGY

## Strength in Numbers

THE PAPER

M. Jagannathan et al., "A conserved function for pericentromeric satellite DNA," *eLife*, 7:e34122, 2018.

Between cell divisions, pericentromeric DNA—noncoding, repetitive satellite DNA abundant around centromeres—bunches together across several chromosomes to cluster them into structures called chromocenters.

The function of pericentromeric DNA and of chromocenters had remained largely unknown until cell biologist Yukiko Yamashita and her team at the University of Michigan set out to see what would happen to fruit fly cells without them. Simply mutating or excising pericentromeric DNA, which occurs as long sequences of tandem repeats, would have been impractical. So Yamashita's group instead targeted a protein, D1, that's known to interact with satellite DNA. When D1 was mutated, fruit fly germ cells did not survive for long. The researchers stained these mutant, dying germ cells with an antibody for Vasa, a cytoplasmic protein, and saw dark spots that turned out to be micronuclei, aberrant structures that can form when rogue chromosomes escape from their chromocenters and cause bits of the nuclear membrane to bud off around them.

Yamashita's team proposes that the protein D1 binds to pericentromeric DNA on multiple chromosomes, in effect locking them together in chromocenters. Without this protein or the requisite DNA binding sites, the chromosomes simply float away from each other, leaving behind incomplete nuclei. To see if these results held up in mammals, the team carried out similar experiments in mouse cells, and found that knocking down a D1 analog called HMGA1 caused micronuclei to form.

Next, Yamashita and her colleagues expressed the D1 protein from fruit flies in mouse cells and saw that fewer chromocenters formed with higher levels of D1 in a dose-dependent way—indicating that in cells with elevated levels of the fruit fly protein, the chromosomes were forming larger bunches. Even though D1 and HMGA1 bind to different DNA sequences in their native species, the former had evidently interacted with mouse pericentromeric DNA.

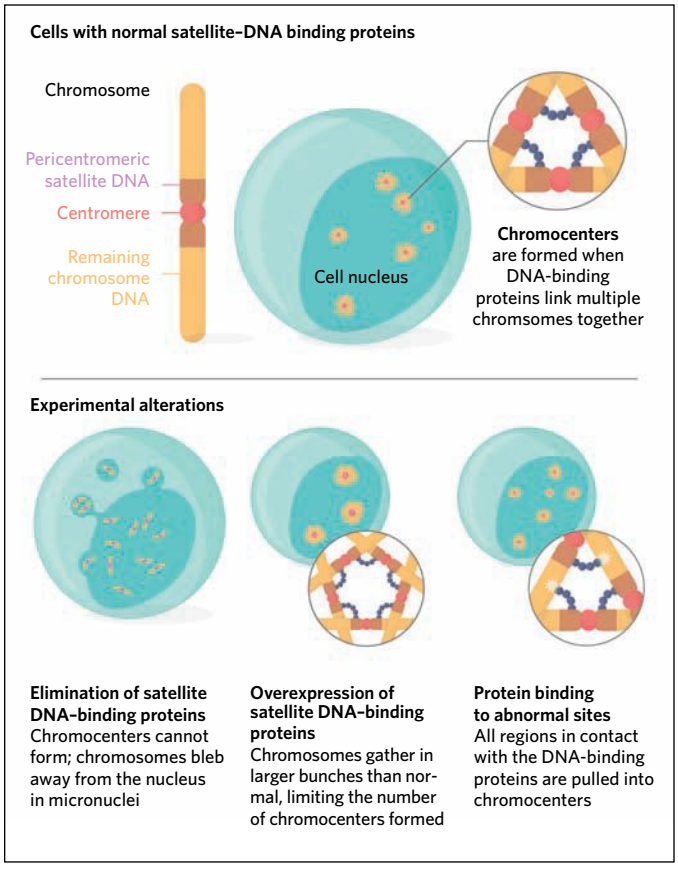
In another experiment in flies, the team attached D1 to DNA sites that don't naturally interact with the protein and saw that those regions were pulled into the chromocenters.

Thus, pericentromeric DNA's function may be to ensure that all the chromosomes that make up a genome stay together.

Aaron Straight, who studies chromosome biology at Stanford University and was not involved in the study, says the disruption of the nuclear membrane and budding of micronuclei is an unexpected result of knocking down the proteins that bind pericentromeric DNA. "That, I don't think, could have been predicted," he says. He adds that whether the absence of chromocenters leads to micronuclei formations remains to be seen.

Yamashita notes that the makeup of such satellite DNA diverges significantly even between closely related species. The team speculates that incompatibility between satellite DNA and chromocenter-forming proteins may make it difficult for would-be hybrids to form chromocenters, potentially affecting their viability and helping to drive speciation.

—Sukanya Charuchandra




**BOUND TOGETHER:** In cells with normal satellite DNA–binding proteins, chromocenters pull several chromosomes together (top). When the function of the DNA-binding proteins is experimentally altered, chromocenter formation also changes (bottom).

BUZZ BUZZ: Exposing mosquitoes to low doses of ibuprofen may prep their offspring for a stressful life ahead.

ECOLOGY & ENVIRONMENT

# Cause and Effect

### THE PAPER

S.M. Prud'homme et al., "Multiscale approach to deciphering the molecular mechanisms involved in the direct and intergenerational effect of ibuprofen on mosquito *Aedes aegypti*," *Environ Sci Technol,* 52:7937–50, 2018.

### PERVASIVE PAINKILLER

Low levels of the drug ibuprofen are frequently found in rivers and lakes worldwide. Because of its ubiquity, a group of French researchers investigated the long-term effects of an environmentally realistic ibuprofen exposure on mosquitoes and their unexposed progeny.

### WAIT FOR IT

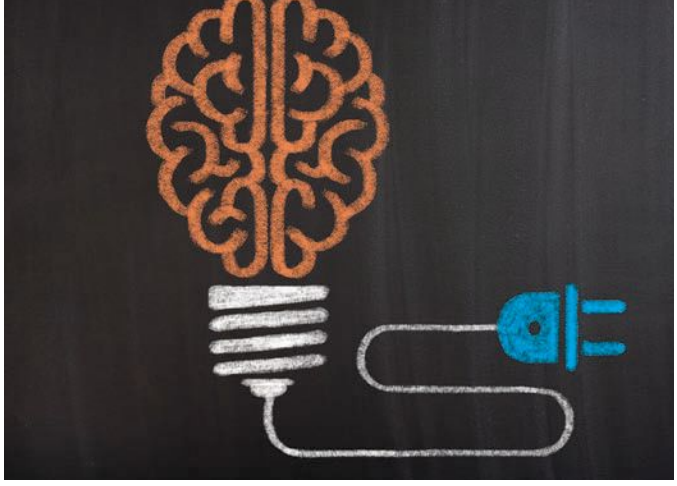
The scientists observed almost no change in mosquitoes that were exposed to the anti-inflammatory drug as larvae, relative to controls, says coauthor Sophie Prud'homme, an ecotoxicologist at the Université Grenoble-Alpes. But the exposed mosquitoes' progeny, which did not themselves get the ibuprofen dose, developed more quickly and were more tolerant of starvation than controls.

### ARMORED UP

The researchers used transcriptomics and metabolomics to take snapshots of molecular processes playing out in mosquitoes. They observed a decrease in certain amino acids, carbohydrates, and other metabolites in the eggs of exposed mosquitoes. The larvae hatched from those eggs contained changes in a signaling pathway involved in mosquito growth that may prepare the unexposed progeny for a stressful life ahead. The researchers suspect that epigenetics might regulate some of these changes.

### CONTAMINANT CONUNDRUM

"The females were stressed and . . . probably the eggs were not as well provisioned," says Immo Hansen, who studies mosquitoes at New Mexico State University and was not involved in the research. It's unclear how the ibuprofen-induced changes would affect mosquitoes' fitness and potential to transmit disease. But the intergenerational changes would have escaped notice if the researchers hadn't examined offspring of exposed insects. Many toxicology studies investigate effects at only one life stage, but they might be missing something in the rest of the life cycle or in the next generation, says Prud'homme. —Carolyn Wilke



ENERGY EFFICIENCY: A gene linked to Alzheimer's risk turns out to be involved in the brain's glucose metabolism.

