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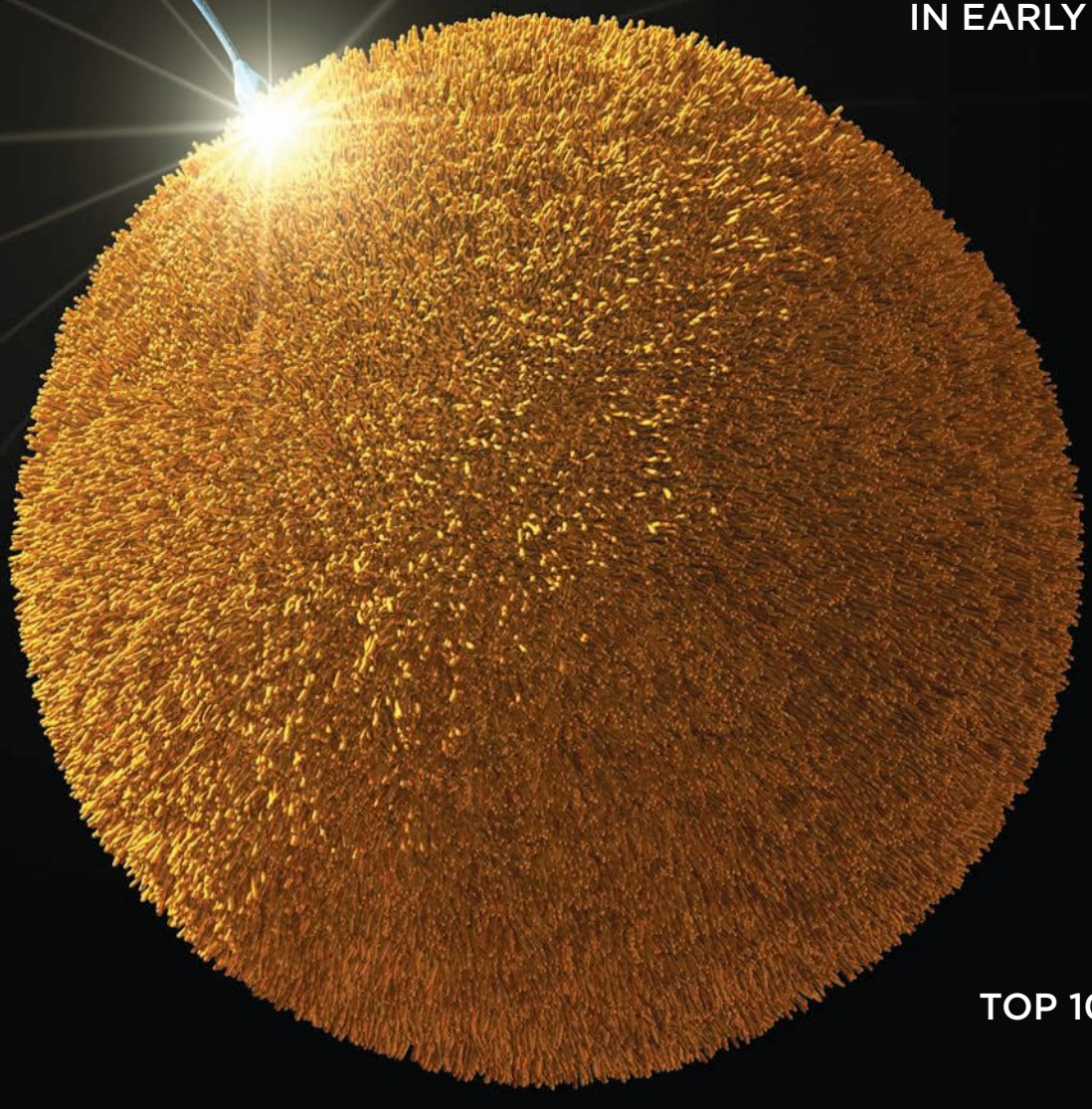

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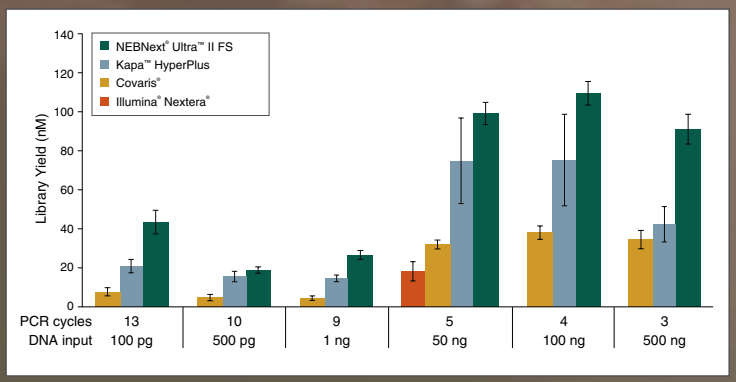

Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. For NEBNext Ultra II FS, a 20-minute fragmentation time was used. For Kapa HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time was used. Illumina recommends 50 ng input for Nextera, and not an input range; therefore, only 50 ng was used in this experiment. "Covaris" libraries were prepared by shearing each input amount in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Error bars indicate standard deviation for an average of 3–6 replicates performed by 2 independent users.

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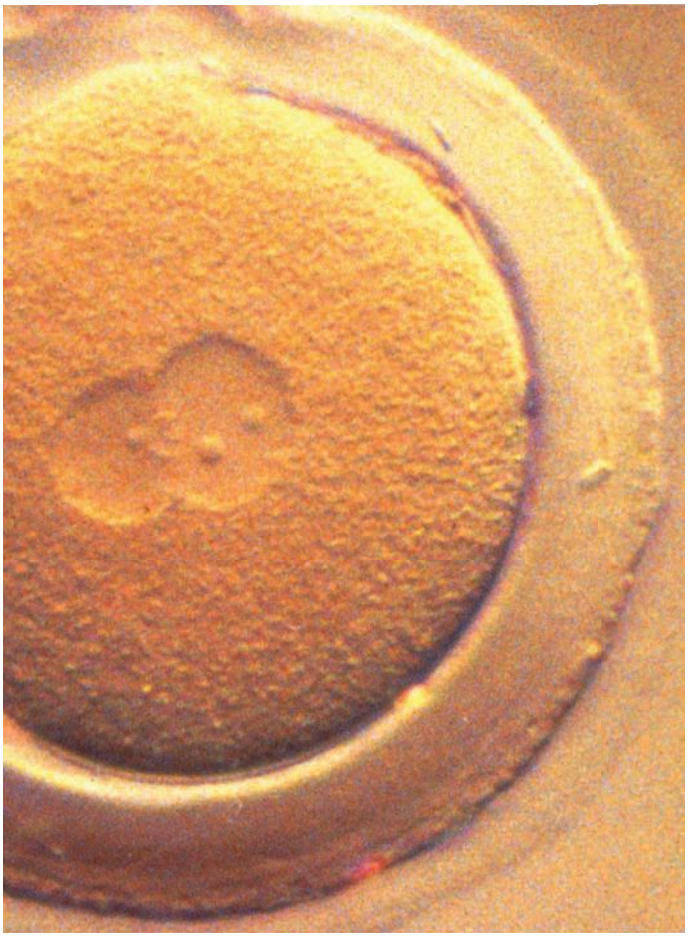
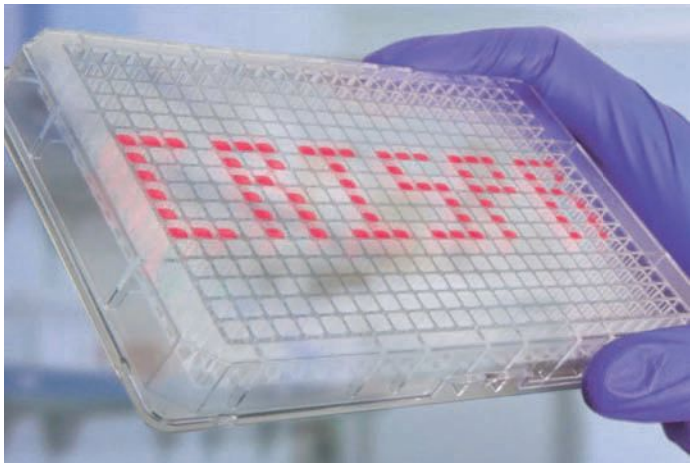




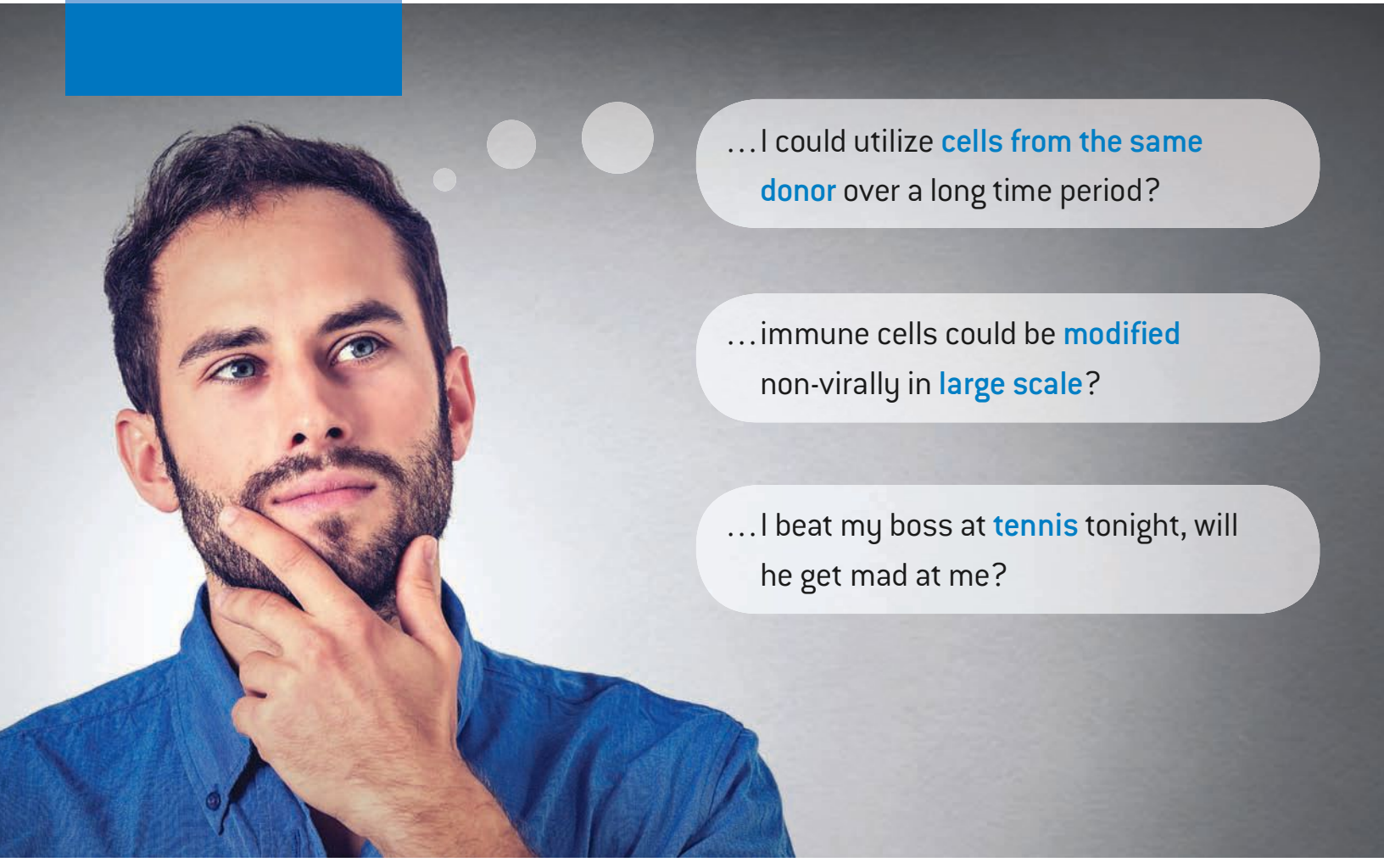
Features

ON THE COVER: © DAVID MACK/SCIENCE SOURCE

BioResearch

Lonza

What if...



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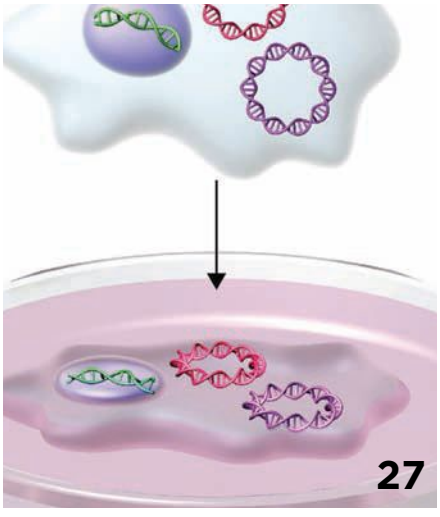

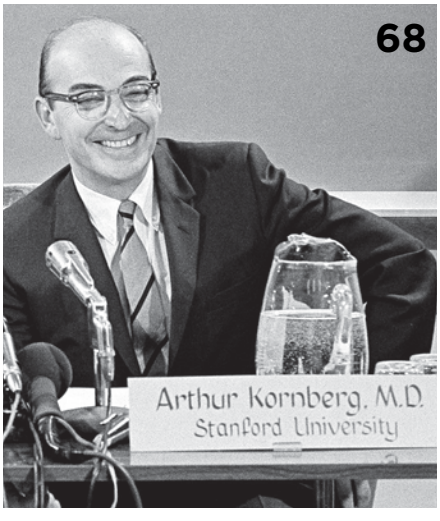

LUIZ ROSA, UFMG; © GEORGE RETSECK; CHUCK PAINTER/STANFORD NEWS SERVICE

CORRECTION:

In "Misconduct Under the Microscope" (*The Scientist*, November 2017), the National Academies of Science, Engineering, and Medicine was misidentified as the National Academies of Science, Engineering, and Math.

The Scientist regrets the error.

PUZZLE ON PAGE 12

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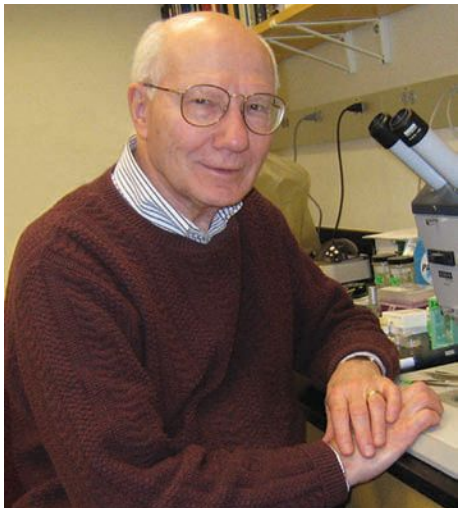


ROY CALDWELL, UNIVERSITY OF CALIFORNIA, BERKELEY; COURTESY OF JOE GALL; EILEEN HEBETS, UNIVERSITY OF NEBRASKA-LINCOLN; EIVIND UNDHEIM

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Kung Fu Shrimp
Watch a mantis shrimp punch its prey into submission using its specialized hammers.

VIDEO
In Situ Hybridization Explained
December profilee Joe Gall of the Carnegie Institution describes the technique, which he developed in the 1960s.

VIDEO
Whipping Boys
See whip spiders use their curious antenniform legs to spar in the lab.

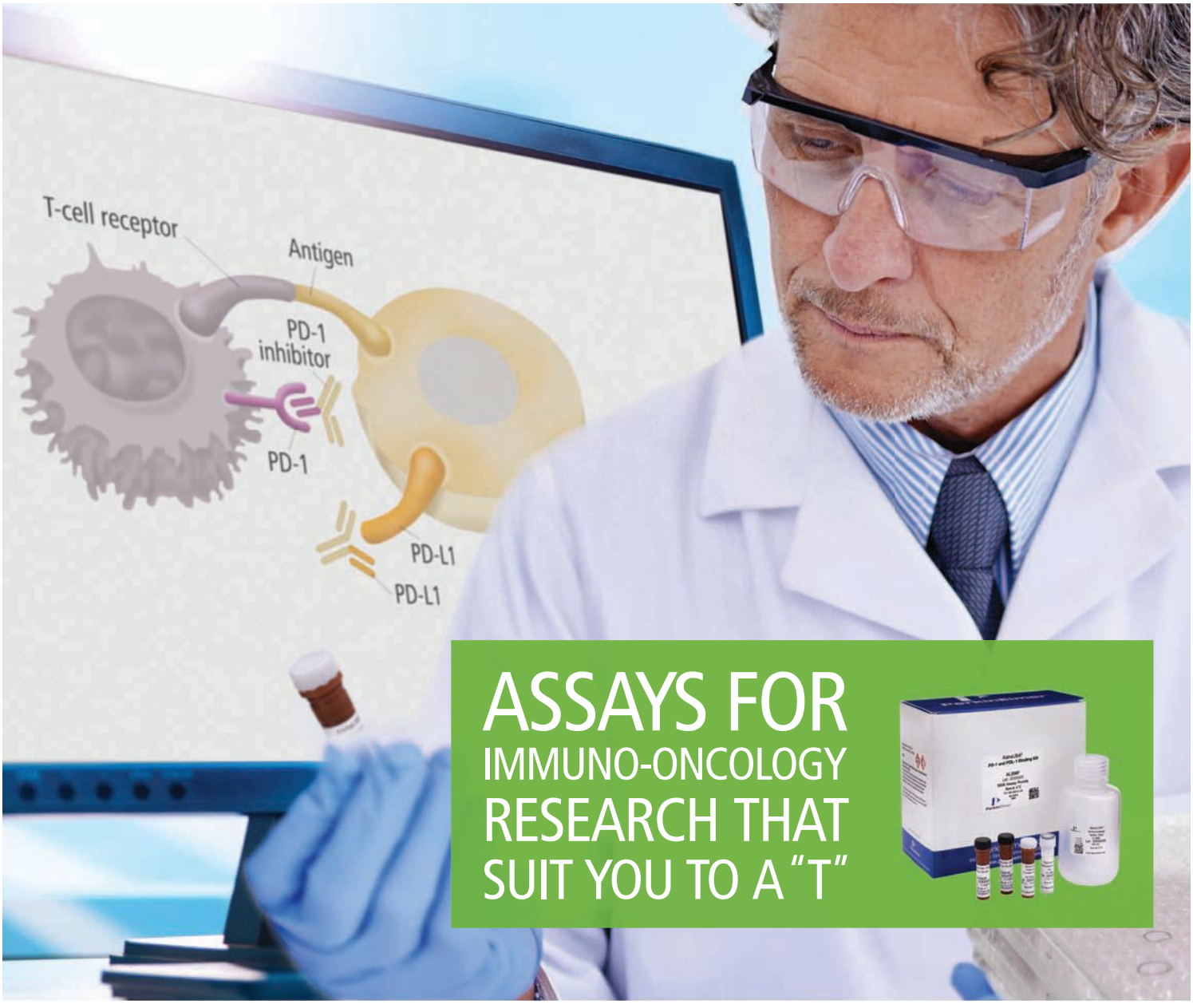
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in January

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

- Glial cell involvement in pain
- Targeting sodium channels for pain relief
- Animal toxin–inspired analgesics
- Bringing safer opioids to market
- Profile: David Julius

AND MUCH MORE



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Contributors

Wendy Jones's interest in neuroscience and psychology grew out of a long-standing love for literature. In what she considers her "first career," Jones earned a PhD in English literature and then taught English literature and writing at Cornell University. During that time, she came across the term "cognitive literary criticism," the practice of applying cognitive psychology to the interpretation of literary work. She was fascinated and started to take courses in neuroscience and psychology. Jones can pinpoint her "great crossing-over" to science to when she dissected a sheep's brain: "I felt like a real scientist," she laughs.

Jones began to publish work exploring the intersection of cognitive science and literature, and taught courses such as "Literature and the Mind-Brain" at Syracuse University and "Cerebral Seductions" at Cornell. Her most ambitious work focuses on her favorite author, Jane Austen. In *Jane on the Brain: Exploring the Science of Social Intelligence with Jane Austen,* Jones takes a deep look at the evolution of social intelligence and the psychology behind the unwavering popularity of Austen's work. "Even though she's been dead for 200 years, she understood us incredibly well," Jones says. Jones guesses that the 18th-century novelist would probably have approved of her new book, describing Austen as an equal enthusiast of science and observation. "I'd really like her feedback, actually!" she says. Read Jones's essay about the vagus nerve's role in social intelligence on page 64.

Amber Dance decided in elementary school that she wanted to be a scientist. It wasn't until she was in grad school at the University of California, San Diego, studying cell biology and microbiology that she started to second-guess her decision. Dance realized she liked talking and thinking about science more than actually doing it, and began to pursue a career in science writing. "Somebody else spends years and sweat and time doing all the hard experiments, and then I get to show up at the end and tell everybody what they found," she says. She ended up completing her PhD, but took a science writing class and began freelancing for the local newspaper while she did so. After Dance graduated, she enrolled in the University of California Santa Cruz science communication program, then headed to Washington, DC, for a summer internship with *Nature*. She followed that up with a part-time job with Alzforum.org, freelancing on the side, and about a year and a half ago made the transition to full-time freelance work. Dance is regular contributor of *The Scientist*'s Lab Tools column, with more than two dozen articles bearing her byline.

The amusing drawings of **Andrzej Krauze** have graced the pages of *The Scientist* since February 2004. With his current contribution of two cartoons per issue (one on the editor's page and one in the Notebook section), that's pushing 200 of them—far short of the more than 10,000 Krauze has contributed (and continues to contribute) to the UK newspaper *The Guardian*. This prolific artist was born in Poland, publishing his first drawing at the age of 19. At the Academy of Fine Arts in Warsaw, Krauze concentrated mainly on graphics; the animated film he submitted as a requirement for graduation was immediately censored, perceived as having an anticommunist slant. For five years, his political cartoons were published in *Kultura,* a weekly Warsaw newspaper. He left Poland with his wife and child in 1979 and settled permanently in London in 1982 after being granted political asylum. "Science is more interesting to me than politics," he says. "It's closer to what is really going on in life and more intellectual." Because English is not his first language, the art must say it all, he adds. To arrive at the final cartoons published in *TS* each month, Krauze lets his imagination soar after getting a brief précis of the subject matter from the editors and the art department. Visit andrzejkrauze.com for a full tour of his incredibly varied and enormous portfolio.

WENDY JONES; MARK BENNINGTON; COURTESY ANDRZEJ KRAUZE

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Passing the Torch

Looking back, looking forward

BY MARY BETH ABERLIN

This is my last editorial describing the contents of an issue of *The Scientist*. Beginning in mid-2011, every month that I have had to pen this message to readers, the task never failed to remind me why I love science and how rare a job it is to always be learning something new. How can this be called work? And every month, it delights me no end to see how articles about seemingly disparate areas of life-science research share fundamental connections, both mechanistic and historical.

This past weekend, I saw for the first time a preserved neuron from the jumbo or Humboldt squid (*Dosidicus gigas*). Its giant axon really does look like a piece of spaghetti. I knew the important role these hefty conductors of nerve impulses have played in the development of neuroscience and why researchers had to focus on axons so large: curiosity outpaced available tools. That same handicap led this month's profilee, cell biologist Joseph Gall, to study lampbrush chromosomes, which are so transcriptionally active in amphibian oocytes that they are almost visible to the naked eye. Still working in the lab at age 89, Gall is the inventor of in situ hybridization and the discoverer of what turned out to be telomere sequences. Coincidentally, both discoveries were made with female graduate students, whom he welcomed into his lab in an era when women researchers were not at all common (page 54).

It's been a wonderful run with wonderful colleagues.

Because paradigm-shifting discoveries are so rare, much of what we report at *TS* results from painstaking, incremental improvements in techniques that have allowed researchers to dissect biological processes in ever-more-minute detail. To my mind, today's most extraordinary advances result from methods that provide glimpses of those processes in single cells. In this issue's cover story (page 28), Senior Editor Jef Akst reports on how such techniques have begun to detail the genomic reprogramming that occurs during very early embryonic development, after that most amazing of single cells—the zygote—forms by the union of a sperm and an oocyte.

Testaments to the value of single-cell analysis are legion, and this year our Top 10 Innovations first-place award goes to a new, commercially available tool, the IsoCode Chip, that can characterize thousands of single cells by assaying close to four dozen of the protein types each secretes. Another winner, 10x's Chromium system, allows precise single-cell transcriptome and whole-genome analysis. Check out all 10 winners on page 44.

Before I cap my pen, I want to reiterate that, for *TS*, the lives of scientists are as important to cover as the results of their research. Working as a scientist can be a hard job, and these days stagnant funding, career pressures, and waves of change in science publishing are making it harder still. But worst of all seems to be a devaluation of science and scientists by those in the highest echelons of our federal government, with a mandate to ignore basic science in favor of research with direct commercial benefits. I hope that this spring's nascent activism continues to motivate scientists to speak out. This issue's Careers column (page 61) covers the importance of philanthropic support of science, not only to counter actual and threatened cuts to basic-science research, but to fund riskier, outside-the-box studies.

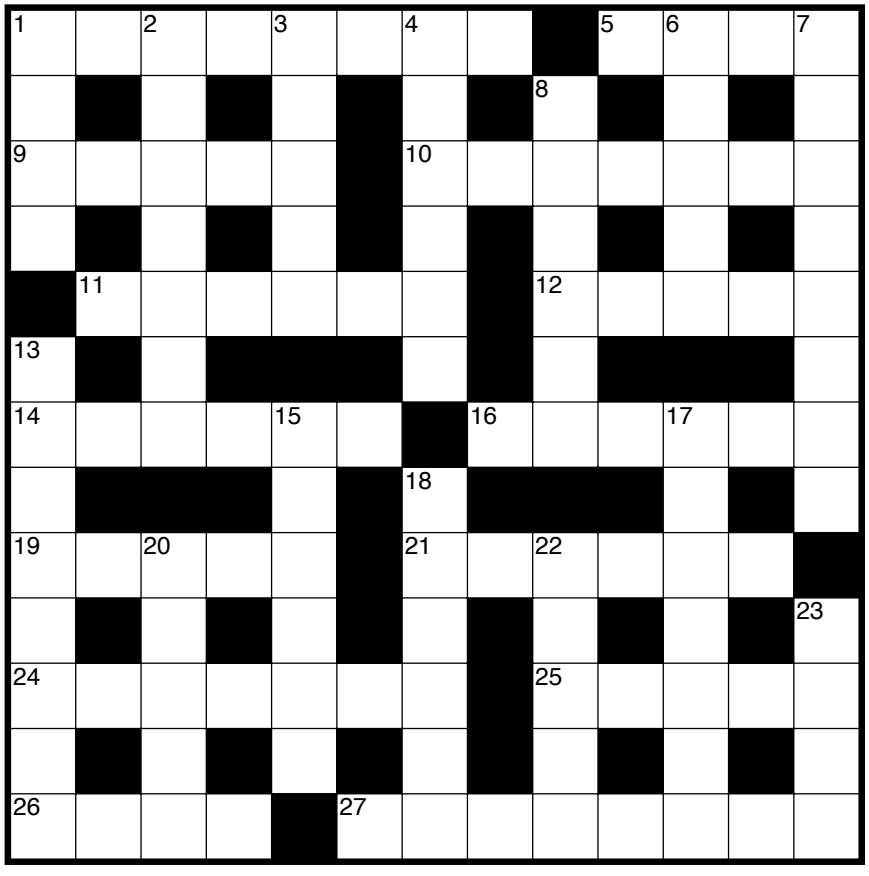
The author of that Careers column is Senior Editor Bob Grant. It is to him that I pass the torch as I retire. Bob is uniquely suited to take over as editor-in-chief. He knows *The Scientist* intimately, having been on staff since 2007. He is not only a hard-nosed reporter but also an award-winning feature writer. And he is passionate about *The Scientist*'s mission.

For me, it's been a wonderful run with wonderful colleagues. I look forward to reading Bob's words in this space in the new year and beyond. ■

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

ANDRZEJ KRAUZE

Speaking of Science



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

BY EMILY COX AND HENRY RATHVON

ACROSS

1. With 27-Across, a pioneer in DNA research (2 words)
5. Notable feature of great whites
9. Shade for a panther
10. "I went to the woods because I wished to live deliberately" author
11. Possible source of mother of pearl
12. One cubic decimeter
14. Like a harvest moon in hue
16. Age of human history
19. First name in radium research
21. Chipped flint from the Tertiary Period
24. Lepidopterist who also wrote fiction
25. Glassware brand found in many a lab
26. Sasquatch's Asian cousin
27. See 1-Across

DOWN

1. Color of the throat of some hummingbirds
2. Shoulder blade
3. Bodies studied by limnologists
4. *Urtica* stinger
6. Producer of a reaction; catalyst
7. Mammal whose name means "shadow-tailed"
8. Catkin-bearing tree
13. State of hibernation or inactivity
15. Hippocrates and Galen, e.g.
17. Word preceding selection and disaster
18. Dweller in a lodge
20. Subject of three Asimovian laws
22. Rabbit, to Lamarck
23. Transmitter of neural impulses

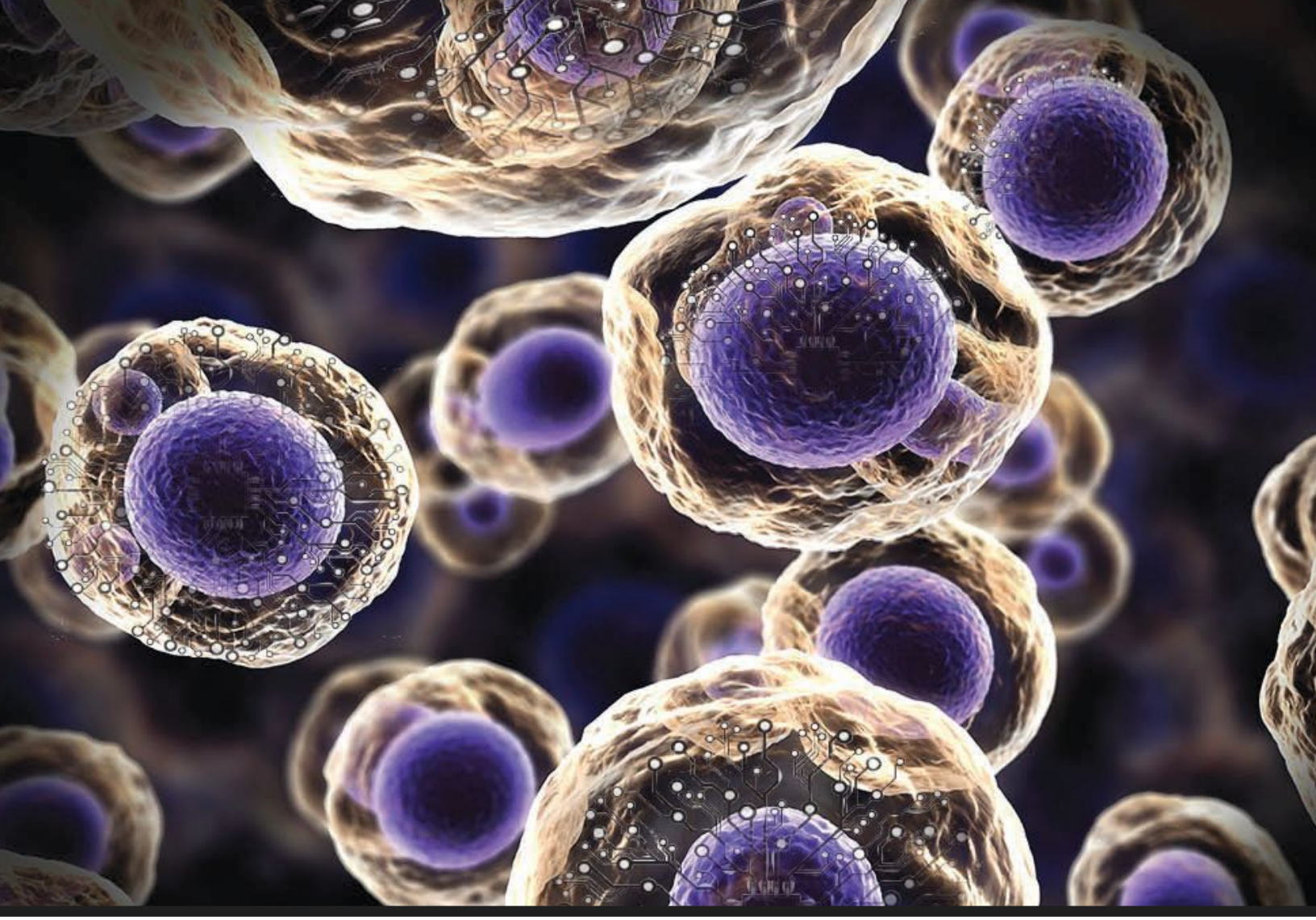
Answer key on page 5

Trying to manage the research community, many people have concluded, is really like herding cats. And it is like herding cats, but guess what? I've got a big bag of cat food—it's called the NIH budget.

—National Institutes of Health Director **Francis Collins,** on the challenges of getting researchers to share data, during a Q&A session at the recent annual meeting of the American Society of Human Genetics (October 18)

We have got to get beyond this point of stifling science, of muzzling good science, and speak to the facts as they are. This shouldn't be about a Democratic or Republican issue. It's about protecting the planet.

—Representative **Jim Langevin** (D-RI), speaking about the Trump administration's recent decision to prevent scientists from the US Environmental Protection Agency from presenting climate change–related research at a conference focused on the state of Narragansett Bay and its watershed (*The Washington Post,* October 23)



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Notebook

DECEMBER 2017

ROY CALDWELL, UNIVERSITY OF CALIFORNIA, BERKELEY

COLORFUL QUARRY: The purple-spotted mantis shrimp (*Gonodactylus smithii*) is strikingly patterned, but proves difficult to catch in its coral reef habitats.

Who You Callin' "Shrimp Brain"?

Mantis shrimps are not the easiest animals to work with, as neuroanatomist Nicholas Strausfeld knows firsthand. Not least, there's the challenge of capturing the crustaceans in the wild. Also known as stomatopods, mantis shrimps live in burrows in shallow seawater and have earned the descriptive nickname "thumb splitters," thanks to their tendency to use their sharp, powerful claws to slash at prey and pursuers.

"At low tide, you wade around and you try and catch these things," says Strausfeld, who has plenty of experience chasing after the purple-spotted mantis shrimp (*Gonodactylus smithii*) with a small handheld net in the tropical waters around Lizard Island, Australia. "They're incredibly fast—it's very difficult."

For Strausfeld and other neurobiologists, however, all the trouble is well worth it, as these feisty little marine predators are yielding unique insight into the evolution of the arthropods—the most species-rich animal phylum on the planet, containing around 85 percent of all described animal species.

"We knew [these shrimps] were very interesting," says neuroanatomist Gabriella Wolff, previously a PhD student in Strausfeld's lab at the University of Arizona and now a research associate at the University of Washington in Seattle. In addition to a complex visual system that receives inputs from independently moving eyes, "mantis shrimps have very advanced behaviors that we haven't necessarily seen in other crustaceans so far." Research has also suggested they are sophisticated navigators, regularly finding their way home from distant feeding sites. Plus, they recognize other individual mantis shrimps, and remember whether their interactions were confrontational or not.

In 2016, Wolff revisited Strausfeld's lab for a summer project to explore the structure of mantis shrimp brains. "We weren't really sure what we were going to find," she says. Almost immediately,

however, the pair discovered something that was wholly unexpected: a mushroom body—a key neural structure most famously associated with visual and olfactory learning and memory in insects. "It was a huge surprise," says Wolff, noting that the two lineages are separated by hundreds of millions of years of evolution. "We were really excited because we'd never seen a mushroom body so much like an insect's mushroom body anywhere outside of insects, especially not in crustaceans."

To learn more about the similarities between the structures in these disparate taxa, Wolff, Strausfeld, and collaborators drew up a list of 13 traits to describe the insect-type neural mushroom body in detail, ranging from the presence of particular fibers and cell clusters to the expression of certain insect proteins involved in learning and memory. Then, the researchers painstakingly worked their way through brains of mantis shrimps, model insects such as *Drosophila*, and a handful of other insect and crustacean species, cataloging the traits.

"We asked, how many of these traits are present in the stomatopod?" says Strausfeld. "And they all are. That was pretty exciting." The team concluded that mantis shrimps possess a mushroom body that is essentially equivalent to the one found in insects (*eLife*, 6:e29889, 2017).

Strausfeld and Wolff are not alone in their excitement. Wake Forest University neurobiologist Susan Fahrbach, who studies the neuroanatomy of social insects such as honeybees, remembers her reaction on reading the paper for the first time. "My jaw just dropped," she says. Looking at one figure in the paper that illustrated a mushroom body feature called the microglomeruli, "if you didn't tell me I was looking at a mantis shrimp, I could have been looking at a honeybee brain," she says. "I had that feeling of, 'Wow, that's what I study—only it's in a shrimp.'"

The unexpected presence of this structure in a crustacean lineage raises the question of where the mantis shrimp mushroom body came from. One possibility, which the authors explore in their paper, is that the feature was present in an ancestor of both insects and crustaceans, and was subsequently lost from crustacean lineages that didn't make use of it. "There are spectacular losses in certain lineages," says Fahrbach. "It's certainly not an outlandish suggestion."

Possible support for that view comes from the researchers' discovery of mushroom body–like structures in three other crustacean groups known for complex behavior. The groups, which might have retained some parts of an ancestral mushroom body, include the pistol shrimps, the only crustaceans to have evolved eusociality; the cleaner shrimps, which nibble parasites off larger animals at marine "cleaning stations"; and the semi-social land hermit crabs. "Like the stomatopods, [these animals] know where they are," Strausfeld says. "They visit the same places for various tasks."

For now, however, the evidence is primarily "circumstantial," notes Tom Cronin, a biologist at the University of Maryland, Baltimore County, who studies arthropod vision. "There are a lot of things that tie all this together, and I think they made a really strong case." But, he adds, to say with certainty that the trait has been conserved between insects and stomatopods, and is not instead a particularly impressive example of convergent evolution, "you'd like to see a transcriptomic analysis to know whether it has the same molecular profile."

Strausfeld says he is keen to carry out exactly that analysis. He and Wolff are hoping to secure funding for a transcriptomic screen of mantis shrimp and insect brains, to search for signs of common ancestry. "That would be the final arbiter to tell us whether these extraordinarily similar centers are in fact homologous or not," Strausfeld says, adding that either way, the structure discovered in mantis shrimps offers a new window into the biology of arthropod brains. "Even if it's convergent evolution, that would be absolutely fascinating, right?"

—Catherine Offord

SOPHISTICATED SHRIMPS: Like many insects, mantis shrimps have sophisticated visual systems and display complex behaviors, from navigating long distances to remembering social interactions with other individuals.

ROY CALDWELL, UNIVERSITY OF CALIFORNIA, BERKELEY

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Robo Calls

Out in the field one day observing Australian magpie-larks (*Grallina cyanoleuca*), bioacoustics researcher Pawel Rek heard the telltale trilling of the birds' territorial defense duet. Magpie-lark pairs, like those of many bird species, sing coordinated duets to warn potential rivals to keep off their turf. But Rek could only spot the male of the pair. "I was really confused when I found that the female was actually sitting on the nest, on the nearby tree, and that it was only the male singing his and the female's part," he writes in an email.

The observation raised questions. Were rival magpie-larks actually fooled by such deceptive duets? If so, why bother with real ones? Intrigued by the fact that the male magpie-lark seemed to be hiding as it sang its deceptive song, Rek also wondered how rivals' actually seeing the birds played into duet communication, especially because the duets are usually accompanied by displays that include wing-lifting and shoulder-shrugging. To find out, he would need the help of some robotic birds.

Fortunately Rek, who has his home lab at Adam Mickiewicz University in Poland but was then on a fellowship at Australian National University (ANU) in Canberra, already had the robots on hand. The animatronic birds were made from a male and a female magpie-lark that had been found dead, taxidermied, and fitted with mechanical parts. Rek and Rob Magrath, a behavioral ecologist at ANU, had used the lifelike robots to compare magpie-larks' reactions to silent wing-lifting displays versus displays coupled with song (*Animal Behav*, 117:35-42, 2016). Now Rek just needed to make some small adjustments to get the setup ready for the next experiment.

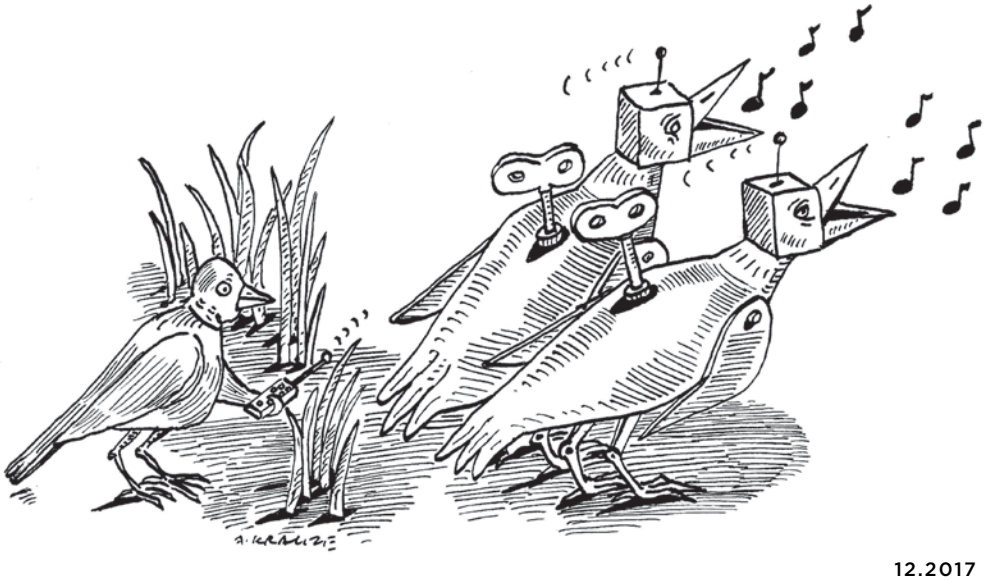
Because it takes two to duet, you might have thought of it as an unfakeable, honest signal of there being more than one bird present, but these guys have shown that it's actually not that.

—Mike Webster, Cornell University

Rek's previous studies on the magpie-larks also proved handy when it came to finding unsuspecting study participants. "When you get to know the birds, you often have a pretty good idea of where they're likely to be," says Magrath. For one experiment, Rek tried playing duet recordings to the birds, with no robots present. He and Magrath then counted the number of songs sung by the hoodwinked males as a measure of how threatened they felt their territories to be. The deceptive duets sung by just one bird induced male listeners to sing as effectively as real ones sung by a male-female pair (*Proc Royal Soc B*, 284:10.1098/rspb.2017.1774, 2017).

The avian listeners reacted about as strongly when the recordings were accompanied by the sight of two robot birds performing corresponding displays. But they only sang about half as many songs when the recordings were accompanied by a single robot bird. "This study adds a new (specific for cooperative signals) level for the classification of multimodal signals," Rek writes to *The Scientist*. "In this particular case the role of the visual component is peculiar—it informs that the message transferred by songs is honest." Rek's earlier observation that male birds singing "duets" alone tended to do so from hiding suggested intent to deceive.

There are other known instances of avian deception, such as deceptive alarm calls, but this is the first study to take on deceptive duets, says Mike Webster, a behavioral ecologist at Cornell University's Lab of Ornithology who was not involved in the study. To him, the results indicate that "animals can use deceptive signaling more flexibly, and possibly more commonly, than we give them credit for. . . . Because it takes two to duet, you might have thought of it as an unfakeable, honest signal of there being more than one bird present, but these guys have shown that it's actually not that."



ANDRZEJ KRAUZE

But Katherine Gentry, a behavioral ecologist at Purdue University, writes in an email to *The Scientist* that the recorded duets the researchers used may have been relatively unconvincing because they were played from one speaker, not two. She cites an earlier study in which magpie-larks reacted more aggressively when the male and female parts of a duet were played by separate speakers rather than just one (*Behaviour*, 141:741-53, 2004), and says, "it makes me wonder just how discernible the sequential-duet playbacks were from the pseudo-duet playbacks."