DISEASE & MEDICINE

# Brain Boost

### THE PAPER

L. Wu et al., "Human *ApoE* isoforms differentially modulate brain glucose and ketone body metabolism: Implications for Alzheimer's disease risk reduction and early intervention," *J Neurosci*, 38:6665–81, 2018.

### GOOD GENE, BAD GENE

Humans carry three different isoforms of the *ApoE* gene, which affects Alzheimer's risk. Liqin Zhao of the University of Kansas and her colleagues previously found that the gene plays a role in brain metabolism when expressed in mice; in a new study, they looked for the pathways involved.

### LEAVING AN IMPRESSION

Zhao's team engineered female mice to express the human versions of either *ApoE2, ApoE3,* or *ApoE4,* and analyzed expression of 43 genes involved in energy metabolism in their cortical tissue.

### BLOCKADE

Mice with *ApoE2* showed higher levels of proteins needed for glucose uptake and metabolism in their brains relative to animals harboring the most common isoform in humans, *ApoE3*. Mice with *ApoE4* had lower levels of such proteins. The brain tissue's glucose transport efficiency also varied across the genotypes, and levels of a key glucose-metabolizing enzyme, hexokinase, were reduced in *ApoE4* brains. However, *ApoE2* and *ApoE4* brains contained similar levels of proteins involved in using ketone bodies, a secondary source of energy, while *ApoE3* brains had lower levels of those proteins. "Brain glycolytic function may serve as a significant mechanism underlying the differential impact of *ApoE* genotypes," Zhao says.

### ENERGY SHOT

*ApoE4* brains' reduced ability to extract energy from glucose may contribute to Alzheimer's pathology by reducing synaptic activity and increasing susceptibility to cellular stress. While *ApoE4* increases risk of developing Alzheimer's, *ApoE2* lowers it. "One of the many explanations for Alzheimer's disease is dysfunctional bioenergetics," says McLean Hospital's Kai-Christian Sonntag, who was not involved in this work. The study's authors suggest that developing therapies that provide more energy to the brain could help keep Alzheimer's at bay. —Sukanya Charuchandra

PROFILE

# Genome Collector

Charles Rotimi works to ensure that genetic epidemiology and population genetics studies include DNA from African—not just European—subjects.

BY ANNA AZVOLINSKY

Not long after starting a job as the head of a chemistry lab at a high school in Benin City, Nigeria, Charles Rotimi told his parents that he wanted to leave his native country to pursue a graduate degree abroad. He applied to a petrochemical engineering school in the UK and to the University of Mississippi for a health care administration degree, at the advice of a Nigerian friend working there. Rotimi chose the US school because of the cheaper tuition. His mother, who ran her own business, offered Rotimi $10,000, enough for a year in the States. "That was a huge amount of money for my family and a validation that she had confidence and trust in my succeeding," says Rotimi, now director of the Center for Research on Genomics and Global Health at the US National Institutes of Health.

Rotimi's adventures abroad started in January of 1982. His entire family—parents and five siblings—saw him off at the Benin City Airport as he boarded a plane to the States. "I had never been outside of Nigeria and at the airport, suddenly leaving dawned on me, and I couldn't hold back my tears," he recalls. The trip wasn't easy. "It was absolutely traumatic," Rotimi says. He took a flight to Gatwick Airport, which is roughly 50 kilometers south of London, and then boarded a helicopter to Heathrow Airport, near the city center. "It was winter, and I had never experienced winter and didn't even have a coat on me, nothing close to adequate," he says. "The hostess in the chopper saw that I was freezing and gave me a blanket, which was a lifesaver."

From Heathrow, Rotimi flew into New York City and then to Memphis, Tennessee, about an hour from the University of Mississippi. His Nigerian friend picked him up from the airport and took him to a McDonald's to introduce him to hamburgers. "I couldn't eat it! I took a bite and couldn't swallow," says Rotimi. "It was just so different from the food I was used to. I would never have thought to put meat and lettuce with sauce between bread."

Rotimi's cultural immersion didn't stop under the golden arches. At the University of Mississippi—where children from some of the wealthiest Southern families go to college, and which has a history of social unrest and discrimination—he would get his first taste of one of the most unfortunate aspects of American culture. "I really started to learn about disparity quite quickly. Other than knowing about apartheid in South Africa, which felt far away, I had no idea about racism. I had no appreciation of what it was and its intensity," he says.

Yet Rotimi never regretted his decision to study at the university. It was where he met his future wife, Deatrice, who is originally from Chicago and was then an undergraduate at Mississippi, and where he completed his master's degree in health care administration in 1983.

Never intending to stay in the US, Rotimi flew back to Benin City to be with his family in 1985 and finally, after a six-month search, landed a job as an administrator at a local health ministry. "The job was not fulfilling, I was starting to run out of money, and suddenly I couldn't see a future for myself in Nigeria," says Rotimi.

He applied and was accepted to the University of Alabama's Master of Public Health epidemiology program and again left the country of his birth, this time for Birmingham. There, Rotimi says that he did only three things—sleep, eat, and study—hoping to secure a scholarship to continue on as a PhD student. The effort paid off, and after a year with perfect grades, he received a full scholarship. By then, Deatrice had joined him and the couple had gotten married.

Following his PhD and a postdoc in California that lasted less than a year, Rotimi landed his dream position at Loyola University—as an epidemiologist studying cardiovascular disease, obesity, and diabetes susceptibility in individuals across the African diaspora. Since then, Rotimi has been studying genetics and health disparities, making it his mission to generate genetic epidemiology data on African populations and to make sure that African genomes are represented among large-scale genomics projects.

## AN INQUISITIVE MIND

Rotimi was born in 1957 in Benin City, the second oldest of six children. "I was one of those kids that drives their parents crazy because they are always asking 'why' about everything and never satisfied with grown-ups' answers," he says. "My mother would ask jokingly, 'Do you have to question everything?' I just wanted to know how things worked." Rotimi says that his mother, Mary, had no formal education but was among the most brilliant people he has known. She had her own wholesale business, supplying goods such as candy, sugar, milk, and toiletries to local stores. His father, Alfred, was a high school English teacher and later became a foreman for a timber and plywood company. Rotimi excelled at math and science from an early age, had supportive teachers, and took chemistry, physics, and biology laboratory courses. He was also an avid soccer player, although not good enough, he says, to play in college.

As an undergraduate, at the University of Benin, Rotimi majored in biochemistry, and in his final year, in 1978, he conducted an experiment that tested for the presence of aflatoxin—a compound produced by a fungus—in poorly stored Nigerian foods and alcoholic beverages, such as palm wine and a millet-based drink called burukutu. Aflatoxin has been linked to liver cancer, and in hindsight, Rotimi says, he was already starting to address a health disparity issue—the lack of refrigeration and other properly functioning, temperature-controlled food storage facilities—early in his career.

NHGRI

## CHARLES ROTIMI

Director of the Trans-NIH Center for Research on Genomics and Global Health at the National Institutes of Health, Bethesda, Maryland, USA

Senior Investigator within the Intramural Research Program, National Human Genome Research Institute (NHGRI), NIH, Bethesda, Maryland, USA

NIH Director's Award for leading the establishment of the Human Heredity and Health in Africa (H3Africa) Initiative (2012)

First person of African ancestry to be elected to the Board of the American Society of Human Genetics

Recipient of HudsonAlpha Life Science Prize (2018)

### Greatest Hits

- Along with Richard Cooper and colleagues, determined environmental causes of hypertension and diabetes among urban and rural populations of West African descent—and showed that African diaspora populations can be used to do large epidemiology studies
- Led the African genome component of the International HapMap Project, which became part of the 1000 Genomes Sequencing Project
- Analyzed the genomes of almost 3,000 individuals to determine the evolutionary origin of the sickle cell anemia mutation, which confers resistance to malaria, and calculated that the mutation emerged about 7,300 years ago in West Africa

## A TAILOR-MADE POSITION

During his PhD in Alabama, Rotimi examined whether foundry and engine plant workers in Ohio were especially susceptible to lung and stomach cancer. The study, published several years after he completed his graduate studies, didn't show a strong link. His study lasted three years, and once he earned his PhD, in 1991, he drove his Honda Civic to Loma Linda University in California to start his postdoc, studying Alzheimer's disease. Rotimi says he thought researching the neurodegenerative disease would interest him, but not long after he arrived, he saw an advertisement for an epidemiology assistant professor position at Loyola University in Chicago to study cardiovascular diseases in individuals of African descent.

"I called about it, and the department chair, Richard Cooper, picked up the phone. 'Dr. Cooper, did you write this ad with me in mind?' I told him. He thought I was crazy, I think, but we both laughed," Rotimi recalls. "This is exactly what I want to do in my career, I told him."

Rotimi went to Chicago for an interview. Cooper, a tall, blond cardiologist from Arkansas who had turned to epidemiology, had witnessed firsthand the discrimination against black people around him while growing up. "The experiences stayed with him, and he was socially conscious and wanted to do research that would shed light on the disease disparity of minorities in the US that likely had more to do with societal structure than genes," Rotimi says. At the end of the interview, Cooper offered Rotimi the position.

Together, and in collaboration with several international scientists, they successfully enrolled more than 10,000 individuals for a study of hypertension prevalence among seven populations of West African origin, including groups of people in Nigeria and Cameroon, on three islands in the Caribbean, and in Chicago. "Cooper was the brain behind this groundbreaking cardiovascular research project," Rotimi says.

"A study this large was unheard of at the time, and it was difficult to conduct," he adds, "I traveled a lot for this work." The effort was worth it. The team found that the rates of hypertension and diabetes dramatically increased from rural Africa to urban centers in the Caribbean and to the African American population in Chicago. A significant proportion of the observed differences could be explained by individual lifestyle factors, including weight, salt consumption, and levels of physical activity.

At Loyola, Rotimi also received funding from the National Institutes of Health (NIH) to study whether genetic differences among those who shared African ancestry could explain additional variation in hypertension, obesity, and cardiovascular diseases. The study dem-

onstrated that coronary heart disease, hypertension, stroke, and diabetes tend to occur in some African-American families and not others, providing evidence for heritable components of these diseases.

"All of this epidemiology and genetic research on African-descent individuals was unique at the time. No one else was really studying this," Rotimi says. "It was fortunate for us that the research proposals made sense to our reviewers, because most of the grants we wrote were funded." He credits Cooper with teaching him "to write well and to ask good questions," which helped Rotimi solidify his approach to designing studies on African genomes to understand gene-environment interactions.

## GENETICS TO TACKLE RACE ISSUES

In 1999, after seven years at Loyola, Rotimi was persuaded by microbiologist Georgia Dunston to move to Howard University in Washington, DC. There, he would help Dunston establish the university's National Human Genome Center to study the diversity within African and African American populations and to be the center's director of genetic epidemiology.

The move and setting up the center proved more challenging than Rotimi had expected. "I had a strong belief in my capabilities, but looking back, I was quite naive," he says. "I had to create the kind of support that I needed and to do that, I had to work four times as hard as I probably would need to elsewhere, as I didn't have as much mentorship. But the experience helped me to see what I was capable of on my own."

Rotimi began to study the implications of genetics for identity and the disparity created if neither African American nor African populations are included in genomic datasets. After attending an international meeting on genetic variation and identity, Rotimi wrote a 2004 *Nature Genetics* perspective on how best to use genetic variation as a tool to help understand disease across different populations. In another *Nature Genetics* perspective he wrote with colleagues and published the same year, Rotimi argued for the use of genomic tools to better understand human biological variation, how to classify our various genetic backgrounds, and what genetics can teach us about breaking down social definitions of race. "Although there is genetic variation in our genomes, the key is that these differences don't rise to the level of being able to parse socially defined racial groups or racial self-identity," he says. "Using genetics to define race is like slicing soup: you can cut wherever you want, but the soup stays mixed."

Around the same time, Rotimi joined the International HapMap Project, which sought to identify patterns of DNA variation in the genomes of individuals around the world, the first spin-off of the Human Genome Project. He engaged three African communities in Kenya and Nigeria to ensure that African genomes were represented. According to the fossil record, modern humans originated in Africa about 200,000 years ago, and 100,000 years later, some left the continent and spread throughout the world. Many of the genetic evolutionary changes in the human genome occurred before the out-of-Africa migration, and "because those that migrated out only carried a subset of the genetic diversity that existed then, it is critically important to include as many African populations in genomic studies [as possible] to fully capture human genetic diversity," says Rotimi.

"The HapMap analysis reaffirmed what my lab was already very conscious of, which is that African genomes, because they are oldest, have among the highest degree of variation, and that you cannot fully appreciate population variation without studying and understanding these most ancestral human genomes," says Rotimi.

He also continued to study diabetes, both for personal reasons (his father died of complications of the disease, and several of his siblings are diabetic) and because economic and other disparities that certain populations and communities face have been implicated in rising diabetes rates.

In 2007, Rotimi joined forces with Kári Stefánsson, an Icelandic neurologist and cofounder of deCODE Genetics. The company studies the genetics of disease, and Rotimi contributed his lab's West African genomic dataset to further understand a diabetes risk gene variant, *TCF7L2*, which was originally identified in a European genomics study. "Because the African genome is older, has undergone more recombination, and is therefore more fragmented, we could show with more certainty that *TCF7L2* and a variant (*HapA*) of the same gene are associated with diabetes risk because the gene functions in energy metabolism."

## THE SAME, YET DIFFERENT

In 2008, Rotimi moved his lab to the NIH in Maryland. He became the founding director of the Trans-NIH Center for Research on Genomics and Global Health, following several brainstorming meetings with Francis Collins, then the director of the NHGRI, on ways genomics studies could be more inclusive of the world's populations. "It's been wonderful," says Rotimi. "One of the best career moves I have made. My lab group here is like the United Nations! Whenever we do a potluck, it is food from all over the world."

Recently, Rotimi and his lab members compiled genomic data from almost 6,000 individuals, representing 13 language families, to come up with 21 different global genetic ancestries. The research showed that more than 97 percent of humans have mixed ancestry, demonstrating that race labels such as "black," "white," and "Hispanic" are far from adequate ways to classify people.

"There are differences among people, but this study again shows that these differences don't cluster into social groups and race. We have these categories for people that we try to justify in biological, cultural, and social ways, but in the end it all breaks down, because one thing humans do very well is to share their DNA," Rotimi says.

Reflecting on his work, Rotimi says he is absolutely surprised that he now finds himself with a distinguished scientific career, in the largest biomedical research institution in the world. "I came from a very humble background, and I had no clue as to how life would play out for me when I first arrived to study in the US," he says. "I've come a long way, and I think it speaks to hard work, having key people in your life who can help shape your opportunities and provide support—and then a little bit of luck here and there." ■

# Rotem Sorek: Bacterial Immunologist

Associate Professor of Molecular Genetics, Weizmann Institute of Science, Age: 43

BY SUKANYA CHARUCHANDRA

Rotem Sorek got his PhD studying human genetics, but he soon learned his true affinity was for microbes. Sorek started his doctoral research around the time that the human genome was being decoded, and he found the field exciting. But as he was finishing his dissertation, scientists were just beginning to crack the genetic code of numerous microbial genomes. The sheer number of microbes with genomes still to be explored proved more enticing to Sorek than the singular human genome.

"There's just so much to discover there, it's like a treasure trove of genomic information," says Sorek, now a geneticist at the Weizmann Institute of Science in Rehovot, Israel.

Sorek completed his undergraduate, master's, and PhD degrees at Tel Aviv University, and in 2006 moved institutions and countries to do his postdoc with geneticist Edward Rubin at the Lawrence Berkeley National Laboratory in California. Rubin was then the director of the US Department of Energy's Joint Genome Institute, with which he is still affiliated, and had led the organization's involvement in the Human Genome Project. Genome sequencing technology was somewhat rudimentary then, in addition to being both expensive and time-consuming. But Rubin's lab was a hot spot of sequencing, and that proved a strong motivation for Sorek, who was excited about using his bioinformatics skills to analyze the newly deciphered microbial genomes and identify the functions of previously unknown bacterial genes. "Sorek is a rare combination—somebody that is able to look at big datasets with biological insights," Rubin says.