Magrath says that the robo-birds were only half a meter apart in the study, and that the study subjects would likely have been unable to differentiate between the sound of a single speaker versus two placed so close together. At any rate, the difference between the birds' reactions on seeing one robo-bird or two "occurs while keeping everything else the same, including the number of speakers," he writes to *The Scientist*.

Gentry is more impressed by an observational component of the study, in which Rek and Magrath found the magpie-larks do deploy deceptive duets, and are most likely to do so during the nesting season, when pairs are often separated.

Emilie Perez, who researches animal communication at Columbia University, says the study suggests "a clear trade-off here between producing pseudo-duetting too often and risking [being] discovered as mimickers." More broadly, she writes in an email, "this paper shows one more time that birds have very high cognitive abilities and are indeed very smart creatures."

—Shawna Williams

Polar Fungi

For the last 11 years, when austral summers arrive, microbiologist Luiz Rosa trades the sunny hills of Belo Horizonte, Brazil, for the cold plains of Antarctica. With its monotonous landscape, Antarctica might seem devoid of life, but despite the low temperatures, dry air, and extreme solar radiation, many microscopic organisms have learned to call this place home. For three months, Rosa, whose home lab is at the Federal University of Minas Gerais, and his team sample rocks, ice, and seawater in search of fungi. By studying them, the researchers hope not only to shed new light on their ecology and evolution, but also to find new candidates for drug discovery.

LUIZ ROSA, UFMG

SCIENCE ASEA: Researchers Luiz Rosa and Gracile Menezes filter Antarctic seawater in a laminar flux cabin in the Polar Microbiology Laboratory aboard the oceanographic research vessel *Almirante Maximiano*.

In their latest paper, published a few months ago in *Extremophiles*, Rosa and his collaborators provided a novel peek at the long-mysterious fungal biodiversity of Antarctic seas. And they observed that the extremely cold waters are actually home to a moderate diversity of fungi (doi:10.1007/s00792-017-0959-6, 2017).

One of the most abundant species Rosa and his colleagues observed was *Penicillium chrysogenum*, the globally common fungal species from which the antibiotic penicillin is derived. In fact, previous work from Rosa's group noted that *P. chrysogenum* is also very abundant on the surface of rocks from Chile's Atacama Desert, another extreme environment.

While *P. chrysogenum* had already been observed in Antarctica, other species were found there for the first time. For example, the team reported the presence of *Rhodotorula slooffiae*, a pigmented yeast species that is known to protect itself from UV radiation by producing a mycosporine—a type of compound often called "microbial sunscreen," which has obvious biotechnological potential.

Rosa and his collaborators also noted the presence of *Exophiala xenobiotica*, a fungal species usually associated with polluted environments. While the introduction of pollution to Antarctica is a worrying trend, the polar region is also especially prone to disturbances wrought by other environmental changes. "Antarctica is an environment which is extremely sensitive to climate changes," Rosa explains. "Thus, ancient microorganisms trapped on the Antarctic ice could be released due to global warming."

Rosa points out that many unknown—and potentially noxious—microorganisms could be slumbering beneath Antarctic ice. As this ice melts and falls into the sea, deep ocean currents will likely transport the microbes for thousands of miles into new marine ecosystems. "These currents are the reason why sometimes penguins are found on the Brazilian coast," Rosa says.

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Itamar Melo, a microbiologist from the Brazilian public research institution Embrapa, was surprised to see Rosa's team reporting land species in the seas ringing Antarctica. "Many of these fungi were mainly known in the terrestrial environment," he says. "For example, *Lecanicillium* is a fungus that is often found parasitizing plant pests, and *Acremonium* is found in soils, where it degrades organic matter."

Melo says it is possible that these microorganisms were originally terrestrial inhabitants of continental Antarctica and were dragged into the sea along with melting continental ice masses. But it is also plausible that these fungal species normally live in marine environments, and are simply more ubiquitous than researchers had appreciated. Rosa says he wants to sample water along the route from Brazil to Antarctica in a future expedition, which will likely shed more light on this issue.

Many of these fungi were mainly known in the terrestrial environment.

—Itamar Melo, Embrapa

Most of Rosa's work on Antarctic fungi had to be done in the field, using an onboard laboratory, as the ecological composition of seawater samples can be affected by freezing. The researchers collected samples using equipment that allows them to simultaneously measure different parameters, such as temperature and salinity. Then, in the sterile environment provided by laminar flow cabinets, they filtered the water samples and placed the filters in dishes with marine agar, a substance that mimics the marine environment. Once the fungi grew, the researchers extracted their DNA and sequenced some genomic regions to identify species.

In addition to answering basic science questions, Antarctic fungi may also possess commercial potential. For example, on a previous expedition Rosa's team found species that demonstrated activity against neglected tropical diseases such as dengue fever and leishmaniasis. Identifying the molecules responsible for these effects could lead to the development of new drugs.

For Melo, though, the biggest potential of Antarctic species may lie in compounds that confer resistance to extreme conditions. "Many of these microorganisms synthesize essential fatty acids, such as omega-3 and omega-6, that protect their cell walls. Others produce exopolysaccharides, [large molecules made of sugars that are secreted into the environment] to protect them from hostile conditions, or antifreezing proteins," says Melo. "Antarctica is a great place to prospect for these kinds of molecules." —Ignacio Amigo

Whip It Good

Whip spiders, also known as tailless whip scorpions, are actually neither spiders nor scorpions. These strange creatures belong to a separate arachnid order called Amblypygi, meaning "blunt rump," a reference to their lack of tails.

Little was known about whip spiders before the turn of this century, but a recent flurry of behavioral and neurophysiological studies has opened a window into their unique sensory world. Researchers have discovered that some of the more than 150 species engage in curious behaviors, including homing, territorial defense, cannibalism, and tender social interactions—all mediated by a pair of unusual sensory organs.

Like all arachnids, whip spiders have eight legs. However, they walk on only six. The front two legs are elongated, antennae-like sensory structures called antenniform legs. These legs, three to four times longer than the walking legs, are covered with different types of sensory hairs. They constantly sweep the environment in a whiplike motion, earning whip spiders their common name. Whip spiders use their antenniform legs the way a blind person uses a cane—except that in addition to feeling their environment, whip spiders can smell, taste, and hear with their antenniform legs.

All aspects of a whip spider's life center on the use of these legs, including hunting—whip spiders are dangerous predators, if you're a small invertebrate that shares the arachnids' tropical and subtropical ecosystems. When Eileen Hebets, a biologist at the University of Nebraska–Lincoln, recorded the prey capture behavior of the whip spider *Phrynus marginemaculatus*, she observed a well-choreographed pattern. First, the whip spider aimed one of its antenniform legs toward the prey. Next, it placed an antenniform leg tip on either side of the prey. Finally, it swung its antenniform legs out of the way and struck with its spine-covered pedipalps, a pair of grasping appendages in front of the mouth. "The way they move their legs is so graceful," Hebets says. "Their movements seem intelligent. And they have this incredible repertoire of sensory capabilities along with interesting behaviors."

One of those behaviors is territorial sparring. *P. marginemaculatus* battle by vibrating their antenniform legs at each other. The animal that keeps at it the longest wins the contest. Initially, it was thought that the opponents actually touched each other. But using high-speed video, Hebets showed that the antenniform legs do not come into contact. Rather, whip spiders position their antenniform legs just over the "knees" of their opponents' walking legs, an area containing long, thin sensory hairs in a socketed base. Electrophysiological studies demonstrated that these sensory hairs are near-field sound receptors, able to detect moving air particles generated by an opponent waving its leg. When Hebets clipped off the sensory hairs, the duration of antenniform leg waving no longer predicted who won the contest (*PLOS ONE*, 6:e22473, 2011).

Other sensory hairs on the antenniform legs detect odors in the air, an unusual ability among arachnids. Recent experiments suggest whip spiders use their sense of smell to find their way home. Hebets, with Verner Bingman and Daniel Wiegmann of Bowling Green State University, captured *Paraphrynus laevifrons* whip spiders in Costa Rica. The researchers deprived some of them of vision by painting over their eyes with black nail polish. For another group of arachnids, sensory input from the tips of the antenniform legs was blocked with either nail polish or by trimming with scis-

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sors. Then the researchers glued miniature radio transmitters to the animals' backs and released the experimental groups 10 meters from their home refuges. The whip spiders could generally find their way back without the use of their eyes. However, sighted individuals with compromised antenniform leg tips experienced a complete loss of homing ability (*J Exp Bio*, 220:885-90, 2017).

"On the tips of the antenniform legs are specialized olfactory receptors that respond to chemicals dispersed in the air," says Bingman. "The most important sensory system for navigation appears to be olfaction, but it is unlikely that olfaction can explain the entirety of this remarkable navigational ability."

Whip spiders are eager to return to their refuges after a night out to avoid their many predators, including their peers. Cannibalism is rare in some species, while in others up to 20 percent of laboratory interactions end with one opponent eating the other.

University of California, Davis, biologist Kenneth Chapin found that a Puerto Rican species named *Phrynus longipes* is highly territorial. "They claim a small patch, maybe half a meter, and defend it from other whip spiders, just like a tomcat or a wolf pack might," he says.

Much whip spider research supports the view that the arachnids lead solitary, aggressive lives. However, some research has painted these fearsome predators as gentle lovers. The whip spider courtship ritual can last up to eight hours and involves ample antenniform leg stroking by each member of the pair.

I think they could provide a gateway into our understanding of the mechanisms underlying complex behavior and the neural structures important for learning and memory.

—Eileen Hebets
University of Nebraska-Lincoln

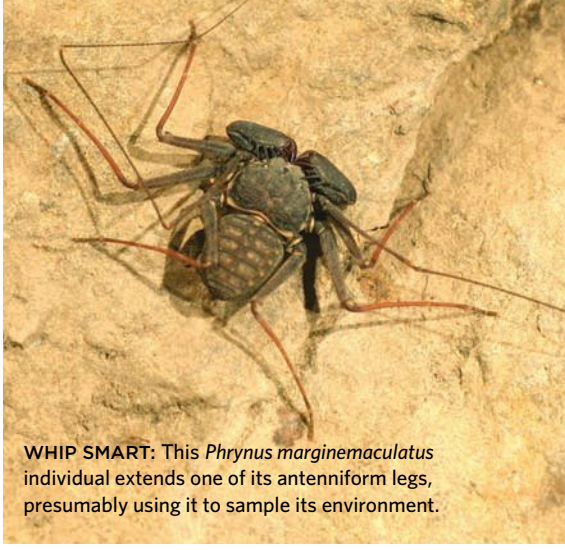
Working with captive mother-offspring groups of whip spiders, Linda Rayor, an entomologist at Cornell University, has shown that some species are surprisingly social. After encountering a whip spider in Costa Rica, Rayor began keeping several species in her office. One day, Rayor noticed a mother sitting in "a sea of the waving whips of her youngsters." The group gently interacted using their sensitive antenniform legs. "I had never seen arachnids do what was essentially a totally amicable behavior," she says. "I was charmed and hooked."

Rayor's research on two species—*P. marginemaculatus* from Florida and *Damon diadema* from Tanzania—suggests mothers and siblings form close groups for about a year before the young reach sexual maturity. "They largely sit within whip length of one another so that they are in constant contact," she says.

Despite all the recent studies detailing whip spiders' fascinating behaviors, little is known about their brains. A structure called the mushroom body is particularly large and convoluted in whip spiders. Mushroom bodies are higher-order brain regions that, in insects and other invertebrates, are associated with information processing, learning, and memory. Whip spiders have the largest mushroom bodies, relative to their size, of any arthropod. But it is not clear exactly what these structures do in whip spiders or how sensory information from the antenniform legs is involved.

"The fact that whip spiders have this unusual central nervous system and associated sensory systems makes them excellent study subjects," says Hebets. "I think they could provide a gateway into our understanding of the mechanisms underlying complex behavior and the neural structures important for learning and memory."

—Mary Bates



WHIP SMART: This *Phrynus marginemaculatus* individual extends one of its antenniform legs, presumably using it to sample its environment.

PEEP THOSE PEDIPALPS: In close proximity to its antenniform legs and mouth, *Phrynus pseudoparvulus*, has a formidable set of pedipalps, grasping appendages used to secure prey.

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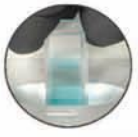

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One-Step Stem Cell Knockouts

Performing gene editing and stem-cell induction at the same time improves the efficiency of functional genetic analyses.

BY RUTH WILLIAMS

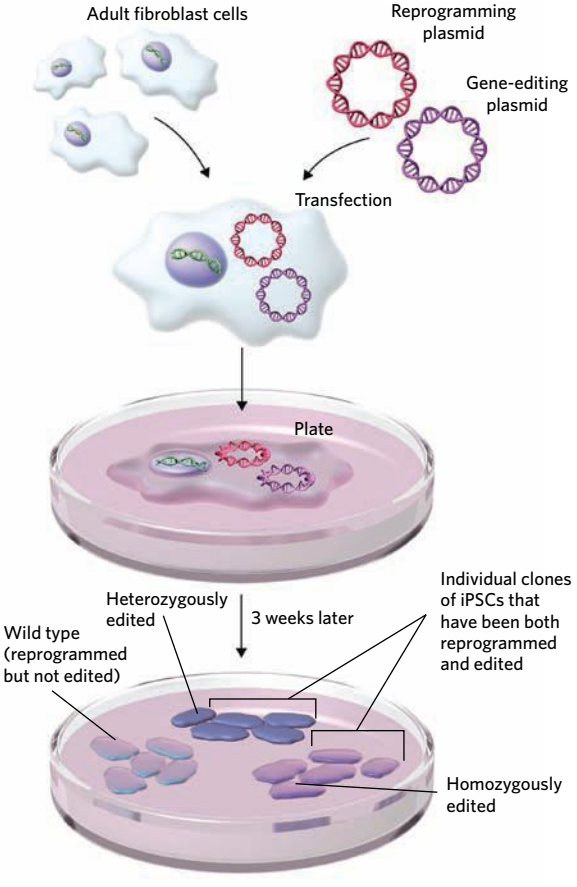
In theory, mutating a gene of interest inside stem cells enables researchers to analyze the effects of that mutation on the development of particular cell types. In the laboratory of Jack Parent at the University of Michigan Medical School, for example, postdoctoral researcher Andrew Tidball is using such an approach to investigate how gene mutations associated with epileptic encephalopathy affect brain cell development. But while trying to introduce the specific mutations into human induced pluripotent stem cells (iPSCs), he ran into difficulties.

A major problem, Tidball says, is that after transfecting iPSCs with gene-editing plasmids, individual cells need to be isolated, but "stem cells don't like to be [alone]. They die unless you add some components to help them along." Even then, he adds, "a very low percentage survive." Furthermore, says Parent, "not all genes are amenable to gene editing [in] iPSCs."

Tidball realized that instead of first transfecting cells with plasmids containing reprogramming genes and then later adding plasmids with CRISPR-Cas9 components, he could combine the two steps into one. Because iPSC induction already involves the production of single cell–derived colonies, he could, in one fell swoop, create gene-edited stem cell lines.

Tidball, Parent, and colleagues have now used the technique to generate multiple cell lines containing epilepsy-associated mutations, and have found that not only is the combination strategy more time-efficient and reproducible than the sequential approach, it is also more successful: more clones carry the intended mutations. The team's investigations suggest that this improvement may be due to increased chromatin accessibility at the time of reprogramming, allowing the gene-editing machinery to reach its target DNA more easily.

The technique also preserves genome integrity, says the University of Wisconsin's Anita Bhattacharyya, who was not involved in the work. "We know that these pluripotent stem cells, over time, tend to acquire chromosomal abnormalities," she says, so doing both processes at once reduces the likelihood of aberrations. "For those people who work in disease modeling of single-gene mutations, this is a really important move forward." (*Stem Cell Rep*, 9:725-31, 2017) ■



DOUBLING UP: Plasmids encoding reprogramming factors and plasmids encoding gene-editing machinery are transfected together into fibroblast cells. Approximately three weeks later, induced pluripotent stem cell (iPSC) colonies grown from single cells are apparent. These clones can be individually picked from the dish for further isolated growth and study.

AT A GLANCE

GENE EDITING OF iPSCS	HOW IT WORKS	SUCCESS AND REPRODUCIBILITY	CONTROLS
Subsequent to iPSC creation	An established iPSC line is transfected with a gene-editing plasmid and isolated into single-cell clones for sequence analysis.	Between less than 1 percent and 20 percent of the clones that survive single-cell isolation (very few) carry desired mutations.	The nontransfected iPSC line is often used as an isogenic control, but it will not have been subjected to the exact same culturing conditions as the mutant line.
Simultaneous with iPSC creation	Differentiated cells are transfected with both reprogramming and gene-editing plasmids. Plating of the transfected cells results in single iPSC clones which are picked for sequence analysis.	Approximately 45 percent of clones carry at least one mutated allele.	Homozygous mutants, heterozygous mutants, and wild-type isogenic controls are all made during the same experiment.

BIRTH OF AN EMBRYO: The haploid nuclei from an egg and a sperm in the zygote following fertilization

In the Beginning

New technologies reveal the dynamic changes in mouse and human embryos during the first week after fertilization.

BY JEF AKST

Last May, to much fanfare, an international group of researchers published two papers describing a new in vitro system that had maintained human embryos in culture for 13 days.[1,2] The experiments could have continued beyond two weeks, if not for the "14-day rule"—a widely recognized limit to how long scientists are permitted to maintain human embryos for research purposes. Bioethicists first proposed the rule, which was subsequently enshrined in the laws of several countries and as a guideline in the U.S., in 1979. Three and a half decades elapsed before the technology existed to keep embryos alive outside of a womb past the implantation stage, which typically occurs about a week after egg and sperm cells fuse. Now, the rule was finally coming into play.

"The decision to stop this beautiful amazing structure that [was] moving forward with self-organization . . . was the toughest I'd ever done in my professional career," says Rockefeller University embryologist Ali Brivanlou, a senior author on one of the papers. "I did it because of respect for guidelines."

The researchers stopped the experiment by flash-freezing the human embryos in liquid nitrogen, suspending them in time. "I have no idea if we will be able to thaw them again and have them come back. But my hope is that one day—hopefully within my lifetime; if not, the next generation of my students and postdocs and others—we'll have the opportunity to go back to the liquid nitrogen and thaw these embryos and ask a very simple question as to how far this self-organization can sustain itself [in culture]. Because it's impossible to imagine that this can go on much farther than 14 days."

The research has reinvigorated the ethical discussion concerning the culturing of human embryos for scientific study, while providing the means to study embryos postimplantation—a period of development that has remained largely mysterious until now. "What happens [during the second week and] later has been the black box of development, because we could not successfully culture embryos beyond implantation," says Magdalena Zernicka-Goetz, a developmental and stem cell biologist at the University of Cambridge in the U.K. whose lab developed the new system. Meanwhile, other technological advances are yielding major insights into the very first week of embryonic development—a period that involves the reprogramming of two highly differentiated cells, a sperm and an egg, into a totipotent cell from which an entire organism will form.

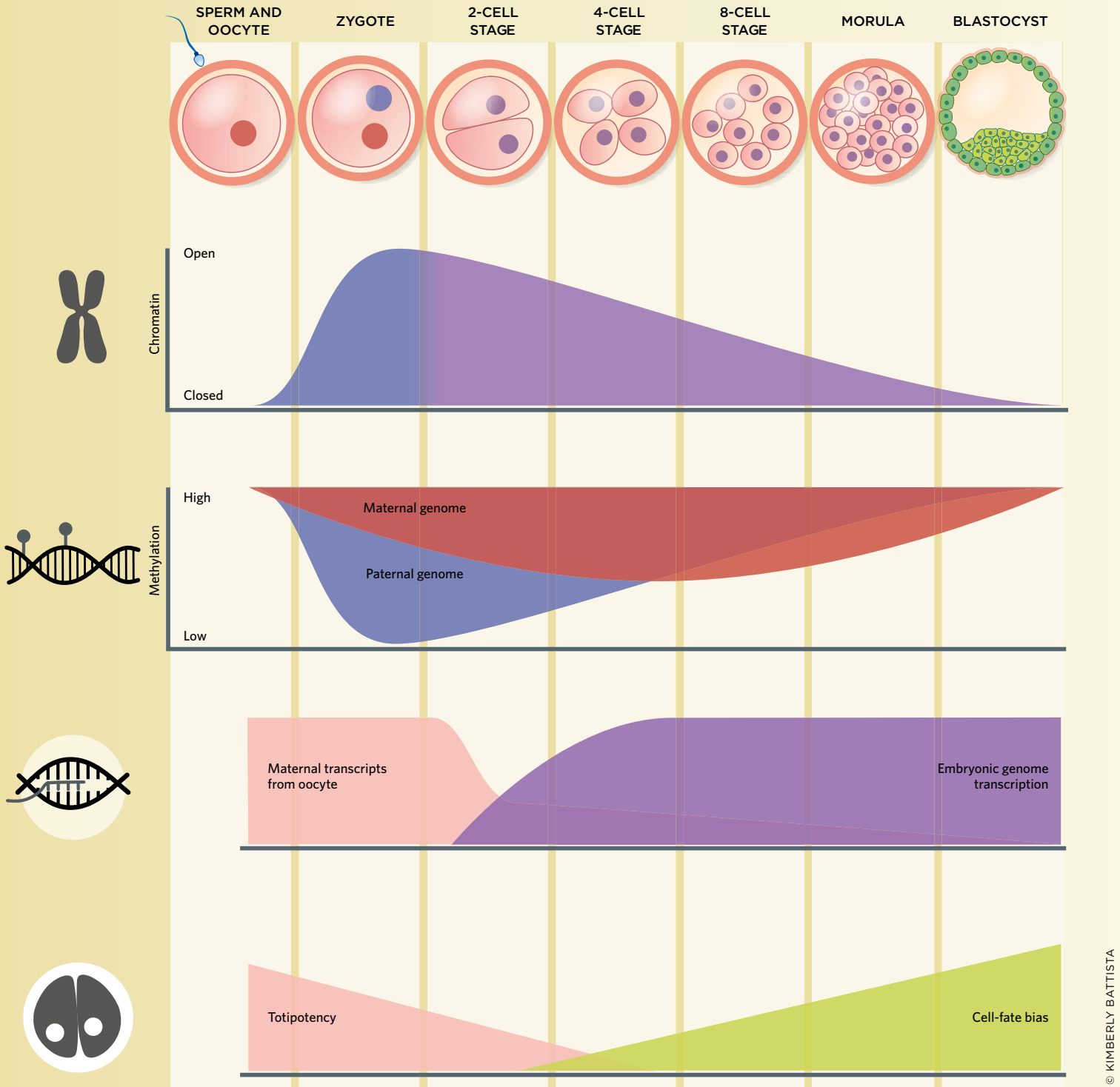
> It's only now that we start having a glimpse of what takes place in the first hours and days.
>
> —Didier Trono
> École Polytechnique Fédérale de Lausanne

"It seems to be a very hot area of research, I think in part because we're trying to understand what creates this very interesting tabula rasa state of the genome where it's totipotent—it can turn into anything," says MIT biophysicist Leonid Mirny.