After working in Rubin's lab for two and a half years, Sorek set up his own lab back at the Weizmann Institute to study CRISPR, and then became interested in studying phage biology and bacterial immune systems more broadly. In 2010, Sorek began collaborating with bacteriologist Pascale Cossart of the Pasteur Institute of Paris, and in 2016, they published a paper describing a technique to analyze bacterial transcriptomes and identify RNAs that regulate microbial gene expression. Sorek and Cossart used the platform to find RNAs that respond to antibiotics by ramping up the expression of genes responsible for resistance and helping bacteria avoid death.[1]

Sorek "is among the young microbiologists who have introduced new concepts in bacterial gene regulation," Cossart writes in an email to *The Scientist*. "His competence is internationally recognized."

In a separate line of research, Sorek and his colleagues have been examining phages that attack bacteria. Certain phages are known to switch between two modes—dormancy or all-out attack—while inside a cell. One group of phages uses a six amino acid–long peptide to communicate with the next generation of virus particles: depending on the abundance of these molecules, the progeny determine what mode to assume.[2]

"Each [type of] phage speaks in one language and can only listen to its own language," Sorek says. "They don't hear each other." His team is now trying to understand the "evolutionary principles that guide this behavior" to prevent cross-talk between different phage types.

Sorek and his labmates have also developed a program to scan many thousands of bacteria, and the researchers have used it to identify 10 previously unknown immune systems that bacteria employ against phages.[3]

Understanding the molecular underpinnings of these newly identified bacterial immune systems might lead to new tools for genetic and genomic engineering, Sorek says. Adding tools to the gene-editing toolkit could help researchers tease apart the genetic codes of organisms in new and varied ways. ■

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DAVID VAAKNIN

# A Multi-omics View of Single Cells

New techniques integrate information from individual cells' DNA, RNA, and proteins.

BY SANDEEP RAVINDRAN

A defining shift in molecular biology over the past decade has been the application of whole genome and whole transcriptome sequencing methods to single cells. With advances in cell isolation and next generation sequencing, researchers no longer need to average out the signal from multiple cells in a population, but can instead study the DNA, RNA, proteins, and chromatin cell by cell.

Single-cell genomics, epigenomics, transcriptomics, and proteomics studies have revealed just how much variation there is in gene and protein expression even between genetically identical cells in the same tissue. But most such studies examine only a single layer of information from each cell, which may give a skewed picture, says Pier Federico Gherardini, a biologist at the Parker Institute for Cancer Immunotherapy in San Francisco. "You cannot just measure RNA and assume that things will look the same with proteins."

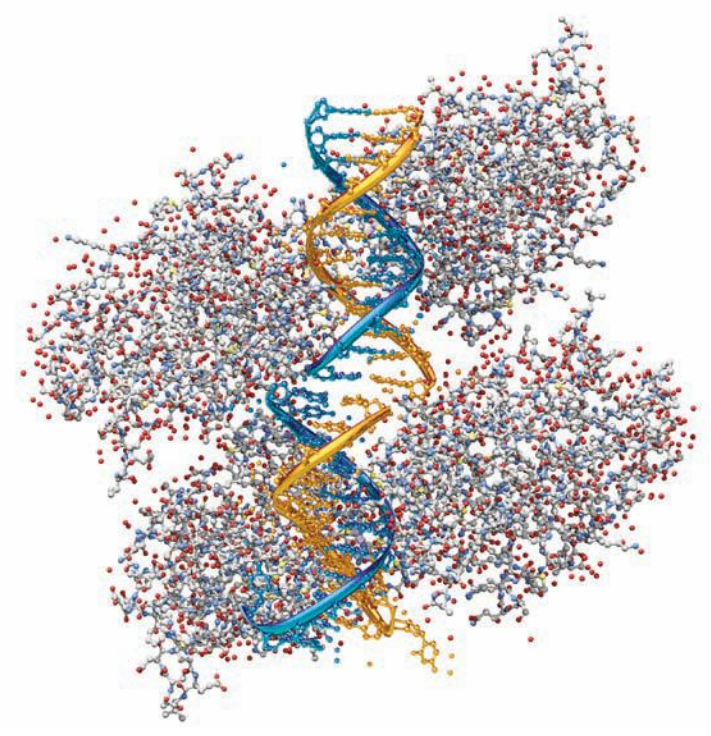
Researchers have started to combine multiple layers of information at single-cell resolution. These "multi-omics" techniques can provide a closer look at the variability between cells and more clearly identify specific cells and their functions. Analyzing genomic DNA reveals the single-cell genome, methylome, or chromatin, while analyzing RNA and proteins yields transcriptome and proteome data, respectively.

"Multi-omics is much more powerful than a single layer alone," says Lia Chappell, a molecular biologist at the Wellcome Sanger Institute in the UK. "You begin to untangle what all that heterogeneity really means and are able to dig deeper into biological mechanism."

Single-cell multi-omics is particularly useful for examining cells undergoing rapid changes, such as activated immune cells, or cells in very heterogeneous tissues, including tumors, says Christoph Bock, a genome researcher at the CeMM Research Center for Molecular Medicine in Vienna, Austria.

The approach can also identify rare but biologically important cells that are masked in a large population. "A classic example would be those few drug-resistant cells that were already there, but [that] you cannot see with bulk techniques because they're drowned out a thousandfold to one by more abundant cells," says Chappell.

Single-cell multi-omics is no cakewalk. There are no commercial kits available yet for any single-cell multi-omics techniques, and many are technically challenging. Researchers must modify existing single-cell protocols so that they're compatible with multiple types of molecules and take great care to minimize the loss or contamination of samples. Yet the extra effort is worth it, experts say.



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*The Scientist* asked researchers developing single-cell multi-omics techniques to guide us through the available options.

## GENOME AND TRANSCRIPTOME

Simultaneously sequencing both DNA and RNA from the same cell can reveal how genomic variation between single cells might explain variations in their transcript levels. Doing so can also detect DNA mutations with greater accuracy.

In **DR-seq** (DNA-mRNA sequencing), single cells are lysed and the DNA and RNA in the lysate are simultaneously amplified. The lysate is then split into two halves, one for RNA sequencing (RNA-seq) and the other for genome sequencing. Keeping DNA and RNA together during amplification minimizes the loss of nucleic acids, but could lead to potential cross-contamination.

**G&T-seq** (genome and transcriptome sequencing) physically separates mRNA and DNA from a fully lysed cell using magnetic beads coated with a short oligonucleotide sequence that binds mRNA. DNA and mRNA are then amplified and sequenced separately. Keeping mRNA and DNA separate allows researchers to use their protocol of choice for analyzing each, but could poten-

tially lead to the loss of nucleic acids. G&T-seq has been automated and is relatively high throughput.

## EPIGENOME AND TRANSCRIPTOME

Techniques that assay a cell's epigenome and transcriptome can reveal how methylation and chromatin accessibility regulate gene expression. "During complex biological processes such as tumorigenesis, heterogeneity will exist in genome, epigenome, and transcriptome simultaneously, and profiling them separately may not work," says Fuchou Tang, a molecular biologist at Peking University. Genetically identical tumor cells may have different DNA methylation or gene expression patterns, and multi-omics techniques may be needed to unambiguously classify them into subpopulations, he adds.

**scM&T-seq** (simultaneous single-cell methylome and transcriptome sequencing) is based on G&T-seq, and uses the same procedures to isolate DNA and RNA from a single cell and to amplify and sequence RNA. Genomic DNA is subjected to bisulfite treatment to convert unmethylated cytosines to uracils, and is then amplified and sequenced to assay the methylome.

**scNMT-seq** (single-cell nucleosome, methylation, and transcription sequencing) builds on scM&T-seq, but single cells are isolated and treated to also probe genome-wide chromatin accessibility. How accessible or protected different genomic locations are can affect gene expression, and researchers using scNMT-seq have uncovered new associations between the epigenome and transcriptome in mouse embryonic stem cells.

> During complex biological processes such as tumorigenesis, heterogeneity will exist in genome, epigenome, and transcriptome simultaneously and profiling them separately may not work.
>
> —Fuchou Tang, Peking University

In **scMT-seq** (another method of simultaneously sequencing single cells' methylomes and transcriptomes) and **scTrio-seq** (single-cell triple omics sequencing), the cell membrane is selectively lysed to separate mRNA in the cytosol from genomic DNA in the intact nucleus. In scMT-seq, the cell nucleus is collected using a micropipette, whereas in scTrio-seq it is separated by centrifugation. In both cases, genomic DNA is subjected to a modified bisulfite treatment and sequencing method to reveal the methylome, while mRNA from the cell lysate is amplified and sequenced in parallel. "Essentially there is no cross-contamination between the genome and transcriptome data," says Tang, who helped develop scTrio-seq. scTrio-seq uses the methylome sequence data to computationally assess genomic copy number variation, and the technique has already been used to analyze heterogeneity in human colorectal cancer samples.

## PROTEOME AND TRANSCRIPTOME

Several techniques can simultaneously assay transcripts and proteins from a single cell. These approaches offer scientists a look at post-transcriptional processes that can result in differences between protein and transcript levels.

In **PLAYR** (proximity ligation assay for RNA), proteins are labeled with antibodies conjugated to distinct metal isotopes. At the same time, RNA transcripts are bound by isotope-labeled probes. A mass-spectrometry-based method known as mass cytometry is used to measure the isotopes and can simultaneously quantify more than 40 different mRNAs and proteins in thousands of individual cells per second.

**CITE-seq** (cellular indexing of transcriptomes and epitopes by sequencing) uses oligonucleotide-tagged antibodies to target cell-surface proteins. Single cells are isolated and lysed, and their mRNA and oligo-tagged antibodies are bound to magnetic beads coated with short oligonucleotide sequences. The RNA and antibody tags are amplified and separated by size, and proteins and transcripts are quantified using sequencing. CITE-seq can simultaneously quantify about 100 proteins along with tens of thousands of RNA transcripts, says Marlon Stoeckius, a molecular biologist at the New York Genome Center who helped develop the technique. Stoeckius is working on extending the method to intracellular proteins, although that will require fixing and permeabilizing the cells, which may degrade RNA quality or cause it to leak out of cells.

**REAP-seq** (RNA expression and protein sequencing assay) is similar to CITE-seq, and also uses oligonucleotide-conjugated antibodies to measure both cellular protein and transcript levels using a sequencing-based readout. Both REAP-seq and CITE-seq can assay a larger number of transcripts, but on fewer cells at a time, compared to PLAYR. "I think they're complementary approaches," says Gherardini, a lead developer of PLAYR. "If you have a big cohort or a clinical study, something like PLAYR is much, much more cost effective," he says.

One advantage of CITE-seq is that the protein quantification is performed on a fraction that is normally discarded in single-cell RNA sequencing prep, so "there is no detriment to the quality of the RNA sequencing library," says Peter Smibert, manager of the Technology Innovation Lab at the New York Genome Center. "We think that any sort of situation where people are using RNA-seq as a readout, we see no reason not to use CITE-seq instead."

## MULTIPLE CHOICES OF MULTI-OMICS

With so many assays to choose from, researchers will have to decide which ones to use based on the biological question they're asking, as well as on how expensive, labor-intensive, and technically demanding a given technique is. "It typically takes a team of technologists, a computational person, and a biologist who knows the experimental system to do these types of projects well," says Bock.

## AN OVERVIEW OF SELECTED SINGLE-CELL MULTI-OMICS TECHNOLOGIES

| Method | Measurement | Isolation technique | Potential loss of nucleic acids | Cell Throughput | Automation | Website/Paper |
|---|---|---|---|---|---|---|
| **DR-seq** | Genome, Transcriptome | Mouth pipette | Low risk of losing DNA or RNA | Low | No | www.nature.com/articles/nbt.3129 |
| **G&T-seq** | Genome, Transcriptome | Flow cytometry | Some risk of mRNA and DNA loss | Medium | Yes | www.nature.com/articles/nmeth.3370; www.nature.com/articles/nprot.2016.138 |
| **scM&T-seq** | DNA methylation, Transcriptome | Flow cytometry | Some risk of mRNA and DNA loss | Medium | Yes | www.nature.com/articles/nmeth.3728 |
| **scMT-seq** | DNA methylation, Transcriptome | Microcapillary pipette | Loss of some cytoplasmic and all nuclear mRNA molecules | Low | Partial | genomebiology.biomedcentral.com/articles/10.1186/s13059-016-0950-z |
| **scTrio-seq** | DNA methylation, Copy number variation, Transcriptome | Mouth pipette | Loss of nearly half of cytoplasmic and all nuclear mRNA molecules | Low | No | www.nature.com/articles/cr201623 |
| **scNMT-seq** | Chromatin accessibility, DNA methylation, Transcriptome | Flow cytometry | Some risk of mRNA and DNA loss | Medium | Partial | www.nature.com/articles/s41467-018-03149-4 |
| **CITE-seq** | Transcriptome and Proteome | Drop-seq and 10x Genomics Chromium | Low | High | No | citeseq.com; www.nature.com/articles/nmeth.4380; satijalab.org/seurat/ |
| **REAP-seq** | Transcriptome and Proteome | Flow cytometry and 10x Genomics Chromium | Low | High | No | https://www.nature.com/articles/nbt.3973 |
| **PLAYR** | Transcriptome and Proteome | Mass cytometry | Low | Very High | No | www.nature.com/articles/nmeth.3742 |

Protocols vary in how they dissociate tissues into single cells. Mouth pipetting and serial dilution are relatively quick, easy, and low-cost methods that can minimize the risk of RNA or protein degradation, but they're also relatively low throughput. FACS, robotic manipulation, and microfluidics are high throughput, but they're expensive and can be rougher on cells.

Cost is another factor. The techniques outlined above can range anywhere from a few dollars to a few hundred dollars per sample based on the protocol and the volume of reagents—such as enzymes and antibodies—needed. That doesn't include the costs of sequencing, which can be limiting as well. "Not many scientists will be able to afford to sequence 100,000 single genomes from single cells," says Chappell.

**The ultimate goal is to capture information from all molecules in a single cell—a sort of "omni-omics"—but that's still several years away.**

Single-cell multi-omics assays can take anywhere from a little more than 24 hours to nearly a week for one batch of samples. Protocols that involve manually separating the nucleus from the cytoplasm tend to be slower and more labor-intensive, whereas FACS-based methods are more convenient and higher throughput.

Bioinformatics expertise is a plus. "For all these types of new assays, you need a little bit of a computational background and little bit of experience with R," a programming language and software environment for statistical computing and data analysis, says Stoeckius. That may soon change, as more people start to use such assays and companies develop easy-to-use software for analyzing single-cell multi-omics data. For now, computational packages such as SEURAT and MOFA have been built to integrate data from two or more omics layers for a single cell.

As single-cell single-omics techniques become more sensitive, accurate, and high throughput, the corresponding multi-omics techniques are likely to improve as well. Researchers are also working on integrating single-cell techniques with additional layers of data, such as spatial information (such as CODEX) and functional assays. Linking a cell's spatial information to other omics layers could help researchers map different cell types and functions within a tissue.

The ultimate goal is to capture information from all molecules in a single cell—a sort of "omni-omics"—but that's still several years away. For now, single-cell multi-omics may be well on its way to changing how scientists approach molecular biology. "The take-up [of these techniques] has been impressively quick, so I think in five years' time this will be seen as obvious and the standard thing to do," says Chappell. ■

# Data Rush

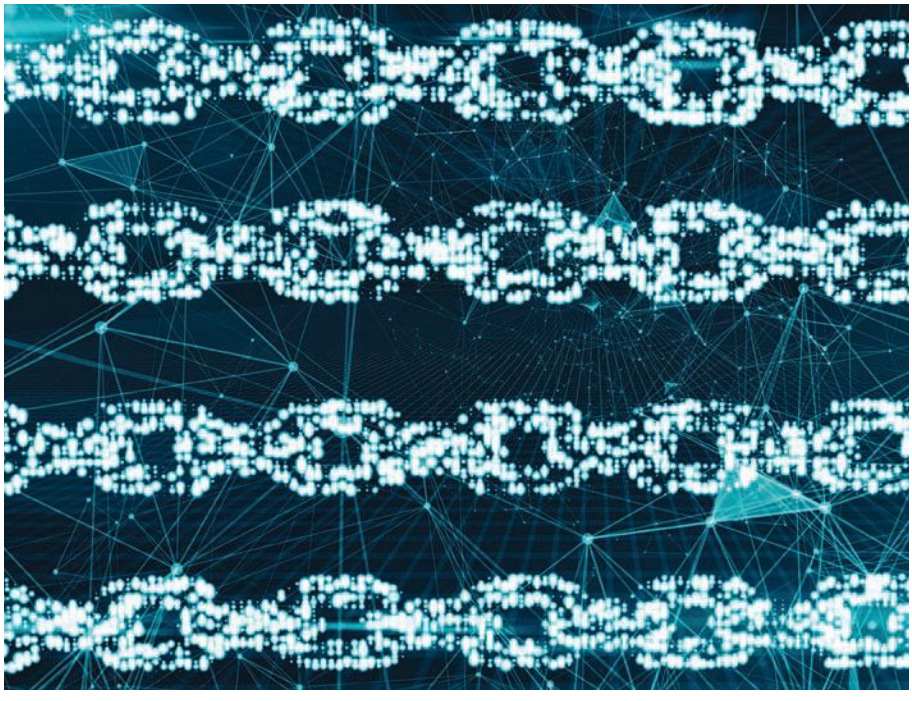
Startups aim to use blockchain technology to let individuals control and directly profit from their genomic and medical information.