With the advent of single-cell technologies, scientists are, for the first time, able to take a peek inside the individual cells of two-, four-, and eight-cell embryos, as

TAKING CONTROL

In the first hours after fertilization, maternal factors residing in the oocyte cytoplasm dictate early development. But soon, the zygote's genes start to take over. This maternal-to-zygotic transition involves massive epigenetic reprogramming, from the overall structure of the chromatin to the complete resetting of methylation on the genome. (Note: Most of the information depicted below is based on studies of mouse embryos; there are some differences in the timing of these events in human embryos.)



CHROMATIN CHANGES

In sperm, chromatin is very compact; the overall accessibility of the chromatin in the oocyte, which is still undergoing meiosis, is unclear. Shortly after fertilization, chromatin in both pronuclei undergoes major restructuring, taking on an open configuration before reestablishing local and global organizational features.

METHYLATION CHANGES

Following fertilization, the vast majority of methyl marks on the genome are removed. The paternal genome undergoes rapid, active demethylation, while the maternal genome loses its methylation passively over the first couple of cell divisions. Simultaneously, the embryonic genome begins to acquire tissue-specific DNA methyl marks as the cells start to differentiate.

TRANSCRIPTION CHANGES

Messenger RNAs packaged in the oocyte are gradually depleted over the first week of development. Meanwhile, the zygotic genome undergoes multiple rounds of activation, with the genes expressed early on playing key roles in embryonic organization and cell-fate determination.

CELL-FATE DETERMINATION

By the four-cell stage, some cells begin to express genes that drive them to become the embryonic lineage that will form the fetus, while other cells begin to express genes associated with the extraembryonic lineage that becomes the placenta.

well as inside the individual pronuclei—one from mom and one from dad—of the initial one-cell embryo, called a zygote, formed upon fertilization. Just in the past few years, experimental results have begun to reveal how the zygotic genome is reorganized and reprogrammed to transfer control of development from maternal factors harbored by the egg to the embryo's own genes. "Given how few of these cells there are, it's really amazing we can now look into these early stages of development," says Mirny. "This progress is totally driven by the single-cell techniques."

"It's only now that we start having a glimpse of what takes place [in the first hours and days]," agrees Didier Trono of École Polytechnique Fédérale de Lausanne (EPFL) in Switzerland. "These last couple of years—and in the few years to come—we're making tremendous progress in understanding what happens during this period."

Chromatin reorganization

In the hours and days that follow fertilization, the genomes of the newly united egg and sperm cells begin to express genes important in early development. Prior to this activation, maternal factors packaged in the oocyte are in charge. But changes to the overall chromatin structure of the paternal and maternal genomes, which are housed in separate pronuclei within the zygote, permit access by transcription factors shortly after fertilization—at about 13 hours in mouse embryos. The exact nature of these dynamics, however, has remained shrouded in mystery for decades.

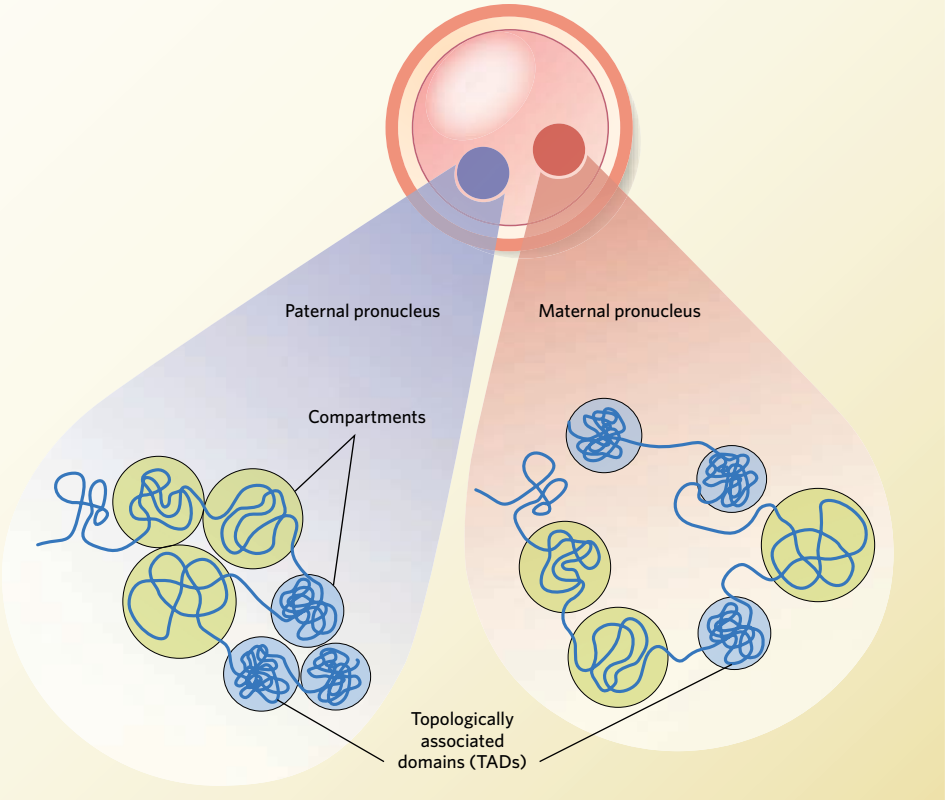
This March, Kikuë Tachibana-Konwalski, a cell biologist at the Institute of Molecular Biotechnology (IMBA) of the Austrian Academy of Sciences, and her colleagues published the first in-depth look at how chromatin structure changes from the oocyte to the single-cell embryo in mice. Her group teamed up with Mirny's lab at MIT to refine a method known as Hi-C (high-resolution chromosome conformation capture) so it could be applied to individual nuclei. During Hi-C, pieces of DNA that are close in space—regardless of their genomic distance—are glued together at contact points, or contacts, before enzymes digest the DNA. The glued pieces are then chemically ligated into single DNA fragments. These hybrid DNA molecules are sequenced, and researchers use computational techniques to map the sequences to determine the higher-order, 3-D structure of the intact genome. (See "Nuclear Cartography," *The Scientist*, October 2014.)

The problem was that traditional Hi-C approaches require thousands or millions of cells. This is because researchers would filter out those reads believed to be hybrids, and they needed to retain enough material to generate a complete map of chromatin conformation. Mirny's team found that they could skip this filtering step, isolating as much DNA as possible from a single cell, and then sort out computationally those reads that are productive. "So you spend more money on sequencing but you're trying to minimize DNA loss," Mirny explains. "As a result, we got 10 times more contacts per cell" than the only other published single-cell Hi-C technique. In total, the method yielded "about a million contacts per individual cell," he says, which "gives you enough information to reveal major features of chromatin organization."

Applying this approach to paternal and maternal pronuclei of mouse zygotes, Tachibana-Konwalski's team analyzed the chromatin structure of the two genomes. According to their results, both the paternal and maternal genomes appeared to have already reestablished local features known as loops and topologically associated domains (TADs)[3]—a finding in conflict with two other studies published this summer, which did not detect these structures until the embryo reached the eight-cell stage or became a blastocyst, a hollow ball of cells that implants in the uterine wall.[4,5] Tachibana-Konwalski says she and her colleagues "are confident that TADs and loops form within hours after fertilization in zygotes," having found evidence of TADs in an as-yet unpublished reanalysis of the other groups' data "with greater statistical power and appropriate controls."

CHROMATIN CHANGES

After fertilization, the genomes donated by the sperm and the egg lose many of the organizational features of their chromatin, which must be reestablished in the early embryo. One recent study showed that the paternal pronucleus of the single-cell zygote contained global features known as compartments, in which more-active regions of the genome associate with other active regions, while less-active regions associate more closely with one another. The maternal pronucleus, however, largely lacked compartments. In this study, both pronuclei had local features known as topologically associated domains (TADs), though other studies have failed to identify these organizational characteristics until later in the first week of development.



Tachibana-Konwalski's team also found a surprising difference between the two pronuclei of the zygote. While the paternal genome also contained higher-order formations called compartments, the maternal genome contained only the local structures, but no compartments—global features of chromatin in which transcriptionally active DNA associates more closely with other transcriptionally active regions, while silent stretches associate more closely with one another. That the paternal pronucleus contained these features while the maternal pronucleus did not "was really unexpected," says Tachibana-Konwalski. The paternal genome "seems to be winning the [reprogramming] race."

One area of the genome where restructuring appears important for early development is the heterochromatin—highly compacted regions of DNA that are normally silent but that suddenly become active in the zygote. For example, retrotransposons, one of the main components of heterochromatin, are highly transcribed at this time. "The activation of these retrotransposons is very peculiar for the developmental process," notes Maria Elena Torres-Padilla, an epigeneticist at Helmholtz Zentrum München in Germany. "It only happens otherwise in disease and cancer and very specific situations; in most of our cells these transposons are silent."

Most researchers had considered retrotransposon activation to be a side effect of the overall reprogramming process, says Torres-Padilla—as the chromatin restructured, transposons were freed from their normal repression, the thinking went. But that explanation didn't sit well with her. So she and her colleagues used transcription activator-like effectors (TALEs), a gene-editing technology, to selectively manipulate the transcription of LINE-1 transposable elements in mouse embryos during the first few days following fertilization. When the researchers prevented LINE-1 activation, they observed decreased rates of development. However, adding LINE-1 mRNAs to make up for the lack of transcription did not rescue the phenotype.[6] "That was the most surprising finding," says Torres-Padilla—"that it's not the messenger RNA itself, but it was really what we were doing on the DNA loci at the chromatin level."

Just what's going on remains to be seen, but she suspects that retrotransposon activation somehow initiates zygotic gene expression. "You have thousands of genes that are going to be activated from the genome of the embryo for the very first time," she says. "I think what the LINEs are doing is to help open up the chromatin, so that perhaps other elements that direct transcription in [other] genes can function more efficiently."

Still, whether changes in chromatin structure are driving early embryonic transcription eludes researchers. And there's still another piece of the puzzle that scientists are working to fit in: at the same time that the chromatin of embryonic genomes is restructuring, the vast majority of cytosine methylation on the DNA is lost. But the exact timing and causative relationship of these changes is unclear. "I think the most exciting aspect of zygote biology is to combine these approaches to precisely understand how individual modifications will change overall chromatin structure," Tachibana-Konwalski says. "To me, the next natural step is to merge these two levels of organization."

Methylation overhaul and transcription initiation

While genome-wide DNA methylation analyses have documented the global removal of cytosine methylation from the maternal and paternal genomes in the zygote, as well as the reestablishment of these marks over the first few days of embryonic development, the pathways that control this epigenetic revamp have been hard to pin down. In recent years, analyses focused on individual cells within the embryo, along with the application of gene-editing technologies to selectively block or activate enzymes thought to play a role, have begun to elucidate these enigmatic processes. "At present, our knowledge of epigenetic reprogramming is accumulating at a dizzying pace," one group of researchers wrote in a 2014 review of the field.[7]

In the maternal genome, passive dilution of the methylation marks occurs over a few days, while the paternal genome undergoes active and rapid demethylation—often accompanied by replacement with alternative modifications, including hydroxymethylation and carboxylation—shortly after fertilization. (See "The Role of DNA Base Modifications," *The Scientist*, September 2017.) One proposed mechanism of this active demethylation process, first posited by Azim Surani of the Gordon Institute and colleagues in 2010,[8] is the breaking and repairing of DNA, and several studies over the years have lent support to this hypothesis. "Of course, [inducing DNA breaks] would be very dangerous at this stage when it's a single-cell embryo," Tachibana-Konwalski notes. "It's not exactly what one would expect evolution to do."

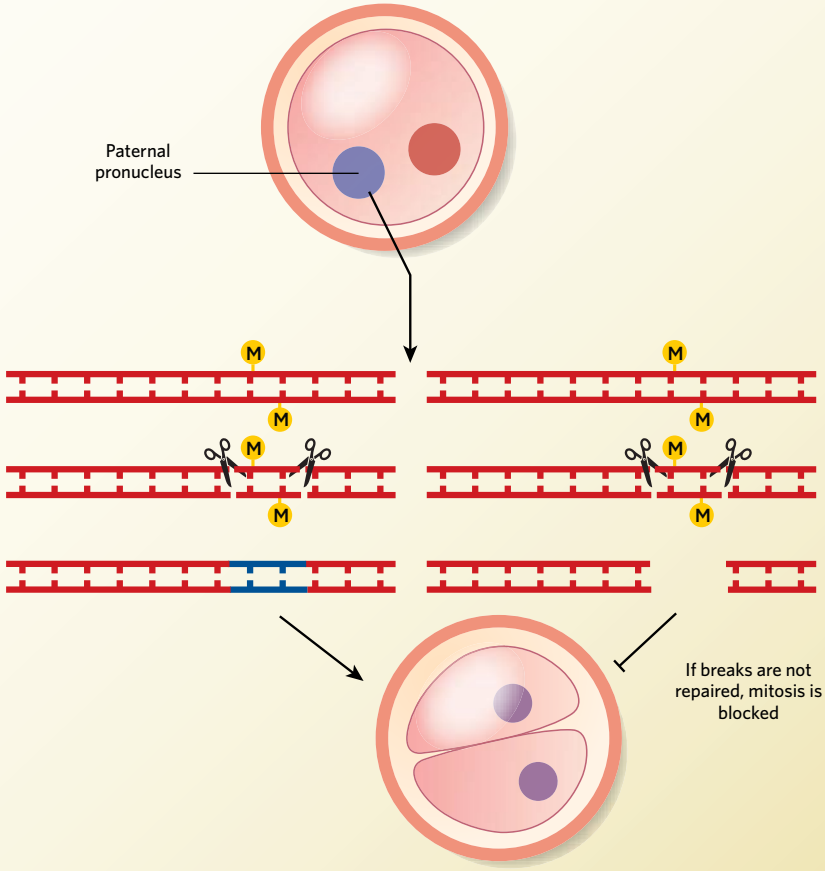
Luckily, as she and her colleagues discovered last year, the cell has a surveillance mechanism to ensure that development does not continue if the breaks go unrepaired. By knocking out key components of the DNA repair pathway, Tachibana-Konwalski and a colleague found that when lesions remained, the zygote did not undergo its first cell division.[9] "This was the first evidence that epigenetic reprogramming is monitored in the context of the cell," she says. "So if reprogramming is delayed, then the zygotes will not enter first mitosis."

Although many questions remain, continued study of the reprogramming process—both at the level of overall chromatin structure and of DNA methylation—will be important for understanding exactly what controls the initiation of embryonic transcription. While transcriptomic surveys over the past several years have begun to document which genes are expressed very early in development, what triggers those transcriptional changes remains a key question in the field. This year, taking a closer look at one of the first genes turned on, EPFL's Trono and colleagues identified what they think might be an important clue.

It all started with the discovery in the 1990s that patients suffering from facioscapulohumeral muscular dystrophy harbor mutations in a gene called *DUX4* that cause the gene to be overexpressed. Then, in 2012, Stephen Tapscott of the Fred Hutchinson Cancer Research Center and colleagues forced the production of DUX4 protein—which is normally epigenetically repressed—in cultured human myoblasts and observed the upregulation of a suite of genes known to be active during early embryonic development.[10] This caught the attention of Trono, who decided

DNA DEMETHYLATION

There are likely many mechanisms governing the global demethylation of the zygotic genome following fertilization. One mechanism at play in the paternal pronucleus involves the excision of the methylated DNA by breaking and repairing the double helix. As those breaks are repaired, nonmethylated cytosines are inserted where methyl marks used to reside. One recent study showed that if these breaks are not repaired, the embryo delays the first cell division.



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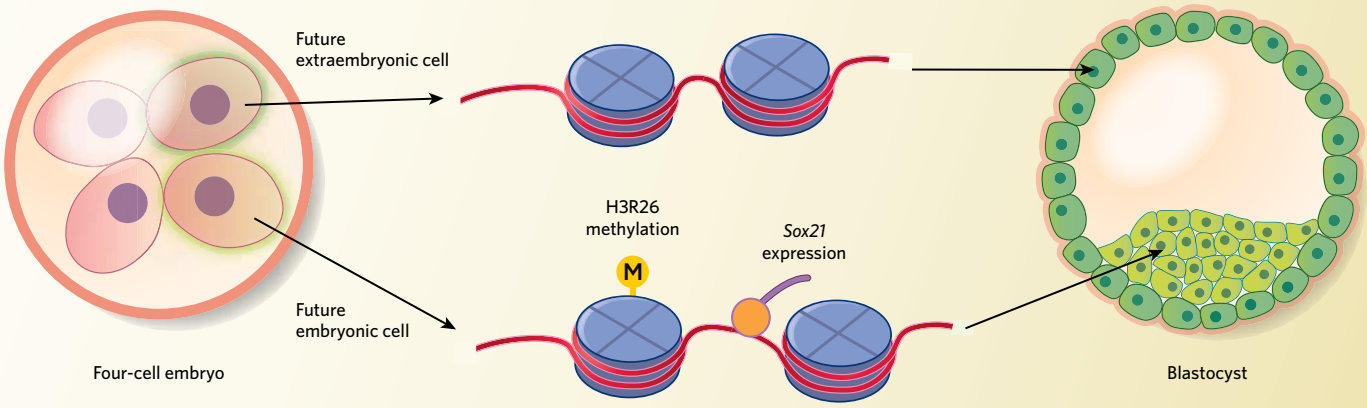
CELL-FATE DETERMINATION

Recent research has shown that cell-fate bias stems from methylation of arginine 26 on histone 3 (H3R26), which lengthens the time certain transcription factors remain on the DNA. Longer binding promotes expression of genes such as *Sox21* that drive cells to become the embryonic lineage (blue) that will form the fetus, while cells with shorter binding form the extraembryonic lineage (green) that becomes the placenta.



to probe deeper into DUX4's potential role in embryonic genome activation.

Existing data on gene expression in human and mouse embryos confirmed *DUX4* is expressed just before full embryonic genome activation. When Trono and his colleagues overexpressed the gene (known simply as *DUX* in mice) in mouse embryonic stem cells, they also saw an induction of the expression of other genes active in early development. The team further demonstrated that DUX bound to the promoters of some of these genes. Finally, deleting *DUX* in mouse embryos just before the two-cell stage—a tricky methodological feat achieved using the CRISPR-Cas9 gene-editing system—the researchers blocked embryonic genome activation altogether.[11] "That was the nail in the coffin, I would say," Trono says. "What this strongly suggests is that DUX is the gene product that kicks it off."

"With the identification of the DUX transcription factors, this has opened up an avenue to understand the first wave of transcription factors," agrees Tachibana-Konwalski. But the question remains—what initiates *DUX* expression? "Even with DUX, it appears that there must be some upstream factors, and this we are still totally ignorant on," she says. "The jury is still very much out on what the master totipotency factor is in mammals."

Cell-fate decisions

While many groups continue to hash out the molecular factors governing embryonic totipotency (which differs from pluripotency; see box on opposite page), others are looking forward to the next important milestone in embryonic development—determining what dictates which cells will form the baby itself and which cells will form the placenta. "When one follows later lineages, there will be differences that one would like to trace back, and ultimately one will trace them back to the zygote and its initial cell-fate separation," says Rickard Sandberg, a computational geneticist at the Karolinska Institutet in Sweden. Once again, single-cell technologies are allowing researchers to do just that.

Over the past several years, the labs of Zernicka-Goetz at the University of Cambridge and Nicolas Plachta at the A*STAR Institute of Molecular and Cell Biology in Singapore have independently shown that, in mammals, this decision isn't black-and-white. Although cells of mammalian embryos differ from one another early on, they retain flexibility in cell-type specification, Zernicka-Goetz explains. "Those fate decisions happen gradually, starting at the four-cell stage and possibly even earlier."

The big question, then, was how cells became biased toward forming one lineage over the other. Last year, Plachta and colleagues found that transcription factors such as Sox2 bind to mouse DNA for different periods of time at the four-cell stage, and that this correlates with cell fate.[12] In the same issue of *Cell*, Zernicka-Goetz's group published a study that further explained why: those murine cells with longer Sox2 binding start to express genes, including *Sox21*, that repress the expression of transcription factors associated with differentiation.[13] As a result, these cells preferentially form the interior population of cells that give rise to the fetus. "I think that this is one of the important discoveries over the last few years," Zernicka-Goetz says.

> Given how few of these cells there are, it's really amazing we can now look into these early stages of development.
>
> —Leonid Mirny, MIT

Of course, this all ties back to the epigenetic reprogramming that the zygote undergoes during its very first hours and days: the length of SOX2 binding is regulated by

CARM1, an enzyme that methylates arginine 26 on histone H3 (H3R26). "So as far as we know for now, everything starts with this particular epigenetic modification—methylation of histones—and this drives cell-fate specification," Zernicka-Goetz says. But what initiates CARM1's methylation of H3R26? "The situation is complex," she says. "Our group and many others are still trying to discover what it is that breaks the symmetry for the very first time."

Still, the progress that has been made in the past few years toward understanding the first hours and days of embryonic development is promising, Mirny says. "Single-cell techniques are still in their infancy across the board, so these are challenging techniques in general, but I think the picture is coming together."