BY SHAWNA WILLIAMS

Even if you've never heard of IQVIA, the company most likely knows some things about you—if only in anonymized form. As Harvard University fellow Adam Tanner documents in his 2017 book *Our Bodies, Our Data*, IQVIA (formerly IMS Health), along with other companies such as IBM and LexisNexus, regularly pay health care providers, pharmacies, clinical labs, and insurers for patients' de-identified health records, prescribing data, insurance claims, and other information. Then they collate and sell this information to pharmaceutical companies and others who rely on such data for research and marketing. And business is good: IQVIA reported revenue of $2.5 billion in the second quarter of this year.

Until now, the proceeds from the booming health data–brokering industry have seldom been seen by the people who contribute the information in the first place. But that could be about to change, thanks to a new class of startups promising to provide platforms for users to take control over the sharing and interpretation of their genetic and other medical information. These newer companies—sometimes referred to as biobrokers—are championing a business model based on delivering incentives (often monetary) to individuals who agree to share their data, combined with heightened data privacy thanks to the incorporation of blockchain, a security technology developed for cryptocurrency exchange.

A handful of such biobrokers have launched over the past few years, with an eye on the lucrative medical-data market, the growing popularity of consumer genomics—an industry that Research and Markets forecasts will be worth more than $900 million by 2023—and a surge in investor interest in applications of blockchain technology. The most established biobrokers are now beginning to launch limited versions of their platforms, with the goal of building up to trading in genomic sequences and detailed medical and behavioral information—as dictated by their users.



Patient control of data "is an inevitability in my view," says Eric Topol, a physician and precision medicine researcher at the Scripps Research Institute whose Twitter manifesto on individual ownership of health data has been widely shared. While it's not yet clear what technological form this empowerment will take, he says, such ownership "is where it's headed—it's just a matter of when."

## Changing the model

First deployed a decade ago as a way of securely recording transactions in bitcoin and other cryptocurrencies, blockchain technology is now used in traditional banking, contract agreements, and elsewhere. One of the technology's distinctive features is its distributed storage of information, bypassing the need for a central hub or server and making records extremely difficult to modify or hack.

The technology is fundamental to the business model of many biobrokers, providing both an essentially fraud-proof record of each time personal data are accessed and a secure way to pay customers in return for sharing their information. "You can think about this as a database and an ecosystem where the data is controlled by the individuals, and through the power of different technologies, these individuals are able to be rewarded," says Dawn Barry, the president and cofounder of California-based Luna DNA, a com-

pany that launched a year ago with the aim of creating such a database. To further increase security, Luna and similar startups would require academics and pharmaceutical companies to ask their research questions and run the numbers directly on proprietary platforms, she says, eliminating the need to download raw data locally. Individuals could opt to share their data for specific research projects and would be compensated in cryptocurrency.

This is in stark contrast to existing options for customers interested in the scientific potential of their medical data. At 23andMe, the granddaddy of direct-to-consumer genetic testing, the Health + Ancestry package, which combs hundreds of thousands of SNPs for reportable information on everything from ancestry to Alzheimer's risk, is on offer for $199. Users can opt to allow their information to be used in research, which enables 23andMe to sell access to the data in aggregated form—the proceeds from which customers never see. Ancestry.com and MyHeritage—both of which have suffered security breaches in recent years—operate similar programs.

The model has failed to encourage as many people to get involved as researchers might like. "For a while I thought that $1,000 would be a low enough price [for genome sequencing] that everybody would flock to it," says George Church, a geneticist at Harvard University and MIT and a cofounder of blockchain company Nebula Genomics. "But as we get closer to that I realize that we really need to be paying them, rather than them paying us, in order to properly motivate, say, a billion people" to get sequenced.

Biobrokers expect that the monetary incentives that pharmaceutical companies, academic researchers, and others offer via their platforms would give more people a reason to get their genome sequenced and make them available. The data could be paired with information on phenotypes, lifestyle, and health outcomes. On some platforms, users could earn additional compensation for completing questionnaires or uploading data from fitness trackers. And pharmaceutical companies looking to recruit trial participants with a particular gene variant could easily identify them.

That type of customer engagement, as Luna's Barry sees it, is central to moving the science along. "We have not been able to do the research we've wanted to do because we've lacked the scope and scale of data . . . and we haven't had people engaged," she tells *The Scientist*. "The only way to fix that problem . . . is to engage people as the owners, and ultimately the sources, of that data."

## Data empowerment

It's not just about money, though. Indeed, Barry says she doesn't envision the platforms yielding huge monetary payouts for individuals. "I personally don't like a lot of the narratives around 'make money from your DNA.'" She expects that while payments are needed to ensure fairness and fully engage people, users will be motivated mainly by the opportunity to gain and contribute to new insights into human health.

Biobrokers may provide those insights to customers in a variety of ways. Users of Luna's platform "will have their data on file and use it as a resource throughout their life, from planning a baby to making sure the baby has a healthy start to choosing medications that are best suited for their biology [to] understanding disease predisposition," Barry says. In many cases, the platforms would also seek to help users make sense of their data. At Germany-based Shivom, which promises to help users collect monetary rewards for sharing data with researchers or participating in studies, the plan is to devise apps that will run on the platform and help what the company calls "genome donors" find personalized diet and exercise recommendations, and access health care providers via telemedicine.

In Church's view, one industry that stands to benefit from customers' access to these insights is the health insurance sector—even if companies never see individual data. Having their genomic information and sharing it with their doctors will help people make better health care decisions, he says. The cost savings could be dramatic if, for example, more people knew before conceiving a child whether they were carriers for a genetic disease. "For the carrier component alone, it's probably worth fifty thousand dollars per person—that's averaging the million-dollar consequences of the five percent of births [of babies with severe genetic diseases] over a large number of people."

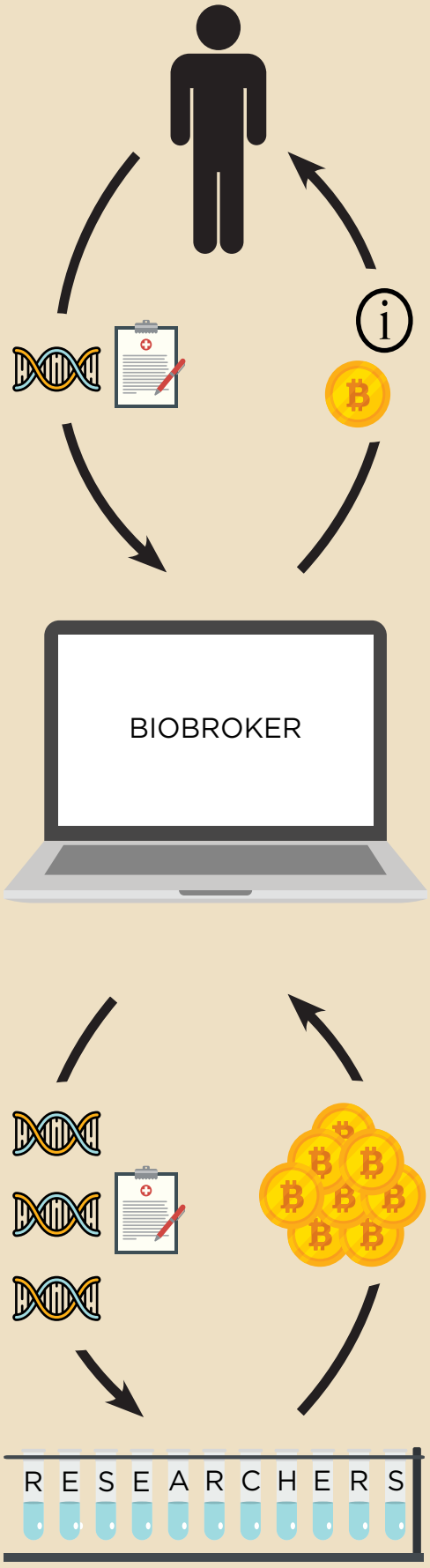
Many fledgling biobrokers are also developing analytical tools to eke more scientific value from patient data. Alongside blockchain, another enabling technology of many of the platforms is artificial intelligence (AI). "We're hoping with the advanced AI, advanced analytics, we're actually going to be able to discover novel insights," such as new biomarkers for disease, says Natalie Pankova, Shivom's chief operating officer.