The next frontier

Developmental biologists appear poised to answer many of the remaining questions about the transition from maternal to embryonic control of development that happens in the first few days after fertilization. The next challenge lies in the weeks that follow, says Zernicka-Goetz, a period into which researchers are just now getting their first glimpses. And so far, her group and others have demonstrated that embryos are more self-sufficient than previously appreciated.

Initially published in 2012,[14] with refinements made a couple of years later,[15] the new culture system designed by Zernicka-Goetz's team has successfully been used to sustain both mouse and human embryos until the point of gastrulation, when the three distinct embryonic cell layers—the ectoderm, the mesoderm, and the endoderm—form following implantation.[1,2] This work has demonstrated that embryos self-organize without input from their maternal host—at least, up to 13 days postfertilization. "Our work and Ali [Brivanlou]'s work show the same thing: that the embryo can organize itself outside the body of the mother," says Zernicka-Goetz. "It doesn't need the maternal information at that stage of its life, which I think is incredible and unexpected."

In addition, these experiments have revealed how the different types of cells in the early embryo interact with one another. This year, Zernicka-Goetz and her group used that knowledge to replicate those interactions using mouse embryonic stem cells and extra-embryonic trophoblast stem cells. Placed in a dish with a 3-D scaffold that resembled the extracellular matrix, the cells assembled to create the first-ever synthetic mouse embryos.[16] While these entities will likely also be the subject of regulations that limit their development in culture, they provide yet another window into the "black box of development" that is the period following implantation, says Zernicka-Goetz.

In combination with advances being made in the study of the first week of development, the study of embryogenesis continues at an unprecedented pace. The next few years should see the publication of new insights into the miracle of life, says Tachibana-Konwalski. "It's an amazing and dynamic field." ■

TOTIPOTENCY VS. PLURIPOTENCY

The zygote and the cells of the two-cell embryo, and to some extent the four-cell embryo, are considered totipotent—each cell is capable of giving rise to a whole organism. Over just a few days, however, they lose this ability. Some cells become destined to form the extraembryonic lineage that forms the placenta, while others are fated to become the fetus itself. Cells in this second group—the so-called embryonic lineage, from which embryonic stem cells are derived—are said to be pluripotent, in that they are capable of differentiating into all the cell types of the body, but not able to form the organism on their own. "Typically people have thought that pluripotent [cells], like stem cells, and cells from early embryo are the same thing," says Maria Elena Torres-Padilla, an epigeneticist at Helmholtz Zentrum München in Germany. "They are very different, not only developmentally, but also now we can molecularly distinguish them very clearly."

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LIFE ON MARS

Growing evidence points to a once-habitable world—
and recent findings suggest that life could exist on the planet today.

BY DIANA KWON

This September, tech mogul Elon Musk unveiled his updated plans for colonizing Mars. By 2024, he said, his aerospace company SpaceX plans to deliver people to our neighboring planet in massive rocket ships, which he hopes to start constructing within the next year. Although perhaps the boldest declaration yet (outside of science fiction) of intent to actually spearhead extraterrestrial habitation, Musk's ambition reflects an age-old curiosity: Can the Red Planet support life? Has it ever before?

In 1976, NASA's Viking 1 and 2 set down on Mars with the primary mission of answering those questions. While the two landers discovered no clear signs of living microorganisms on the planet's barren surface, photographs taken from orbit revealed geological features that suggested a once-watery environment—dry valleys that resembled those created by rivers on Earth. "If you assume that liquid water is all life needs, then this could count as the first evidence that life might have been possible on Mars in the past," says Alfonso Davila, a research scientist at NASA Ames Research Center in California.

Subsequent missions to the planet started to paint a clearer picture of its potential biological history. For example, in the early 2000s, NASA rovers *Spirit* and *Opportunity* discovered sediments and minerals that couldn't have formed without water, as well as materials, such as patches of silica, typically found in hot springs and steam vents, where extremophiles thrive on Earth. Most recently, the rover *Curiosity,* which landed on the planet in August 2012, has detected simple carbon-based organic compounds in the Gale Crater, a large cavity near the Martian equator.

Despite growing evidence that Mars might have been teeming with life eons ago, exploration of the planet has painted a bleak image of its contemporary environment. Because it lacks a thick atmosphere and a magnetic field, which are essential for making Earth a hospitable place to live, Mars is exposed to harmful ultraviolet (UV) light and ionizing radiation from cosmic rays. Those features, along with low temperature and pres-

MARTIAN MISSION: Since landing on the Red Planet on August 5, 2012, NASA's *Curiosity* rover has roamed the environment collecting samples and taking photos in search of signs of life, both past and present.

NASA/JPL-CALTECH/MSSS

sure, "make the environment pretty hostile to life as we know it," says Manish Patel, a senior lecturer in planetary sciences at the Open University in the U.K.

Nevertheless, scientists are uncovering aspects of the planet that indicate Mars could still be harboring isolated pockets of life. Although the chances may be small, these findings have major implications for continued missions to the Red Planet—and, of course, its potential future colonization by humans. (See "A Hostile Planet" on page 41.)

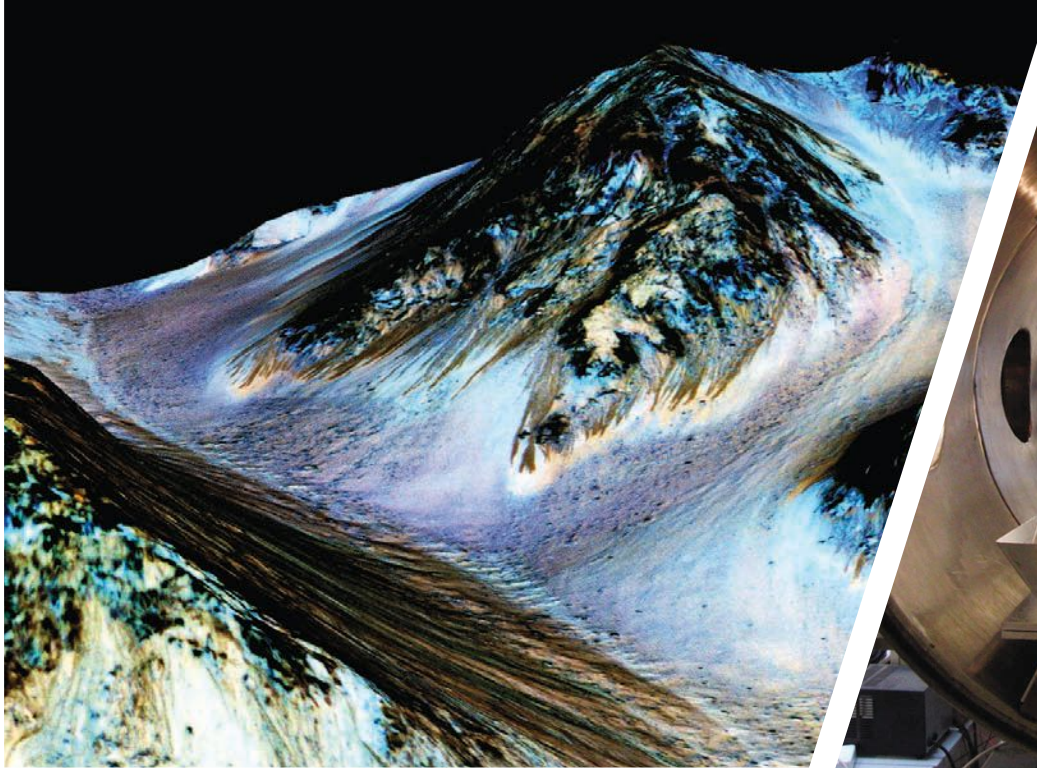
Water marks

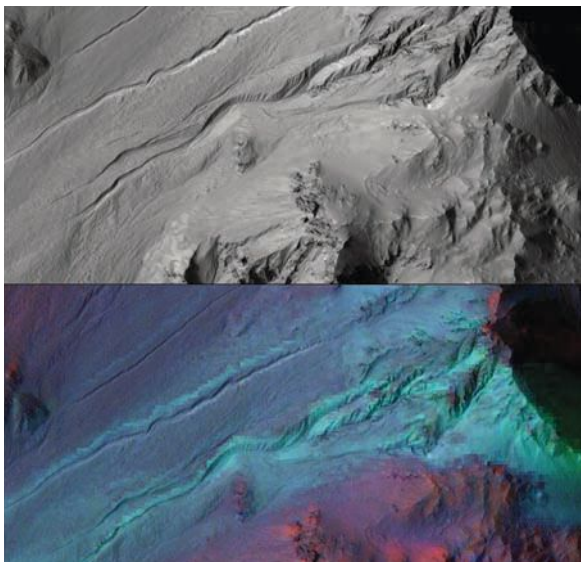
Remnants of a wet Mars remain the clearest hint that the planet once could have harbored life. Data gathered by *Curiosity* point to the existence of a massive freshwater lake in the Gale Crater billions of years ago, and scientists' analyses suggest this environment had habitable conditions: a relatively neutral pH, low salinity, and elements that make up the building blocks of life—carbon, oxygen, hydrogen, sulfur, nitrogen, and phosphorus.[1]

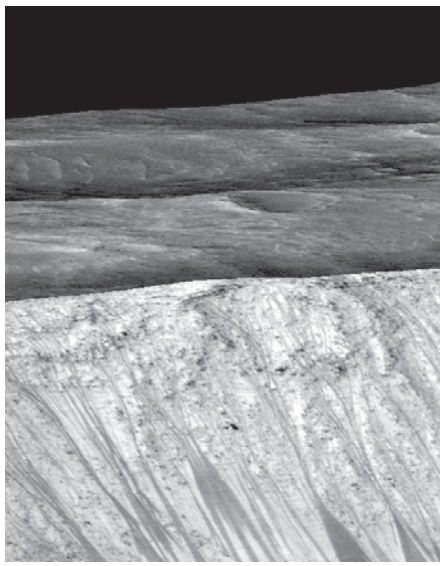
Curiosity has also detected evidence of simple organic molecules in this region, including methane,[2] chlorobenzene,[3] and hints of longer-chain molecules resembling fatty acids[4]—all of which have primarily biological origins on Earth. "The consensus is that Mars had a lot of water in its ancient past, and that life could have existed and grown then," Patel says. (See "Ancient Microbes" on page 42.)

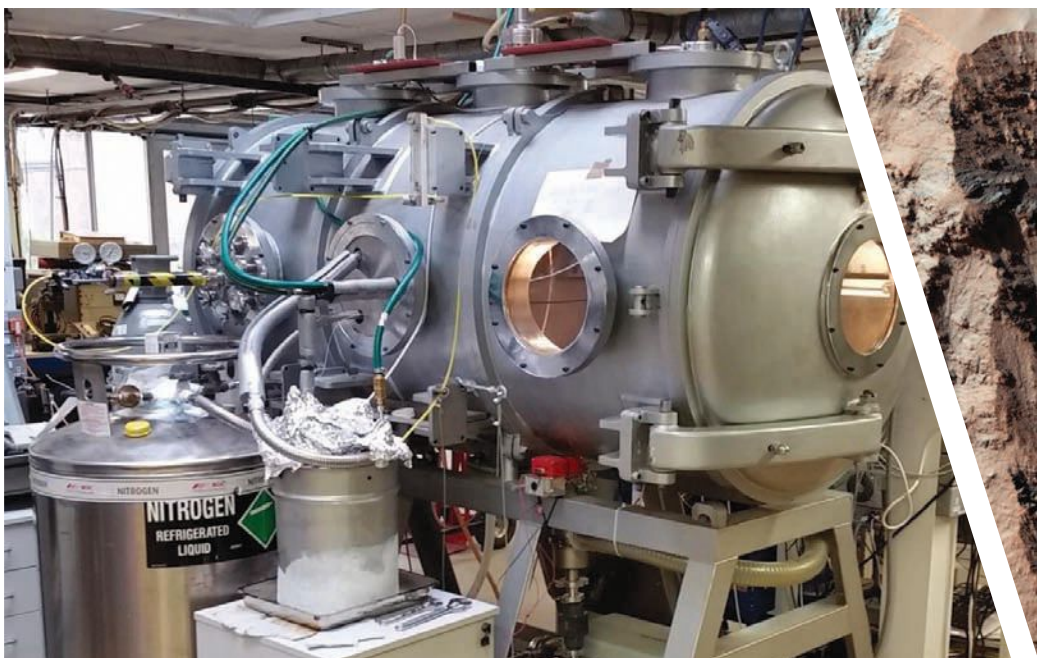
Nowadays, however, confirmed sources of Martian water exist solely as ice, primarily in the planet's polar regions, with very recent evidence pointing to the possibility

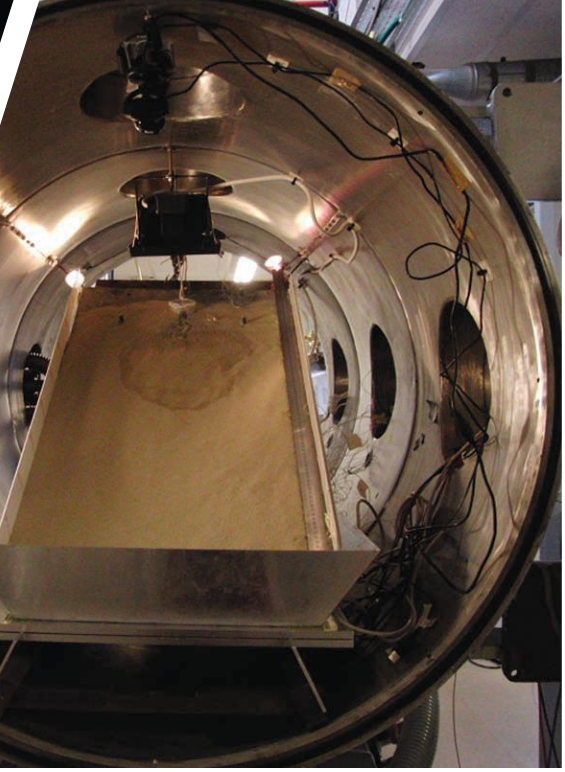
Despite growing evidence that Mars might have been teeming with life eons ago, exploration of the planet has painted a bleak image of its contemporary environment.

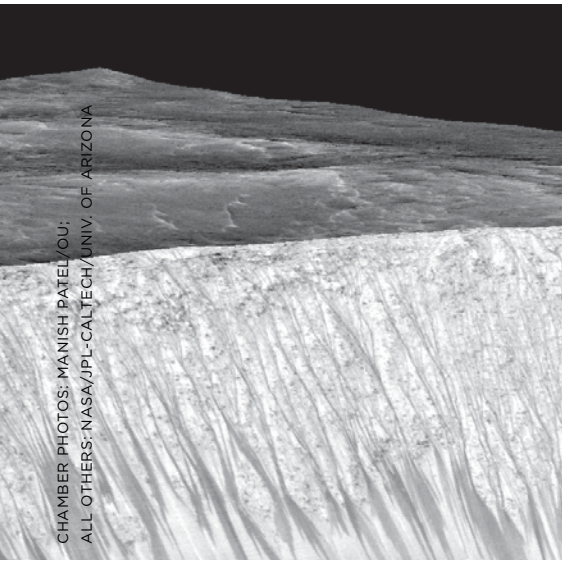












CHAMBER PHOTOS: MANISH PATEL/OU; ALL OTHERS: NASA/JPL-CALTECH/UNIV. OF ARIZONA

A RIVER RUNS THROUGH IT?: Two features of Mars's surface suggest that water may, at times, flow on the planet. Channels known as gullies (left, middle; right, bottom) that appear on steep slopes look comparable to formations created by flowing water on Earth, although recent analyses indicate that these were likely formed by other processes. More recently, researchers have identified recurring slope lineae (RSLs; left, top; right, middle), seasonal streaks also suggestive of flowing water. The primary theory, based on the identification of perchlorates, is that RSLs are formed by brine, or very salty water. Where the water would come from is still a mystery, and alternative theories challenge the idea that water is needed to form such structures. For example, some scientists have posited that dry sand avalanches could result in the same streaking pattern. Experiments in the Open University's Mars chamber (left, bottom; right, top), which simulates the environment on the planet, could help determine the conditions that form these geological structures.

of ice patches at much lower latitudes, near the planet's equator.[5] And life—at least as we know it—needs liquid water to survive.

In 2000, scientists detected Martian gullies, channels traversing the landscape that appear similar to those created by flowing water on Earth.[6] Images that the Mars Global Surveyor spacecraft captured along the sides of craters, pits, and valleys suggested that these formations are relatively young, as they lack geological features such as impact craters or dusty dunes. These images hinted at the possibility that liquid water might have existed in the planet's recent past—and might still sometimes be present on the planet's surface. More evidence for this idea emerged a few years later when researchers reported that new, light-colored streaks in the form of fingerlike branches had appeared in some of the gullies, further signaling recent activity.[7]

Subsequent analyses, however, revealed that the streaks could have been produced through other processes. In 2010, based on images from the Mars Reconnaissance Orbiter (MRO), scientists reported that the streaks appeared only during the Martian winters. During that time of year, water stays frozen and dry ice builds up on the planet's surface, meaning that carbon dioxide, a gas that makes up more than 90 percent of the planet's atmosphere, may have been the cause.[8]

Sure enough, when Patel and his colleagues tested this hypothesis last year, they found it to be a likely explanation. In the Open University's Mars Chamber, which simulates the temperature, pressure, and atmospheric composition of the Red Planet, the researchers deposited carbon dioxide frost onto the surface of soil, then warmed the chamber with a heat lamp to mimic what happens when the sun rises. The resulting process of sublimation—where a solid transitions directly into gas—was enough to create very similar formations.[9] And in another 2016 study, an independent group of researchers reported that data from MRO supplied no evidence of minerals associated with flowing water in those structures.[10]

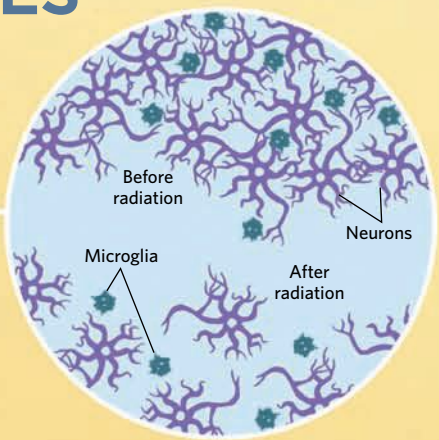
Meanwhile, another feature of the steep Martian slopes, dubbed recurring slope lineae (RSLs), has provided more-tantalizing evidence that the planet could occasionally host liquid water. Unlike gullies, RSLs are dark streaks that appear during the warmest parts of the year, growing in the summer, when ice is most likely to melt, and fading in the winter.[11] And although scientists have never directly detected liquid water, it may not take as much of it as some researchers expect to generate these features. In another Mars Chamber experiment, published last year in *Nature Geoscience,* Patel and colleagues placed a block of ice in the simulated Martian environment and found that a small amount of water, which boiled at much lower temperatures due to low pressure, was able to kick up the soil to create streak-like features.[12] "That showed that if there is water, you need a lot less than originally [thought]," Patel says. Altogether, the presence of liquid H_2O on the planet remains up for debate.

Salty surfaces

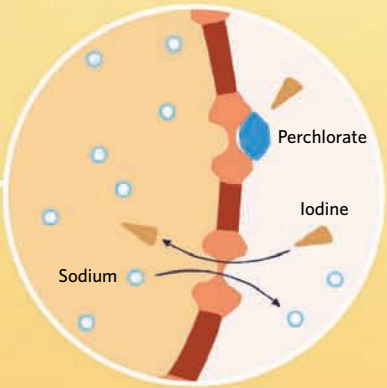
The case for contemporary water on Mars has been bolstered by signs of perchlorates, a type of salt, in the seasonal streaks.[13] Perchlorates lower the freez-

MARTIAN MALADIES

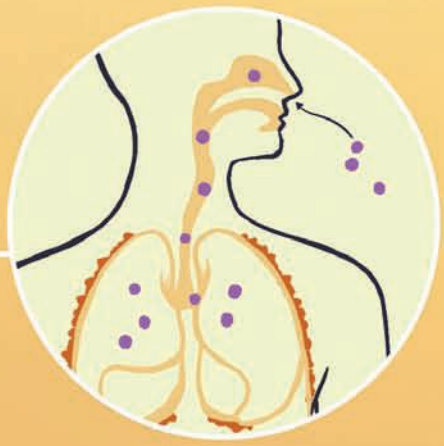
Humans may have grand dreams of colonizing Mars, but before that happens, scientists and engineers will need to devise ways to protect travelers from the planet's hostile environment. Spacesuits can help protect against most environmental harms, such as frigid temperatures and low oxygen. However, high levels of space radiation, which is the biggest concern, will be the most difficult to avoid.



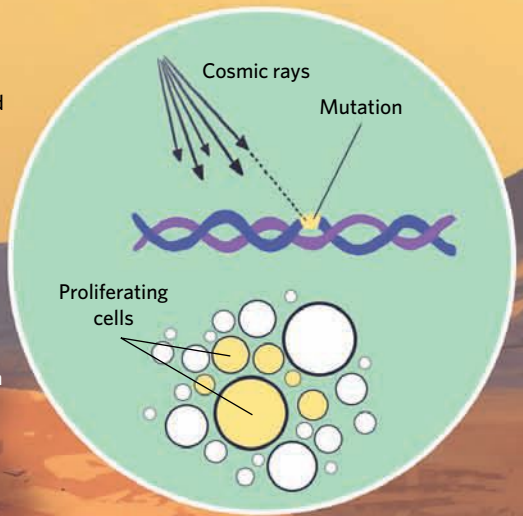
BRAIN
Studies on rodents show that after exposure to cosmic radiation, the neurons in the brain suffer significant damage, primarily in the medial prefrontal cortex, a region involved in key cognitive functions, including decision-making and memory.



THYROID
Perchlorates, a type of salt found in Martian dust, can impair thyroid gland functioning by inhibiting the uptake of iodine, a building block of hormones produced by the organ. If ingested, the salts block the activity of sodium iodide transporters on thyroid cells.