> We really need to be paying them, rather than them paying us.
>
> —George Church
> Harvard University & MIT

Advancing AI was one of the inspirations for founding Longenesis, a partner of Nebula and a company that aims not to launch its own platform but to provide the technologies other firms need to do so, says Chief Science Officer Alex Zhavoronkov, who also serves as CEO of the AI-based company Insilico Medicine. "We realized that there is a lot of value embedded" in datasets used to train AI programs to see patterns in genomic and health data—value that should belong to individuals rather than to hospitals or pharmaceutical companies, he says. Zhavoronkov says AI will also be critical to ensuring data authenticity—for example, by spotting suspicious patterns that might indicate information on the platform doesn't belong to the person who entered it. "That is a major challenge, how to make people remain anonymous and at the same time trust them."

## The Wild West

Any company offering insights into individuals' genomes faces potential ethical quandaries about the information it




**DOLLARS FOR DATA:** Biobrokers would enable academic and commercial researchers to run studies on a secure platform using DNA and health data from subjects who have expressly provided consent. In return, individuals would receive genetic sequencing results and other health information, and potentially money in the form of cryptocurrency.

provides, and those quandaries arguably become more complicated when money's involved. For example, the prospect of a health insurance company sponsoring people to get their genomes sequenced with the expectation that they'll avoid having a child with a genetic disease raises the specter of eugenics. Church says it's important that people are educated in advance about what they might learn from genetic testing, and opt in or out of receiving certain kinds of information.

That said, the idea of selling access to our most personal information is not such a departure in an era in which we already implicitly "monetize our privacy in many ways"—for example, by effectively exchanging our browsing and search behaviors for access to "free" websites, notes Alta Charo, a bioethicist at the University of Wisconsin Law School in Madison. In contrast to such largely hidden exchanges, emerging blockchain-based platforms could provide people "potentially more opportunities to have very specific control over what's given out and in what specific form."

Indeed, biobrokering could represent not a potential ethical problem, but a solution, argues David Koepsell, cofounder and CEO of Florida-based biobroker EncrypGen. "The danger of people wanting to sell their data, and being incentivized by that, seems to me to be less of a problem than what's going on now, where they're giving it away without really realizing it, and somebody else is profiting by it," he says. A philosopher and lawyer, Koepsell became interested a few years ago in issues of privacy and ownership in genomics, and concluded that blockchain could help provide a mechanism for individual control of personal information.

By itself, however, technology can't deliver full individual ownership of medical data—not without legal backing. "There's no unified mechanism or agreement on who gets to own the data and how they get to profit by it," Koepsell notes. Even if blockchain-based platforms can safeguard the privacy of information users upload, in most countries there's nothing to stop entities that somehow get hold of a person's data outside the platforms from selling it. Potentially, they could even use one of the blockchain platforms to do so. "Without having complete control of the chain of custody of the sample, there's really very little you can do."

Koepsell suggests regulatory oversight will be needed to guard against such scenarios. But governing the genomic marketplace of the future could pose a challenge, Charo says, because blockchain's decentralized nature means it's "almost immunized against regulation." A slew of blockchain-based startups are already eschewing initial public offerings—stock market launches that require disclosure of certain financial and business information—in favor of initial coin offerings (ICOs), which are conducted in cryptocurrency and require no such disclosures.

According to ICO listing site CoinSchedule, offerings have raised the equivalent of more than $18 billion so far this year across all sectors combined. That influx of coin has led to a "Wild West" situation for personal data trading, with many would-be genomic data–sharing platforms looking to get in on the action, says Zhavoronkov. But given the head start more-established biobrokers have—EncrypGen says it has about 1,000 users building profiles on its site so far, for example, while Luna is planning to roll out a bare-bones iteration of its platform this year—newcomers starting from scratch with an ICO don't stand much of a chance, he says. "They don't have a platform, they don't have a system, they did not spend a few years piloting and testing, they are just fundraising and investing in PR without having done the work."

The biobroker sector has seen "a lot of hype and overstatement," agrees Koepsell. "People need to be aware of the difference between the claims and the reality in blockchain in general, but also in the genomic blockchain efforts that are going on." ■

*THE SCIENTIST STAFF*

# In Defense of Forgetting

Science tells us that a crucial component of memory is the ability to discard less-important information.

BY HILDE ØSTBY AND YLVA ØSTBY

There once lived a man who claimed he could remember every day of his life since he was nine years and four months old. What day of the week had it been? What had the weather been like? In interviews, nearly blind farmer Daniel McCartney could answer these questions in great detail, and with almost perfect consistency when asked again weeks later. "February 28, 1831?" McCartney would pause, reflect for a few seconds, then answer: "Monday. It was very muddy. We carried sap from the sugar-trees, and two girls came to our house for a visit that evening."

Imagine being able to recall every moment you've ever experienced—every phone number, birthday, fact, or name you'd ever heard—without reminders, calendars, apps, or lists. Some may argue that McCartney was gifted with an exceptional memory, a modern-day superpower most of us can only dream of. But in celebrating this one incredible skill, are we neglecting another?

Both inside and outside the research lab, humans tend to prize remembering so much that we sometimes ignore its counterpart, the equally essential art of forgetting. While writing our book, *Adventures in Memory*, we explored both sides of the memory coin, uncovering fascinating insights about the science of how and why we fail to remember.

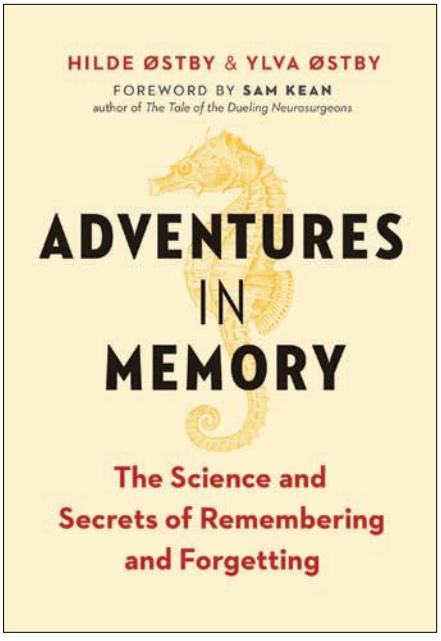
We learned, for example, that a great deal of forgetting occurs shortly after an experience takes place, through processes in our attention and working memory apparatus that select which memories to keep and which to discard. This natural filtering system allows us to focus on the moments that matter most: those with high emotional import and personal significance. But forgetting is important in the long run too.

New research investigating the active cellular processes that contribute to long-term forgetting suggests that neurogenesis—the production of new neurons within the hippocampus—may actually aid the capacity to form new memories by destroying existing memory traces. In memory, as in life, we need to clear out some of the old in order to make space for the new.

Yet, even when we've forgotten most of an experience, our recollections can seem convincingly complete. How can this be? A memory, it turns out, is more than the sum of its traces. Using existing knowledge, our brains fill in for the specifics we've forgotten, recreating the scene with people, surroundings, actions, and emotions. This way, we don't actually need all the details to "remember" a moment—just a vague sense of what took place. The same is true for cumulative memories, recollections of events that have been repeated many times.

Can you recall what it was like to take the bus to work on July 12th? To cuddle your child at bedtime last Wednesday? Rather than remembering each of these instances in detail, our brains construct a compilation of them all—forgetting most of what we've experienced in favor of a kind of "average" memory.

So really, all remembering incorporates, perhaps even requires, an element of forgetting—and thankfully so. What would life be like if you could recall each and every moment? If you could remember precisely how you buttered every slice of toast you ate in February, or the exact hue of the socks you wore on your mother's 37th birthday? It would take you an entire day just to remember what you did yesterday!




*Greystone Books, October 2018*

Perhaps we simply expect too much of our memories. By telling stories, sharing knowledge, and relying on other forms of external memory, could we learn to celebrate a little forgetting? Memories fade, but, thanks to our amazing forgetfulness, the important ones—the ones that resonate, that make us who we are—remain. ■

*Hilde Østby is a writer and editor with a master's degree in the history of ideas and Ylva Østby is a clinical neuropsychologist who researches memory at the University of Oslo. Read an excerpt of* Adventures in Memory: The Science and Secrets of Remembering and Forgetting *at the-scientist.com.*

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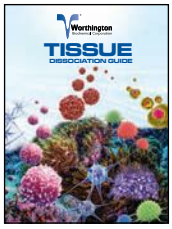


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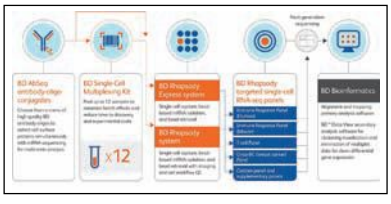


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# INNOVATION *AND* APPLICATION

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**SHORT COURSES: FEBRUARY 2-3**

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## KEYNOTE SPEAKERS:

**Teresa K. Woodruff, Ph.D.**
*The Graduate School at Northwestern University*

**Eran Segal, Ph.D.**
*Weizmann Institute of Science*

**POSTER ABSTRACT SUBMISSIONS FOR STUDENT POSTER AWARD DEADLINE: OCTOBER 29**
**MEMBER REGISTRATION DISCOUNT DEADLINE: OCTOBER 31**
**EARLY BIRD REGISTRATION DISCOUNT DEADLINE: DECEMBER 17**
**FINAL POSTER ABSTRACT SUBMISSION DEADLINE: JANUARY 21**

EVENT DETAILS AT **SLAS2019.ORG**

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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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# Charting Crescents, 1910

BY SUKANYA CHARUCHANDRA

On the day after Christmas, 1904, Walter Clement Noel staggered into Presbyterian Hospital in Chicago, suffering from respiratory distress. An intern, Ernest Irons, carried out routine tests on Noel's blood, stool, and urine. The patient's blood contained "many pear-shaped and elongated forms," Irons noted. Over the next two and a half years, Irons and his supervisor, James Herrick, attended to Noel, an international student from the West Indies island of Grenada, for symptoms ranging from fever to arthritis to anemia. In 1910, Herrick published the first paper reporting on Noel's condition, a case of what would come to be known as sickle cell anemia.

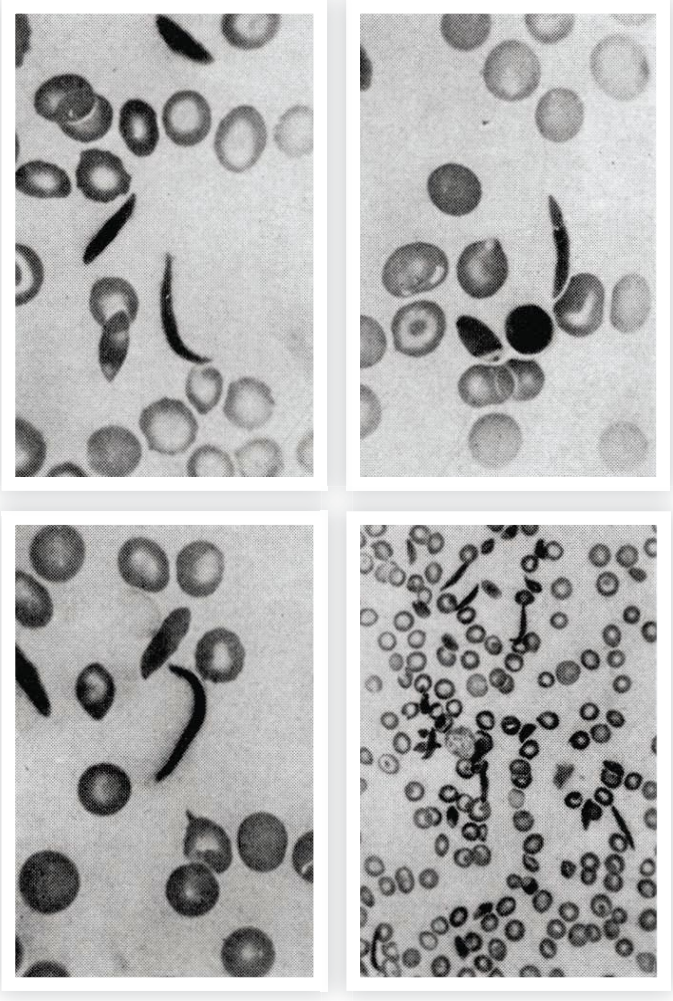
By the early 1900s, researchers had been looking through microscope lenses at microbes and other small structures for centuries. Yet the condition that chemist Linus Pauling called the "first molecular disease" went unmentioned and unidentified for years. "Why did it take a foreign patient for us to discover sickle cell?" asks Todd Savitt, who studies African-American medical history at East Carolina University and tracked down Noel's identity. "There were thousands of patients in Chicago who were of African descent who carried the sickle cell gene."

Noel had traveled to United States to study at the renowned Chicago College of Dental Surgery (since absorbed into Loyola University). Herrick described him in his case study as an "intelligent negro of 20." Noel belonged to a planter class family that was fairly wealthy, enabling him to seek care at Presbyterian, a private hospital now called Rush University Medical Center. Savitt notes that Noel's stay in the US also coincided with a time of increased opportunity for black Americans, including improved access to education and medical care.

Herrick wrote in his paper that he saw a "large number of thin, elongated, sickle-shaped and crescent-shaped forms" in Noel's blood. Herrick and Irons considered diagnoses ranging from malaria to syphilis to intestinal parasites, but those ailments didn't adequately explain what they saw under the microscope, or the patient's strange constellation of symptoms. April 1907 was the last time Herrick and Irons saw Noel, who returned home to Grenada later that year.

Three months after Herrick's paper appeared in the *Archives of Internal Medicine* in 1910, another description of sickle cell anemia, this one in an African-American housemaid and cook, Ellen Anthony, appeared in *Virginia Medical Semi-Monthly*. Another case study was published in 1915, and a fourth in 1922, when the disease was finally given a name by Verne Mason, then a resident at Johns Hopkins Hospital. By that time Noel, a practicing dentist, had died from pneumonia, entirely unaware of his sickle cell status.

In 1949, Pauling reported that patients with sickle cell anemia have an abnormal form of hemoglobin, making it the first condition known to stem from a defective protein. Seven years later, Vernon Ingram and his colleagues identified the specific amino acid change responsible.



**SICKLED CELLS:** Images of Walter Noel's blood that appeared in James Herrick's 1910 paper. Herrick wrote that he reported Noel's case "because of the unusual blood findings, no duplicate of which I have ever seen described."

J.B. HERRICK/*YALE JOURNAL OF BIOLOGY AND MEDICINE*

The disease is more common in people with ancestors from Greece, Turkey, Italy, India, the Arabian Peninsula, parts of the Caribbean, South and Central America, and Africa—1 in 12 African-Americans carry a single copy of the gene variant. While one copy of the gene variant is protective against malaria, two copies cause sickle cell disease. Current treatment options range from bone marrow transplants to pain medication, and the disease is a target of some gene therapies now in development (see "CRISPR Inches Toward the Clinic," *The Scientist*, August 2018).

"What Herrick did was certainly groundbreaking," says Savitt. "He started it—he and Irons." ■

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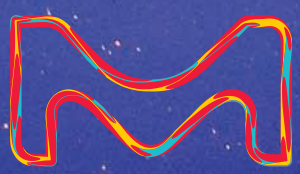



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