LUNGS
Aside from the basic problems associated with breathing in fine-grained particles, Martian dust could contain chemicals hazardous to human health.



CANCER
Extended exposure to cosmic rays can increase the chances of developing tumors by causing carcinogenic mutations and modifying the tissue microenvironment. Cancers that are already common, such as those of the lung, liver, and blood, would see the greatest uptick.

A HOSTILE PLANET

NASA hopes to send humans to Mars by the 2030s, and private companies, such as SpaceX, Mars One, and Lockheed Martin, have grand plans to establish human settlements on the planet. But big questions remain about the plausibility and safety of such missions.

People who land on the Red Planet will face harsh conditions, such as frigid temperatures, low pressure, and an atmosphere with precious little oxygen. Micron-size dust particles may also be a major factor, as they could cause respiratory problems and contain toxic materials. In addition, Martian soil contains abundant amounts of perchlorates, a type of salt that can impair the functioning of the human thyroid, which could be hazardous to scientists digging in the dirt.

On the other hand, perchlorates might actually be extremely useful during a mission to the Red Planet. Not only are they a component of rocket fuel, the compounds could also be a source of oxygen for human consumption: many microbes metabolize perchlorates, generating this element as a by-product, and some scientists have proposed prototypes of portable emergency systems that exploit these microbial pathways to generate breathable air (*Int J Astrobiol*, 12:321-25, 2013).

A much more serious concern about living on Mars is radiation. Without a protective magnetic field like that surrounding the Earth, the surface of the Red Planet is constantly bombarded with galactic cosmic rays—high-energy particles from space that can lead to a variety of health problems. At the doses of cosmic radiation that humans would receive on a trip to the Red Planet, one of the primary problems they will face is cancer. According to analyses by Francis Cucinotta, a radiation biologist at the University of Nevada, Las Vegas, astronauts on the International Space Station can exceed their lifetime limits of radiation, based on NASA's radiation standards, in just 18 months for women and two years for men (*PLOS ONE,* 9:e96099, 2014). And radiation levels would likely be even higher on a trip to Mars, which is far beyond the Earth's protective magnetosphere. (The cancer risk is slightly higher in women because they have the added concerns of breast and ovarian cancer plus a greater risk of developing lung cancer, although the latter association is not well understood, Cucinotta says.)

Rodent experiments have revealed that exposure to radiation akin to that experienced on Mars can lead to an increased risk of cancer in "bystander" cells close to those damaged by radiation, which can release "oncogenic signals" (*Sci Rep,* 7:1832, 2017). Radiation exposure can also alter the tumor microenvironment in ways that promote cancer. Using mouse models of breast cancer, Mary Helen Barcellos-Hoff, a radiation oncologist at the University of California, San Francisco, and her colleagues discovered that when healthy epithelial cells were transplanted into an animal that had been exposed to Mars-like radiation, tumors developed from those unirradiated cells (*Cancer Cell,* 19:640-51, 2011). "You create the seed of the cancer with mutations, but they still have to be in the appropriate soil for the cancer to actually develop," Barcellos-Hoff says. "[We've found that] the kind of radiation found in space likely perturbs [the tumor microenvironment] in a more profound way than radiation that's found on Earth."

More recently, scientists have amassed evidence suggesting that cosmic radiation may have worrisome effects on the brain. Specifically, Charles Limoli of the University of California, Irvine, and colleagues have shown in animal experiments, mostly with rodents, that these galactic particles can cause deficits in learning and memory, reduce the complexity and density of dendritic spines, and lead to persistent neuroinflammation (*Sci Adv,* 1:e1400256, 2015; *Sci Rep,* 6:34774, 2016). "The data suggests that the irradiated brain is never normal," says Limoli. "Now, how precisely these cognitive deficits will manifest and impact astronaut performance is another important question that's very difficult to pinpoint."

While radiation risks are concerning, they are not deal breakers for future Mars travel, Limoli says, and researchers are now working on ways to mitigate these issues. For example, NASA is exploring ways to protect astronauts from radiation with compounds that repair damaged DNA. One such compound is nicotinamide mononucleotide, which scientists recently reported could reverse aging in mice by activating processes involved in DNA repair (*Science*, 355:1312-17, 2017).

In addition, Limoli and his colleagues are developing drugs that could help alleviate radiation effects in the brain. "We're working on a variety of pharmacologic interventions," Limoli says. "[And] we can always hope that our engineering colleagues come up with better and better shielding."

ANCIENT MICROBES

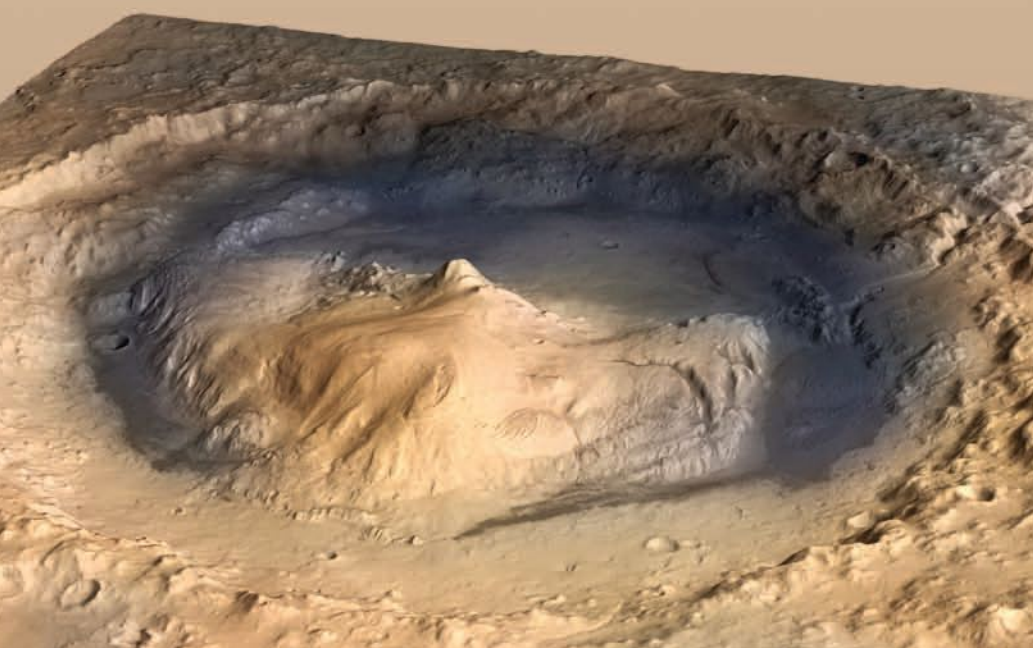
The chances of finding life on Mars today may be slim, but many scientists believe that the planet hosted living organisms at some point during its history. One of the most promising regions for ancient Martian life is the Gale Crater, a large region near the planet's equator. Data gathered from the crater by rovers and orbiters have revealed evidence both of past (and possibly present) water and of simple organic molecules—two essential ingredients for life.

Recently, while examining data collected by the rover *Curiosity,* a group of researchers discovered boron, a chemical element that can stabilize the sugars used to make RNA (*Geophys Res Lett,* 44:8739-48, 2017). Some scientists believe that this element may have even contributed to the origin of life on Earth. "Boron, when it's dissolved in water, has very special properties—it can react with organic molecules to form other types of organic molecules," says Patrick Gasda, a postdoc at Los Alamos National Laboratory. "We found boron in this area [that used to have] lots of water; if there were organics there, that could actually mean that you could do these types of reactions on Mars."

Scientists currently only have speculative estimates about when the Red Planet was last amenable to life. For example, NASA researcher Alfonso Davila and his colleagues have proposed that parts of Mars may have been habitable as recently as 5 million to 10 million Earth years ago (*Astrobiology,* 13:334-53, 2013). They estimate that during that period, the planet was tilted at an angle that may have provided polar regions with enough solar energy to melt the subsurface ice. After completing additional analyses, the researchers also posited that the water composition in the atmosphere during these periods was similar to that seen in the driest parts of the Atacama Desert in Chile, where microbes have been found living in extremely arid soil (*Astrobiology,* 16:159-68, 2016).

"While this does not necessarily mean that Mars was as habitable as the Atacama during those periods, it does suggest that the habitability window near the surface might have closed not billions of years ago, but perhaps tens of millions to several hundred million years ago," Davila says. And the current conditions on the planet, while probably not conducive to modern microbial activity, are promising for researchers searching for signs of living organisms in the planet's history, he adds. "Those same conditions, extreme dryness and extreme cold, that prevent life from being active in the environment are also very good at preserving evidence of life."

A LIVING LAKE?: More than 3 billion years ago, a massive meteor hit Mars, creating an approximately 155-km-wide crater in the planet's surface. Data from NASA's *Curiosity* rover suggest that this area, known as the Gale Crater, was once filled with water, and may even have hosted life. Analysis of the sediments also points to once-habitable conditions, with evidence of simple organic molecules that may have originated from biological sources.



NASA/JPL-CALTECH/ESA/DLR/FU BERLIN/MSSS

ing point and evaporation rate of water, which would allow H_2O to exist as a liquid in Martian conditions. On Earth, perchlorates also act as an energy source for some microorganisms.

"People were getting really excited because they were thinking, well, bacteria can metabolize perchlorates, so perhaps these are potential habitats that we could maybe explore on future missions," says Jennifer Wadsworth, a PhD student in astrobiology at the University of Edinburgh. "So we thought, okay, well let's look at perchlorates and see [whether] bacteria could survive under Martian conditions."

As it turned out, when bathed in UV light, these salts can actually be lethal. When Wadsworth and her advisor exposed the soil bacterium *Bacillus subtilis* to perchlorates while irradiating the cells with UV levels typical for the Martian surface, the microbes died within minutes.[14] "Perchlorate seems to be quite abundant everywhere, and the radiation penetrates quite a few meters [beneath the planet's surface], according to models," Wadsworth says. "So it could mean that the top few meters of soil are in fact uninhabitable." However, she adds, this finding does not rule out the possibility that there might be extremophiles that could survive these conditions, or that more-conventional microbes live farther underground.

Deep below the surface, UV and ionizing radiation are significantly reduced, while pressure and temperature begin to increase. "You can reach a point where you're shielded from all the nasty things, and the temperature and pressure could be high enough to allow a habitable environment," Patel says. "The evidence is piling up that if we want to find these signs of life on Mars, we really need to get down below the surface to get away from nasty oxidants and environmental influences."

Curbing contamination

Of course, the most definitive way to confirm life on Mars would be to collect live or previously living specimens. *ExoMars*, a rover that the European Space Agency plans to send to Mars in 2020, will be equipped with a drill that can extract

soil samples from depths down to two meters, the deepest of any Mars sampling to date. The robot's onboard laboratory will carry out tests on collected specimens. Another upcoming rover expedition, NASA's Mars 2020, plans to collect samples to set aside for future missions to ferry back to Earth.

Without knowing exactly what life-forms, if any, exist on our red, dusty neighbor, it is difficult to predict what people might encounter when they eventually get there. "How do you look for something that you don't know [about]?" Patel asks. "It's a real problem that we face. All we can do is look for what we do know—and even then, it's incredibly difficult to measure everything."

The evidence is piling up that if we want to find these signs of life on Mars, we really need to get down below the surface to get away from nasty oxidants and environmental influences.

—Manish Patel, Open University

Directly probing for life on the Red Planet takes some finesse, as scientists must ensure that they do not accidently misidentify organisms that hitched a ride from Earth as Martian. Although it is not possible to reduce the risk of contamination to zero, researchers can take measures to lower the chances that they will introduce Earthly organisms into their experiments. *Curiosity,* for example, is barred from exploring the RSLs, due to concerns that the rover, which was not completely sterilized prior to launch, might contaminate the suspected water in those regions.

"Being able to clean [spacecraft] well enough to identify Mars microbes if they might be present and distinguish them from the residual contamination from Earth is an extremely challenging problem," says Cassie Conley, NASA's planetary protection officer. Future rovers will be subjected to various sterilization strategies before launch, including wiping down surfaces with sterilizing solutions, baking heat-resistant components at high temperatures, and using highly sensitive biosensors to identify the presence of microbes.

Researchers are also trying to ensure that the human explorers NASA plans to send to Mars by the 2030s do not contaminate the planet—a much more difficult task, as most of the methods used to clean spacecraft cannot be applied to people. "We can be confident about how much contamination we sent on [robots], because we can measure it before launch and be confident that it won't increase," Conley says. "Once humans start landing on Mars, there will be associated microbes that come along."

Monitoring microbial migrants within astronaut communities is also important for managing human health. In a study published earlier this year, Kasthuri Venkateswaran, a senior research scientist at NASA's Jet Propulsion Laboratory who is involved in the Planetary Protection Program, and colleagues found that after four people spent 30 days in an enclosed habitat that mirrored conditions on the International Space Station, the diversity of certain fungi—including those associated with allergies and asthma—in their surroundings increased.[15] In another recent investigation, researchers reported that bacterial communities in a simulated spacecraft changed after hosting six crew members for 520 days.[16] In this case, cleaning agents were able to keep the microbial populations under control, pointing to the importance of maintaining strict sterilization protocols in space.

Keeping any potential life-forms native to Mars from hitching a ride back to Earth is another concern. Scientists and policy makers want to ensure that samples brought back by rovers or human explorers—or living organisms that accidently hitch a ride—will not endanger species on Earth. Such Mars-to-Earth contamination, Conley says, presents "a much more complicated set of questions about public health and the potential for invasive species." ■

Diana Kwon is a freelance science journalist living in Berlin, Germany.

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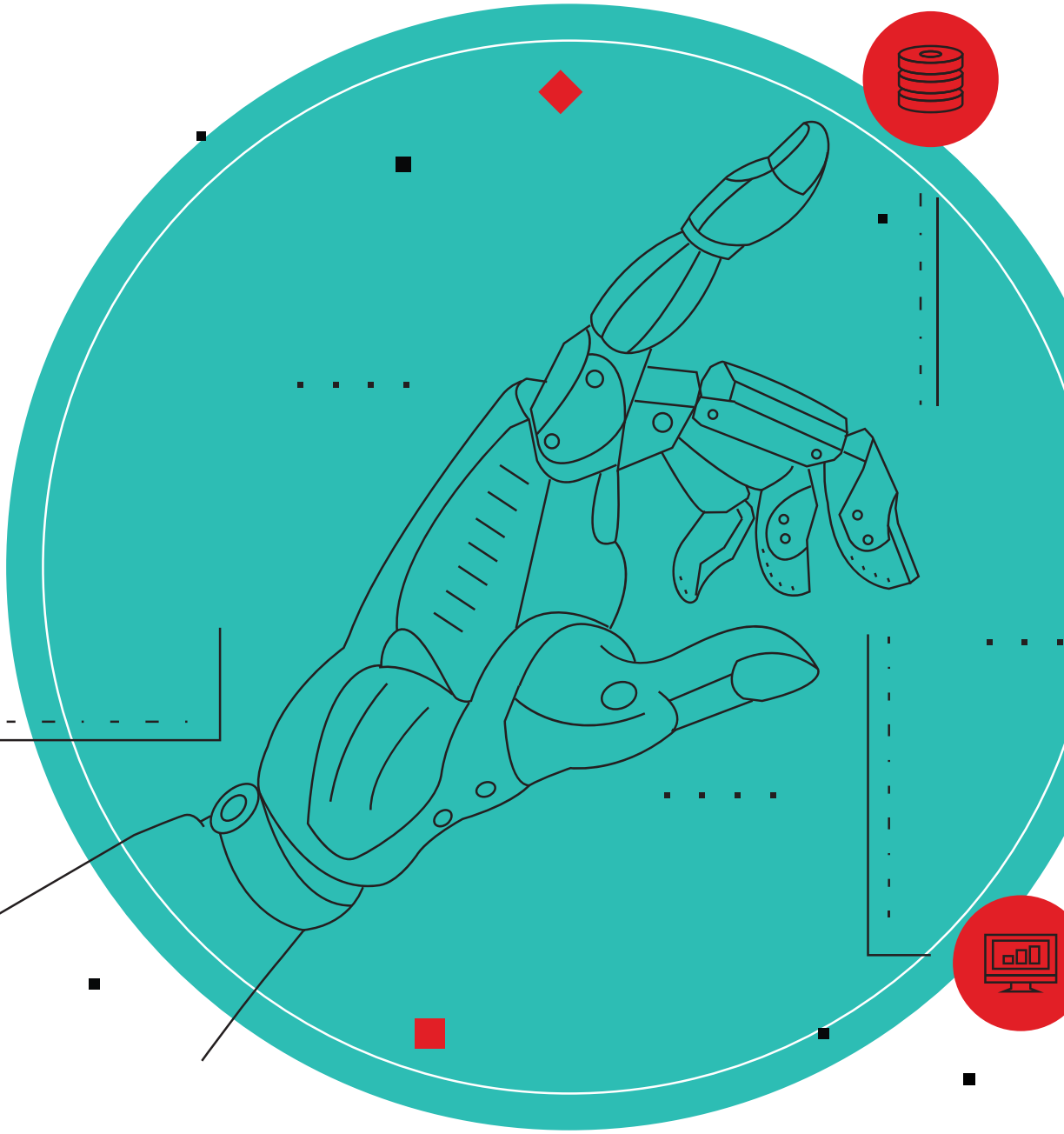


2017

TheScientist TOP 10 INNOVATIONS

From single-cell analysis to whole-genome sequencing, this year's best new products shine on many levels.

BY *THE SCIENTIST* STAFF

Innovation comes in many forms, molded into various outlooks, adapted to shifting time frames. Sometimes, technological and conceptual progress is undergirded by a more expansive view to encompass the bigger picture—think evolutionary theory or the widespread applicability of Sanger sequencing. Other times, innovation, especially in the life sciences, is achieved by zeroing in on the minute components that make biology tick—receptors, cells, organelles.

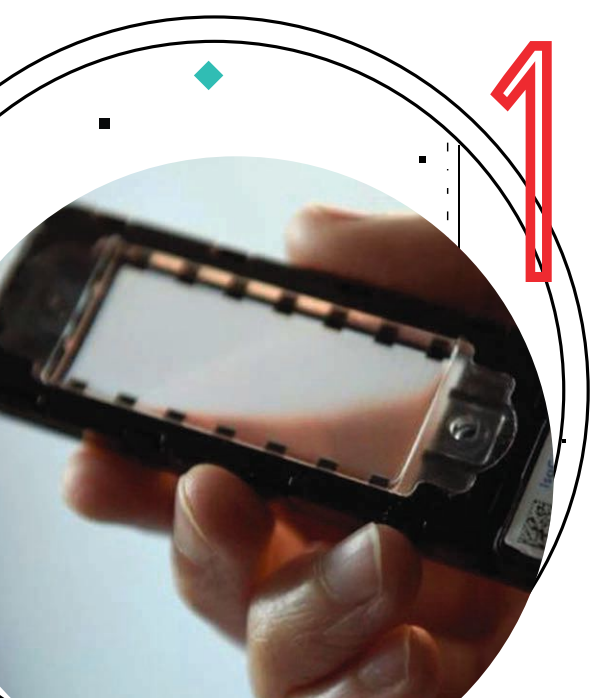
This year's Top 10 Innovations highlight breakthroughs on this fundamental scale. Winning products that include cutting-edge single-cell protein and gene expression analyses, souped-up Cas9 proteins for CRISPR-based genome editing, and culture systems for research organoids illustrate the innovative drilling down into fine-scale biology. Other winners, such as a handheld blood-testing device and a biomarker detection system, underscore the importance of technological development in the clinical laboratory.

In all, 2017 has brought us another bright crop of innovative products, selected by our independent panel of expert judges. *The Scientist* is proud to present this year's Top 10 Innovations.

1

IsoPlexis ◆ IsoCode Chip

This new single-cell technology allows researchers to characterize cells based on the proteins they secrete—as many as 42 different cytokines, chemokines, and other molecule types at once. Commercially launched this February by Branford, Connecticut–based IsoPlexis, IsoCode chips contain thousands of long microchambers that house only single cells. Within each microchamber, 15 spatially separated slots contain up to three different antibodies targeting specific secreted proteins; upon binding, the antibodies fluoresce in three colors, allowing researchers to distinguish the proteins.



"The ability to profile thousands of individual T cells or immune cells at once, the ability to basically, for each of those immune cells, get between 30 and 45 secreted proteins per cell, that's the real innovation," says IsoPlexis CEO Sean Mackay. Existing technologies either measure cells en masse, losing granularity, or look at only a few secreted proteins per individual cell, he notes. "Instead of just a few, you can now look at 40 secreted proteins per cell—that's a real big leap in the field."

Among the potential applications for IsoCode chips is the analysis of CAR T cells, which are currently being developed for various blood cancers. For example, researchers at Kite, a Gilead company, have found that the assay—and the built-in algorithm that calculates the so-called polyfunctional strength index (PSI)—associates strongly with patients' likelihood of response to the company's recently approved CAR T-cell therapy for non-Hodgkin's lymphoma. "It's quite powerful," says John Rossi, director of translational sciences at Kite. "Current assays that rely on a single-plex ELISA or even multiparametric flow cytometry don't give you the level of resolution that the IsoPlexis platform can provide."

IsoCode chips come in 10 different panels, ranging from 24 to 42 antibodies per panel, at a cost of $500–$600. The automated IsoLight imaging and workflow platform can be purchased starting at $200,000. But the IsoCode chips can also be paired with other fluorescence microscopy systems.

CRUICKSHANK-QUINN: *"The IsoLight single-cell technology, with its ease-of-use, has the potential to impact cancer research for both biomarker discovery and patient monitoring."*

Abbott ◆ i-STAT Alinity

Abbott's latest version of its handheld blood-testing device, the i-STAT Alinity, has all the bells and whistles to make point-of-care assays more user-friendly. Roughly the size of a 1980s cell phone, Alinity is packed with technology unthinkable three decades ago. Various cartridges loaded into the device can perform myriad tests on a blood sample of just several drops, including glucose levels and hematocrit, with results delivered to clinicians within minutes.

Narendra Soman, the director of R&D for Abbott's Point of Care Diagnostics business, says one of the improvements in i-STAT is a large color touchscreen, which signals users with audio and visual cues if a patient's levels fall into a concerning range. "The visual display is a fantastic feature," reminiscent of a smartphone, says Geoff Herd, the point-of-care testing coordinator at Whangarei Hospital, New Zealand, in an email. His colleagues use Alinity in the maternity ward and emergency room. "The system has been so well designed it is easy for users to get test procedures right and hard to get them wrong," he says.

"We added a lot more functionality for test results," Soman adds. "Once a blood result is obtained, it can go from the instrument to a patient's medical record."

The gadget's new, ergonomic design better suits the way health-care providers carry it around in the hospital. Before, i-STAT was designed to sit in a large pocket; now, Alinity's curves conform to the shape of an armpit. "What we noticed was nurses, essentially, wanted their hands free to carry other things," says Soman.

Alinity came on to the market a year ago, and is available in about four dozen countries for $7,000 to $12,500 USD, but is not yet available in the U.S. Soman says Abbott is waiting for a few more assays to be cleared by the US Food and Drug Administration before selling it stateside.

CRUICKSHANK-QUINN: *"The i-STAT Alinity can be used in any setting due to its portability and ease-of-use to obtain information on the blood and organs. Only a few drops of blood with results in 2–10 minutes has immediate impact in point-of-care testing."*

QGel ◆ QGel Assay Kit for Organoids

Scientists can use animal-derived extracellular matrix (ECM) to nourish research organoids in their labs. But Switzerland-based QGel makes synthetic human ECM that has several advantages over those nonhuman products, says Colin Sanctuary, QGel's cofounder. For one, QGel's product, which was released in January, is synthesized in many different combinations of protein subunits "tuned" to the cell type of interest, based on what's known about the ECM components of particular human organs or tissues. QGel is also consistent from batch to batch, so it provides better replicability than animal-derived gels. And it's compatible with liquid-handling robots, unlike animal-derived products, which can clog the machines and need to be kept at difficult-to-maintain temperatures. Sanctuary says he hopes to see organoids grown from patients' cancer cells and used to craft personalized treatments. He predicts that if QGel rather than animal-derived media is used to grow the organoids, their use in clinical treatment will have a much smoother path to regulatory approval.

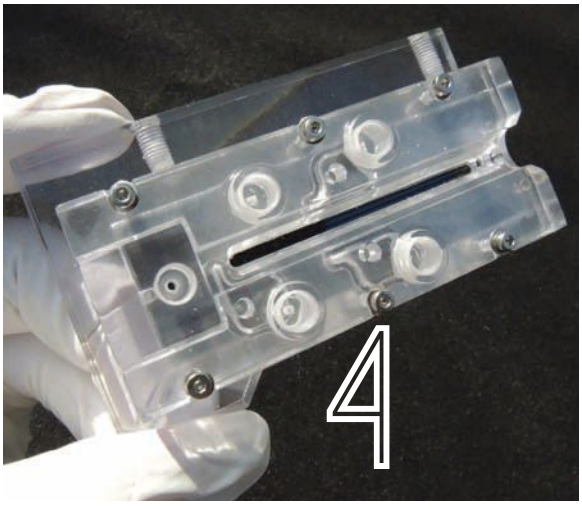
Oncology researcher Silvia Goldoni of Novartis tells *The Scientist* her group uses QGel to grow cancer cell lines, which they plan to use for drug screening, and patient-derived cells. "One of the things we're particularly interested in is the possibility to grow cells that historically have been very hard to grow," she says, given that growing cells in 2-D, or "in the absence of important ECM elements or other supporting cells types . . . really hinders our ability to model certain cancers in vitro."

A QGel Assay Kit for Organoids costs about $4,000 to $5,000, and enables approximately 3,000 experiments, Sanctuary says.

UNGER: *"This clearly has the potential to be transformative at both a scientific level and an economic level to the business of developing drugs and medical device interventions, by providing accurate 3-D, in vitro human tissue such as organs and tumors including the extracellular matrix."*

Intabio ◆ Blaze

Intabio's Blaze system for detecting and identifying protein isoforms aims to save pharmaceutical companies loads of time in laboratory prep work. Protein analytics that ordinarily take a month, says Intabio CEO Lena Wu, could happen in just a day with Blaze.



The system, set to launch within the next few months, would be deployed for quality control in biologics manufacturing. Typically, analysts seeking to find any abnormalities within a biologic sample separate components by capillary isoelectric focusing, then identify any isoforms via mass spectrometry. The two-step process of selecting samples and scaling them up for mass spec is time-consuming, Wu explains.

Blaze speeds things up by integrating detection, quantitation, and identification into one microfluidic system that sends proteins for mass-spec analysis immediately after detection, obviating the laborious process of prepping material for mass spec separately. "It completely changes the paradigm of when you can get this critical information about the quality of the product you're making," says Wu.

John Teare, the director of Late-Stage Development Program Management at Bayer Pharmaceuticals, says he's eager to test it out. He provided some of the biological material Intabio used to develop Blaze. "So many times we do isoelectric focusing and see an unusual peak and ask, `What is this?'" says Teare. "With Blaze you run it, and you say, `What's the mass of that peak?' And boom."

Although pricing is still yet to be set, Wu estimates the device will cost between $70,000 and $200,000, and a reagent kit for 100 samples will run between $5 and $10.

UNGER: *"This offers a truly dramatic increase in research productivity, which can immediately affect budgets and pipeline of products under development."*

Quanterix ◆ SR-X Ultra-Sensitive Biomarker Detection System

This August, Lexington, Massachusetts–based Quanterix brought its Simoa biomarker detection technology to the lab bench, launching the compact SR-X system. The platform offers more than 80 different assays to test samples—typically blood or serum, but some assays are also compatible with cerebral spinal fluid or single-cell lysates—for the presence of cytokines, other markers of neurodegeneration or neuroinflammation, and more.

Simoa, the SR-X's core technology, is also at the heart of the larger HD-1 system (the size of two side-by-side refrigerators), launched in 2014, explains Jeremy Lambert, director of product strategy at Quanterix. Because Simoa uses more magnetic beads relative to the proteins they're targeting, each bead captures only a single protein. Those protein-carrying particles are then pelleted, washed, combined with an antibody detector, and flowed across an array of 200,000 microchambers that can house only a single particle; there, the antibody detector interacts with a fluorogenic reporter molecule. "The ability to count individual beads provides the very high sensitivity that enables detection of very low concentrations of proteins," Lambert says. Researchers can look for up to six different target proteins in a single assay without compromising sensitivity, he adds.

The SR-X uses the same technology, but is much smaller. The size of a large microwave, it fits on a standard benchtop. And the SR-X's assay prep—including the incubation of samples with capture beads, for example, and the washing step—are performed by the researcher before the samples are fed into the machine.

"That gives a lot of flexibility to the end user, where they can vary the conditions of an assay," Lambert says. These steps can be performed using conventional lab devices that are part of a standard ELISA workflow, he notes.

CRUICKSHANK-QUINN: *"This benchtop instrument is able to detect protein and nucleic acid biomarkers directly from blood and tissue without the need for sample extraction and amplification steps."*

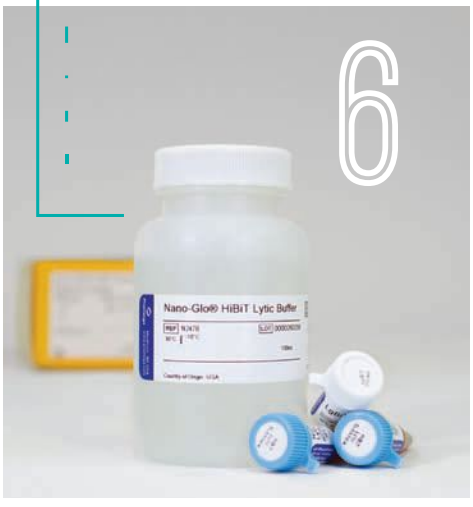
Promega ◆ HiBiT Protein Tagging System

Promega's new protein detection system excels at measuring protein levels across the cell. "The basic idea of the HiBiT Tagging System was to provide a really simple, sensitive bioluminescent method to quantify the abundance of a protein of interest, whether it be in the cell or on the cell surface," says Chris Eggers, a senior research scientist at Promega.

When the small and easily integrated 11-amino-acid tag (High BiT or HiBiT) interacts with the complementary Large BiT (LgBiT) 156-amino-acid component, they bind tightly and release detectable light. Researchers can incorporate the small HiBit tag just about anywhere on a protein of interest using CRISPR-Cas9, another preferred expression system, or one of Promega's plasmids, which can be purchased for $395. Promega also offers the option to license the sequence of the HiBiT tag free of charge. Detection reagents start at $160 and, depending on which reagents and volume are needed, cost as much as $8,925.

Biologist Julien Sebag of the University of Iowa has been using the system to study G protein–coupled receptor (GPCR) trafficking. He is happy with its speed, especially compared to ELISA. His group tags GPCRs with HiBiT and then measures both extracellular levels and total levels of protein to determine what he calls the "trafficking ratio" of the receptor. "The sensitivity is very good as well, so that allows us to express the proteins at lower levels—more physiologically relevant levels—and still be able to detect them," Sebag says.

UNGER: *"Interesting improvements facilitate small-peptide tagging, and are appropriate to CRISPR-Cas9, both very promising areas."*

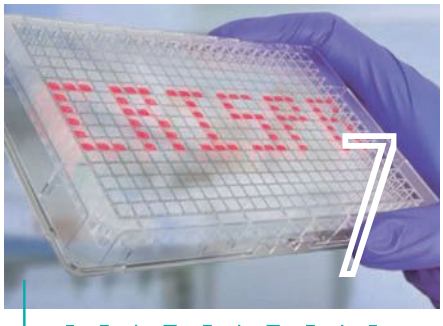


Dharmacon, A Horizon Discovery Group Company ◆ Edit-R crRNA Library—Human Genome

Genome-wide, pooled CRISPR screens can provide researchers with information about the role of specific genes involved in cell function—but are not without limitations. "While this is a powerful, useful format, it does have restrictions on the complexity of the phenotypic assay that can be used," explains Louise Baskin, senior product manager at Dharmacon. "Everything in a pooled screen has to be almost an on-off—it has to be an increase in some sort of reporter signal, or more commonly, it's simply cell death."

Dharmacon's Edit-R CRISPR-Cas9 screening platform, launched on the market in June 2017, instead provides users with an arrayed library of synthetic crRNA guides with a "one-well-per-gene" format, allowing for a much subtler assay, Baskin says. "You can measure 1, 10, 20 variations on a phenotype for a much more complex and rich data set."

Dharmacon provides four distinct guide RNAs per gene, so customers "get a lot of redundancy," Baskin notes. "Having multiple data points per gene really improves statistical power." The catalog libraries, available in 96- or 384-well plate formats, come in sizes that can target from 50 to around 18,500 genes for between $2 and $15 per well, Baskin says, or between $8 and $60 per gene.



The University of California, San Francisco's Judd Hultquist recently used Edit-R as part of a project to investigate HIV-host interactions in primary human T cells. "The ease of use, high efficiency, broad accessibility, and functional adaptability make this platform truly revolutionary," Hultquist writes in an email to Dharmacon. "The work has opened up a lot of new scientific possibilities for us. . . . Having these reagents available to us, in 96-well format especially, made all the difference."

CRUICKSHANK-QUINN: *"This CRISPR library allows for rapid assessment and high-throughput screening of multiple targets across many genes to cover the entire human genome."*

10x Genomics ◆ Chromium

8

With its Chromium system, 10x Genomics aims to make transcriptome and whole-genome analysis more precise than ever. Using the single-cell system's reagents and hardware, researchers partition their samples as single cells (or long DNA molecules), together with reagents and individually barcoded gel beads into individual oil droplets. Reagents lyse the cells and, together with barcoded beads, create a cDNA library of their RNA transcripts, which are then sequenced. The barcodes are specific to each droplet, and after Chromium software crunches the data, users can trace gene expression in individual cells. The result, says Mike Lucero, 10x Genomics's head of strategic marketing, is "a digital count of each gene from hundreds of thousands of droplet compartments."

The controller for the single-cell system costs about $75,000; there's also a Chromium controller that adds in a whole-genome sequencing functionality, available for $125,000. In October of this year, the company rolled out the Chromium Single Cell V(D)J Solution, which analyzes the adaptive immune receptor and antibody repertoires of T and B cells, and measures gene expression from the same single-cell samples. To run experiments, purchasers need the controller plus reagents, chips, and complementary software. Lucero says Chromium has enabled customers to find new cell types and cell states and to track changes in gene expression over time in, for example, a developing embryo.

Michael Schatz, a computational biologist at Johns Hopkins University, says one of his uses for the Chromium system, which originally debuted in May 2016, has been in a project to map the newly sequenced domestic pepper genome. One property that makes the technology unique is its ability to differentiate whether a given allele came from the maternal or paternal chromosome, he says. "It does provide effectively a very new and powerful microscope to see things we've never been able to see before."

KAMDAR: *"Great technology for profiling single-cell gene expression, enabling deep profiling of complex cell populations."*

Thermo Fisher Scientific ◆
TSQ Altis Triple Stage Mass Spectrometer

9

Mass spectrometry continues to march toward ever-greater sensitivity, selectivity, and speed. Thermo Fisher Scientific's TSQ Altis Triple Stage Mass Spectrometer robustly and reliably quantitates most analyte types, even in complex samples such as plasma and tissue. This system can be used widely in analytical, forensic toxicology, and clinical research applications.

The Altis boasts triple quadrupoles, which allow researchers to target specific molecules and affords enhanced ion-transmission consistency. Another advantage of the system is the active-collision cell, where ionized samples collide with a neutral gas and fragment, which ensures fast, selective reaction monitoring and resulting boosts in productivity.

After Jun Qu, who works on the development and analysis of antibody drugs at the University at Buffalo in New York, did extensive beta testing with the Altis in May, he ordered one and awaits its arrival. Qu says he is impressed with the instrument's ability to isolate a narrow window of a sample that includes the peptide of interest. Eliminating the unnecessary parts of samples containing hundreds of thousands of peptides helps avoid what Qu calls "chemical noise," the signal from non-target peptides that interfere with the target's detection—a particularly important step for protein analysis.

"Regardless of the molecule type, from small to large, every organization faces some significant challenges [in] analysis, especially when it comes to achieving more sensitivity to meet today and tomorrow's regulatory standards," Debadeep Bhattacharyya, a senior marketing manager at Thermo Fisher Scientific, writes in an email to *The Scientist*. Bhattacharyya declined to provide pricing information for the Altis.

KAMDAR: *"The new TSQ Altis mass spectrometers can develop quantitative methods for biotherapeutic proteins and target receptors with extreme sensitivity, selectivity, accuracy, and precision."*

Thermo Fisher Scientific
Invitrogen TrueCut Cas9 Protein v2

The Cas9 protein's cutting efficiency can be a limiting step in CRISPR-Cas9 genome editing. Thermo Fisher Scientific's new Invitrogen TrueCut Cas9 Protein v2 has been specially engineered to maximize cleavage efficiency and therefore accelerate the process.

10

"Most of the labor in cell engineering is in isolating clones" that have been successfully edited, says Jon Chesnut, senior director of synthetic biology R&D at Thermo Fisher Scientific. "By improving the efficiency of the cleavage event . . . more cells in the population are going to be properly edited." This makes it easier to identify the edited clones, he adds.

The TrueCut protein can achieve efficient editing not only in standard cell lines but also in stem cells and primary cells. Working with T cells, for example, "in one experiment we knocked out the [PD-1] receptor to 95 or greater percent," says Chesnut. "It's essentially a complete knockout of the receptor in one transfection."

Olivier Humbert, a staff scientist at the Fred Hutchinson Cancer Research Center, uses the TrueCut system to edit blood stem cells with the aim of developing therapeutics for hemoglobinopathies such as beta thalassemia. The protein "allows us to efficiently edit those stem cells, which can be a little tricky to work with," he says. "We can genetically modify over 70 percent of those blood stem cells."

Thermo Fisher Scientific offers TrueCut in two concentrations: 1 μg/μL for standard editing assays and 5 μg/μL for more challenging assays. At the lower concentration, the company offers 10 μg for $85 or 25 μg for $108; 100 μg of the higher concentration costs $230.

KAMDAR: *"This is a next-generation CRISPR-Cas9 protein engineered to deliver maximum editing efficiency across a range of cell types and gene targets."*

THE JUDGES

CHARMION CRUICKSHANK-QUINN
Instructor at the University of Colorado Denver Anschutz Medical Campus. Cruickshank-Quinn was a research fellow at National Jewish Health in Denver performing omics research in lung disease. Before that, she was a graduate student at SUNY Buffalo, where she worked in the departmental mass-spectrometry facility.

BARRY UNGER
Associate Professor of Administrative Services at Boston University. Unger has founded and participated in numerous companies, including Kurzweil Computer Products, Inc., which became Xerox Imaging Systems. He is also cofounder and chair emeritus of the MIT Enterprise Forum.

KIM KAMDAR
Managing partner at Domain Associates, a health care–focused venture fund creating and investing in biopharm, device, and diagnostic companies. Kamdar began her career as a scientist and pursued drug-discovery research at Novartis/Syngenta for nine years.

Editor's Note: *The judges considered dozens of entries submitted for a variety of life-science products by companies and users. The judging panel is completely independent of* The Scientist, *and its members were invited to participate based on their familiarity with life-science tools and technologies. They have no financial ties to the products or companies involved in the competition. In this issue of* The Scientist, *any advertisements placed by winners named in this article were purchased after our independent judges selected the winning products and had no bearing on the outcome of the competition.*

TS WEBINARS

ONDEMAND | Cancer Stem Cells: Getting to the Root of Cancer

The stem cell theory of cancer implies that anticancer therapies must target and destroy all resident cancer stem cells, in order to produce a durable response. Therapies that target cancer stem cells are currently being tested to confirm their safety and efficacy, but research into the weaknesses of cancer stem cells continues. To explore the knowns and unknowns in the field of cancer stem cell research, *The Scientist* brings together a panel of experts to share their results, as well as the lessons they've learned from studying the root cause of cancer.

WATCH NOW! www.the-scientist.com/rootofcancer

IRVING WEISSMAN, MD
Director, Institute for Stem Cell Biology and Regenerative Medicine
Stanford University School of Medicine

ALKA MANSUKHANI, PhD
Associate Professor, Department of Microbiology
New York University School of Medicine

TOPICS COVERED:

- How stem cells become cancer stem cells
- Methods for constraining cancer stem cell proliferation

WEBINAR SPONSORED BY:

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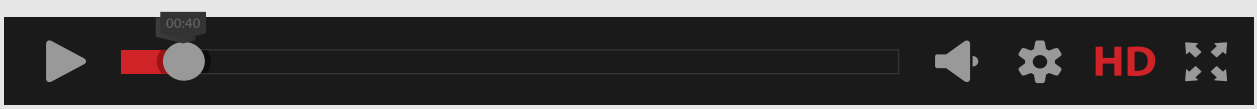
LAB TOOLS
THE SCIENTIST WEBINAR SERIES

ONDEMAND | Power up! CRISPRi & CRISPRa Tools for Genome-Wide Screening

Forward genetic screening with CRISPR-Cas9 has created remarkable new opportunities for biological discovery. The power of complete gene knockout in a pooled screening platform has delivered novel target ID, tackled complex mechanism of action, and driven the design of efficient and economical patient stratification for clinical studies. Using transcriptional regulation with catalytically-dead Cas9 (dCas9), both loss-of-function studies (CRISPR interference, CRISPRi) and gain-of-function studies (CRISPR activation, or CRISPRa) are now possible at a genome-wide level. Horizon Discovery is leveraging these technologies to address novel research questions and deliver insights into essential gene function, hypomorphic expression, and gene dominance.



WATCH NOW! www.the-scientist.com/screeningwithcrispr

BENEDICT CROSS, PhD
Functional Genomic Screening Lead
Horizon Discovery

TOPICS COVERED:

- How to use CRISPRi/a screening for target ID and validation
- Understanding drug MOA and patient stratification

WEBINAR SPONSORED BY:

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The Literature

PHYSIOLOGY

Fat Chat

THE PAPER

W. Ying et al., "Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity," *Cell*, 171:372-84.e12, 2017.

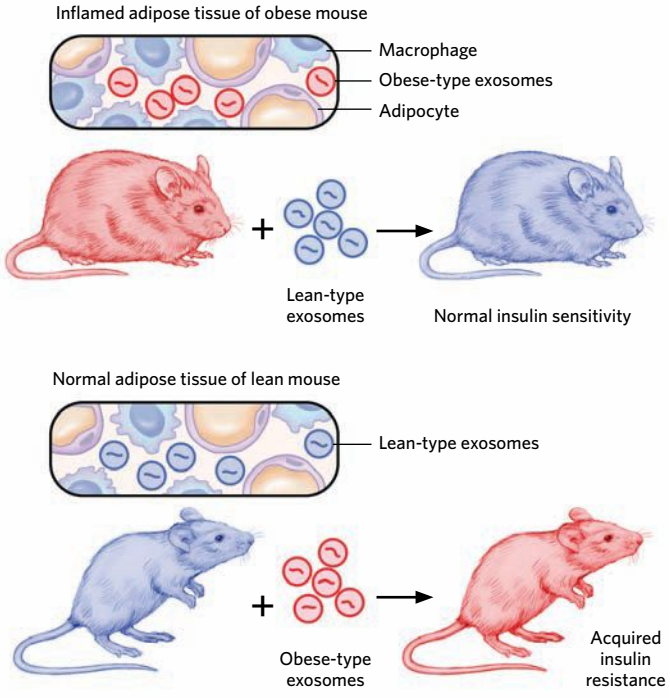
Jerrold Olefsky has spent much of the last decade trying to decipher the connection between obesity and the risk for type 2 diabetes. It's now known that "in obesity, the adipose tissue becomes highly inflamed and fills up with macrophages and other immune cells," Olefsky, an endocrinologist at the University of California, San Diego, explains. "This inflammation is very important for causing insulin resistance," in which cells fail to respond to hormonal signals to take up glucose.

But a crucial piece of the puzzle has been missing. "Insulin resistance is a systemic thing," Olefsky says. For inflamed fat tissue to trigger it, "somehow, all the tissues must talk to each other. We just didn't know how."

Research has not supported a major role for early suspects such as cytokines. But reading a paper a few years ago on the role of tiny vesicles called exosomes in intercellular communication in cancer, Olefsky was struck by the fact that, "Well, gee, all these cells make exosomes." Known to carry microRNAs (miRNAs)—small nucleic acids that influence gene expression—exosomes seemed like plausible candidates for an inter-tissue communication system in obesity.

Olefsky's group isolated macrophages from adipose tissue in obese and lean mice and harvested exosomes produced by the cells in vitro. Then, the researchers added these vesicles to cultured muscle, liver, and fat cells—major insulin targets in the body. While lean-type exosomes made recipient cells "super insulin-sensitive," Olefsky says, obese-type exosomes induced insulin resistance. In vivo work showed a similar effect: lean mice injected with obese-type exosomes became insulin resistant without gaining weight, while obese mice treated with lean-type exosomes stayed obese, but developed normalized insulin sensitivity.

To find the responsible microRNAs, the team searched for differences in the exosomes' contents. One microRNA that was more common in obese exosomes was miRNA 155, which targets *PPARγ*, a gene already well-known to Olefsky's group. "When you stimulate [*PPARγ*], it causes insulin sensitivity; when you inhibit it, it causes insulin resistance," he says. "We ended up showing that miRNA 155 is made by macrophages, does get into exosomes, does get into other tissues, and does inhibit *PPARγ*."

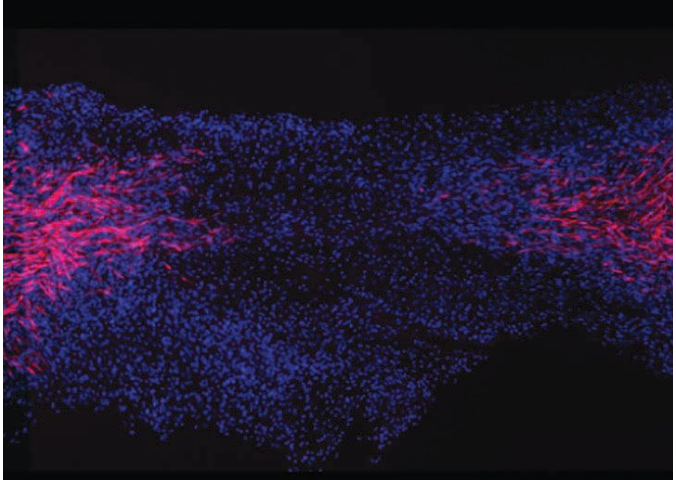


SIGNALLING INSTRUCTIONS: Obesity promotes insulin resistance via exosomal microRNAs, according to researchers at the University of California, San Diego. Macrophages associated with adipocytes in mouse fatty tissue package microRNAs into exosomes, which are released into circulation and are taken up by other cell types. When researchers treated lean mice with exosomes made by macrophages from obese mice, they found that despite remaining lean, recipient mice became insulin resistant. In contrast, treating obese mice with exosomes from lean mice improved the recipient animals' insulin sensitivity, without reducing their weight.

The University of Oxford's Fredrik Karpe, who studies the metabolic effects of obesity, notes that the team's experiments were well carried out, but lack a link to humans. "The obvious thing would be to take a blood sample from humans and see if you have these exosomes," he says, adding that there are likely many processes involved in the development of insulin resistance besides the one suggested here.

Olefsky agrees that microRNA 155 is not "the end of the story." His team is now looking for other microRNAs in macrophage-derived exosomes, and exploring their potential as biomarkers or as inspiration for therapeutics. These tissues "were always talking to each other through exosomes," he says. "We just didn't know how to listen."

—Catherine Offord



REGENERATION: Fluorescently labeled Schwann cells (pink) migrate into the wound site of a severed nerve and lay the foundations for nerve repair.

CELL BIOLOGY

New Identities

THE PAPER

M.P. Clements et al., "The wound microenvironment reprograms Schwann cells to invasive mesenchymal-like cells to drive peripheral nerve regeneration," *Neuron*, 96:98-114.e7, 2017.

TO PROTECT AND REPAIR

In the peripheral nervous system, axons are able to mend themselves after injury thanks to Schwann cells, a type of glial cell responsible for producing myelin, the fatty substance that wraps around some nerve fibers. Schwann cells migrate to the injury site and help guide the regrowing axons through a connective-tissue bridge that forms across the gap.

DUAL IDENTITIES

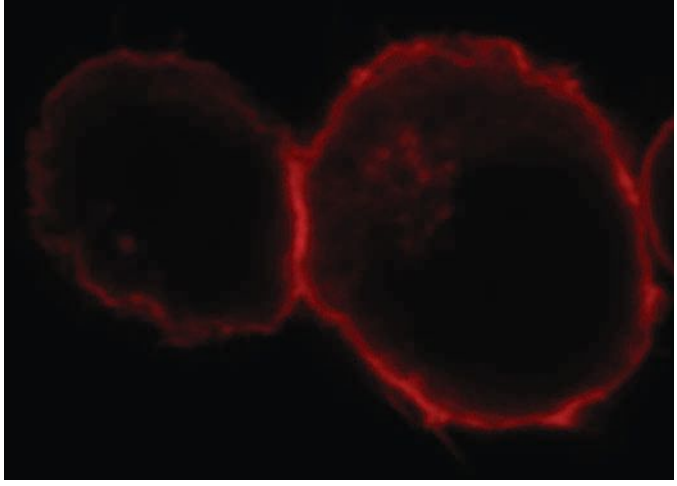
Prior studies have shown that while aiding repair, Schwann cells transition from a myelinating phenotype to a progenitor-like state. This switching is similar to what happens when adult cells are genetically reprogrammed into induced pluripotent stem cells for use in regenerative medicine, says Simona Parrinello, a cell biologist at Imperial College London. "But instead of being forced experimentally, it happens naturally."

MOLECULAR MARKERS

To identify the molecular changes that accompany this transition, Parrinello and her colleagues isolated Schwann cells from both severed and intact mouse nerves and characterized their transcriptomes. The analysis revealed that Schwann cells in the injured area were more proliferative and invasive, and displayed gene expression patterns that were more stem cell–like than those from unaffected parts of the nerve.

CANCER CONNECTIONS

"Schwann cell tumors usually arise from injury sites, and this is probably why," says Haesun Kim, a biologist at Rutgers University who was not involved in the work. These findings could also have implications for regenerative medicine, Parrinello adds. "If we understand how a cell does this as part of a normal regenerative process, we might be able to understand what we need to do [to make] experimental reprogramming more efficient." —Diana Kwon



KNOW YOUR ENEMY: Natural killer cells, like the one attacking this larger cancer cell, can be activated by cell-surface receptors called activating KIRs.

IMMUNOLOGY

Targeted Killing

THE PAPER

M.M. Naiyer, "KIR2DS2 recognizes conserved peptides derived from viral helicases in the context of HLA-C," *Science Immunology*, 2:eaal5296, 2017.

KILLING MACHINES

Natural killer (NK) cells help fight viral infections as part of the body's innate immune response. Activation of these cells depends partly on a set of NK cell-surface proteins called activating killer cell immunoglobulin-like receptors (KIRs). But how activating KIRs recognize pathogens is poorly understood.

SEARCHING FOR A MATCH

While screening for viral peptides that stimulate one receptor, KIR2DS2, hepatologist Salim Khakoo's group at the University of Southampton, U.K., stumbled across an amino acid sequence that appears highly conserved across multiple flaviviruses, from Zika to Japanese encephalitis. "There are about 63 different flaviviruses, and they almost all have this five-amino-acid sequence," says Khakoo. "We were absolutely astonished."

ONE SIZE FITS ALL

Using human cell lines, the team showed that major histocompatibility complex proteins—important components of the vertebrate immune system—on virus-infected cells present this sequence to KIR2DS2, which then activates NK cells to inhibit viral replication. The fact that multiple viruses stimulate the same receptor suggests the possibility of developing broadly antiviral therapeutics, Khakoo says. "We're working on ways of using this knowledge to activate natural killer cells, and develop a natural killer cell–based vaccine strategy."

OUT OF LINES

KIR researcher Marcus Altfeld of the Leibniz Institute for Experimental Virology in Germany says he's impressed by the study's description of KIR2DS2's mechanism of action. However, he notes, "cell lines create a bit of an artificial system. . . . The next challenge will be to see whether these responses can be seen in cells from a patient."

—Catherine Offord

MELANIE CLEMENTS, PARRINELLO LAB; GWENOLINE BORHIS

PROFILE

Captivated by Chromosomes

Peering through a microscope since age 14, Joseph Gall, now 89, still sees wonder at the other end.

BY ANNA AZVOLINSKY

Cell biologist Joseph Gall, who was born in 1928, grew up spending lots of time outside, observing and collecting frogs, butterflies, and other insects. "There was no television when I was younger. After school, I roamed around the neighborhood and the nearby woods," says Gall, now a staff scientist at the Carnegie Institution for Science in Baltimore, Maryland. "My mother used to make me dozens of butterfly nets and made sure I always had science books." Gall attributes his lifelong interest in science to her. "She was the first person in her family to go to college. This was in the 1920s and was rare for a woman. After college, she immediately married my father, a lawyer, had my older brother, and became a homemaker. That was the pattern in those days. Today she would have been a professional of some sort."

"I have been credited, legitimately, with fostering women in the lab at a time when there were not many women in science. It was unusual for the time and it goes back to the fact that I learned science from my mother."

When Gall was 14, his father bought a 500-acre cattle farm in Virginia and hired a farm manager. Gall helped bale hay and did other farm chores in the summer, when not at boarding school, but his real love was science. Through a work connection, Gall's father got him a professional Bausch and Lomb microscope. "I can't remember a time when I wasn't interested in looking through a microscope. I was completely self-taught. My mother got me the right books, including a copy of E.B. Wilson's *The Cell in Development and Heredity*. It was the bible in cell biology for many years," says Gall. "By the time I was 14, I had read that and other cell biology books and had set up a laboratory in my room. I made slides of everything—insects, the protozoa in our pond water—and then progressed to making slides of the organs of the farm animals." Gall's parents got him the tools he needed to fix tissues and make paraffin sections. "I learned this all myself and it made me really independent."

Here, Gall recalls how he invented in situ hybridization, why he has always promoted women in science, and why he never "became" a biologist.

GALL GETS GOING

Professional biologists. For three years, Gall attended a boarding school outside of Charlottesville, Virginia. While he enjoyed the regimented schedule and the language classes, he was less than inspired by the science curriculum. "But it didn't do anything to my scientific interests," he says. The headmaster decided that Gall should attend Yale University and "somehow it was all arranged and it happened. I don't remember even applying." He started at Yale as an undergraduate in 1945, when most colleges had been nearly emptied because of World War II and were looking for students. Gall chose a premed major only because he didn't know that there was such thing as a professional biologist. "I thought that you had to be a doctor, and only in my junior year did I realize that there was graduate school and that the biology professors teaching me weren't MDs. The lack of career counseling would be astounding to anyone today."

Observing chromosomes. Gall graduated in 1949 and arranged with Donald Poulson, a *Drosophila* geneticist and cell biologist in the zoology department, to stay on at Yale as a graduate student. In his home laboratory, Gall had already been making mitotic spreads using fixed tissues, and he wanted to work on chromosomes for his PhD thesis. In a textbook, he came across an image of a lampbrush chromosome—a conformation formed by the unusually high transcription of the meiotic chromosomes in immature oocytes of amphibians and other animals, but not in mammals. Gall couldn't believe the magnification scale on the image and wanted to see them for himself. He ended up analyzing these chromosomes—which had not been well characterized—in newt oocytes. "They are truly gigantic and one of the best-kept secrets in biology, up to 1 mm in length and can almost be seen with the naked eye," says Gall. The phase-contrast microscope had recently been invented, and Yale had just purchased its first one. Gall published a 70-page paper describing lampbrush chromosomes in 1954.

One strand. After obtaining his PhD in 1952, Gall took an instructor position in the zoology department at the University of Minnesota. He was mostly expected to teach, but also was given a microscope and some lab space. "In 1952, the National Science Foundation (NSF) had just been formed and the NIH still only had a meager funding budget," Gall recalls. He was among the first to receive grant funding from the NSF, as one of his colleagues in the department, H. Burr Steinbach, was an assistant director there and told him how to apply. Gall continued to study lampbrush chromosomes and began a decades-long collaboration with Harold "Mick" Callan, a professor at the University of St Andrews who was also studying them. An experiment by Callan's

COURTESY OF JOE GALL

graduate student Herbert Macgregor, using the enzyme DNase to cut lampbrush chromosomes into fragments, inspired Gall to perform a similar experiment, but to control the kinetics of the reaction in order to determine how many DNA molecules make up a chromosome. The experiment showed that there was only one DNA molecule per chromatid within the chromosome and that the brush analogy wasn't really correct: the bristles of the brush were loops. "At the time, it was almost universally believed that chromosomes of higher organisms were multistranded and that larger genomes meant more strands in the chromosomes, even though there was no evidence for this. This is probably the most important early experiment I did, although it's almost never cited," Galls says. "Matthew Meselson and Franklin Stahl, who used the tiny *E. coli* circular chromosome, are given credit for showing that a chromosome is a single DNA strand. For higher organisms, Herbert Taylor used tritium-labeled incorporation into living chromosomes to demonstrate that the label distributed semi-conservatively during replication. Taylor's paper is one of the most important of semi-forgotten experiments in cell biology."

JOSEPH GALL

Staff Member, Department of Embryology
Carnegie Institution for Science, Baltimore, Maryland
1983 American Society for Cell Biology E.B. Wilson Medal
2004 Lifetime Achievement Award of the Society for Developmental Biology
2006 Albert Lasker Special Achievement Award in Medical Research

Greatest Hits

- Using DNase kinetics, showed that amphibian lampbrush chromosomes are not multistranded, but consist of a single, extremely long DNA molecule
- With Mary-Lou Pardue, invented the in situ hybridization technique, which uses labeled RNA or DNA molecules to bind and visualize complementary DNA or RNA within fixed tissues or cells
- Showed that DNA-dense yet gene-free chromosomal regions in mouse and *Drosophila* corresponded to simple DNA repeats called satellite DNA
- With Elizabeth Blackburn, identified tandemly repeated sequences at the ends of ribosomal RNA genes (rDNA) in *Tetrahymena* that later turned out to be telomere sequences
- Characterized Cajal bodies and histone locus bodies, organelles in the nucleus

GALL GOES HIGHER

Poring over pores. Again following on Callan's experiments, this time in flattening and laying out the nuclear envelope on a slide prior to electron microscopy, Gall showed in 1954 that the envelope is peppered with nuclear pore complexes; 13 years later, he showed that these complexes are octagonal rather than circular. "We thought that these pores were so big that anything could get in and out. I never thought at the time that there was regulated transport into and out of the nuclear envelope," says Gall. (See "Nuclear Pores Come into Sharper Focus," *The Scientist*, December 2016.)

Moving on. In 1964, Gall returned to his alma mater, Yale, where he became a professor in the biology department and in the newly formed Department of Molecular Biophysics and Biochemistry. "I realized that there was this new field beginning that would eventually be called molecular biology. It was clearly the future, but there was as yet no way to detect specific DNA or RNA sequences within cells," he says. Researchers were already immobilizing nucleic acids onto nitrocellulose filters and using a radioactively labeled piece of RNA to detect the complementary sequence on the filter and quantitate it. The approach inspired Gall to develop a similar technique for identifying a specific nucleic acid sequence in DNA immobilized inside a tissue preparation.

Gall and others had been studying the phenomenon of "gene amplification"—specifically, the production of massive amounts of extrachromosomal DNA coding for ribosomal RNA that occurs in amphibian oocytes. "I realized that here was the perfect test material for developing a technique to detect specific DNA molecules in fixed tissues." Because there was no cloning yet, Gall and his graduate student Mary-Lou Pardue used this naturally amplified DNA. In 1968, the two developed a method called in situ hybridization, using tritium-labeled RNA as a probe to target the many copies of ribosomal DNA in *Xenopus* oocytes and visualizing the hybridization with autoradiography. The technique worked beautifully.

Gall's lab showed that in *Drosophila* and mouse the densely stained, highly concentrated DNA regions that were found to be free of genes actually corresponded to simple DNA repeats called satellite DNA. "Possibly the most important early discovery to come out of the in situ hybridization technique was the realization that satellite DNA corresponds to heterochromatin," he says. A modified, more sensitive version of the technique, FISH (fluorescence in situ hybridization), now incorporates fluorescently labeled rather than radioactively labeled nucleic acids and employs fluorescence microcopy rather than autoradiography for visualization.

Telomere sequences before telomeres. Gall began to study the chromosomes of the ciliate *Tetrahymena* after he saw images of its multiple nucleoli. After extracting the *Tetrahymena* DNA, he used ultracentrifugation to separate out the multicopy extrachromosomal ribosomal DNA, and then, using electron microscopy, observed that the strands were either circularized or linear in form. "There was something funny about the ends that made them stick together sometimes. Elizabeth Blackburn, who had learned how to do DNA sequencing in Fred Sanger's lab, joined my lab as a postdoc and decided to sequence these ends," he says. Blackburn found that the ends all contained the same sequence, TTGGGG, repeated many times. "That was the discovery of the telomeric sequence, but not the discovery of the telomere because we had no idea at the time that all chromosomes have this sequence at their ends and that they form a specific structure," says Gall. Blackburn, along with Carol Greider and Jack Szostak, went on to win the Nobel Prize in 2009 for research on how telomeres and telomerase work to protect the ends of linear chromosomes.

Nothing unusual. Greider, who was a graduate student in Blackburn's lab, credits Gall with being a fantastic mentor and training many of the prominent female scientists who became leaders in the study of telomeres, among other fields. "I have been credited, legitimately, with fostering women in the lab at a time when there were not many women in science," says Gall. "It was unusual for the time, and it goes back to the fact that I learned science from my mother. It was nothing unusual to me that women should be scientists. It was not that I was positively seeking women in my lab, but to those who wanted to join, I would say 'Yes,' and that wasn't true for many other male professors."

Bodies of confusion. In 1983, Gall moved from Yale to the Carnegie Institution for Science in Baltimore because at Yale he was fending off offers to become an administrator or a dean, and he wanted to remain focused on his lab. More recently, he has been studying nuclear bodies, subnuclear organelles whose functions are still poorly understood. One of these structures, which he named the Cajal body after its discoverer in the early 1900s, Santiago Ramón y Cajal, is thought to be involved in RNA splicing. Gall's lab found Cajal bodies—which are typically identified by the presence of a protein called coilin—in *Drosophila melanogaster* in 2006. Further study of these organelles in *Xenopus* oocytes led Gall's team to conclude in 2010 that a different type of nuclear body, which Gall named the histone locus body, had been confused with Cajal bodies in the literature because both are associated with coilin.

"I will retire when I can't think of anything else to do! I am just as anxious to come to the lab each morning as I ever was."

Mystery introns. The lab is currently focused on stable introns found in the cytoplasm of *Xenopus* oocytes. While most introns are spliced out of pre-messenger RNA and degraded within minutes, the stable, circular introns Gall and graduate student Gaëlle Talhouarne identified in 2014 persist and are transferred to the fertilized egg, suggesting a regulatory role in mRNA translation. (See "Uncovering Functions of Circular RNAs," *The Scientist*, July/August 2017.)

GALL GAZES

Lab rat. "I still do experiments," says Gall. "My name is not on the papers as a courtesy. I typically do the in situ hybridization experiments and someone else does the molecular biology and the bioinformatics. I've also done a lot of the *Drosophila* microscopy and immunostaining."

Book-ish. Gall is an avid collector of biology books and texts, with an extensive library containing items that date back to the 17th century. The most prized part of his collection: "An original copy of the journal containing Mendel's paper." Gall also has most of Theodor Boveri's original papers, and other important 19th-century cell biology books and papers.

Biologist by birth. "When people ask me, 'When did you become a biologist?' I always answer, 'I never became a biologist, I just always was.' I think I am one of those very lucky people who never had to do any soul searching. I always knew what I was from day one."

Going strong. "I will retire when I can't think of anything else to do! For now, I don't have any plans to retire, but it all depends on health. Fortunately, I am quite healthy at this point, but I am not taking on new graduate students because at 89, I don't want to make a five-, six-year commitment. I am just as anxious to come to the lab each morning as I ever was." ■

Neslihan Taş: Digging Microbes

Research Scientist, Climate and Ecosystems Division, Lawrence Berkeley National Lab. Age: 37

BY SHAWNA WILLIAMS

For many undergraduates, an internship at a wastewater treatment plant might not provide the most alluring introduction to the microbial world. But for Neslihan Taş, then at Marmara University in Istanbul, learning how sewage from millions of people was converted into safe wastewater "really made me . . . realize how big of stewards microbes are to our world," she says.

Taş began taking more biology courses as she earned her bachelor's degree in engineering and then enrolled in a master's program in environmental technology at Wageningen University in the Netherlands. It was her microbiology lab coursework there that ultimately enticed her to change career paths. "More and more, I realized that we actually do not understand biological systems well enough to be able to approach them as engineers and use their properties in one way or another to make things better," she explains. "In general, we know so very little about how microbes work and how they interact with each other and do the things that they do for general earth cycles."

So Taş went on to earn a PhD in microbiology at Wageningen, investigating how certain anaerobic bacteria break down chlorinated pollutants in a process known as reductive dechlorination.[1] Then, during a postdoc at nearby Vrije Universiteit, Taş worked on several projects involving microbial processing of pollution and response to climate change.[2]

Taş's skills in molecular biology techniques and her ability to work with researchers in other disciplines helped make her an "exceptional candidate" when she later applied for a postdoc position at the US Department of Energy's (DOE's) Lawrence Berkeley National Laboratory, recalls Janet Jansson, Taş's postdoc advisor there. For her part, Taş was drawn to the big-picture approach of a national lab. "Usually in DOE labs . . . it's really multidisciplinary, large-scope, really ambitious projects. So that has a very nice accelerated feeling to it," she says.

First as a postdoc and then as a research scientist, much of Taş's work at Lawrence Berkeley has focused on microbes' role in the carbon cycle—particularly in the Arctic. "The thing that Neslihan really brought to the fore was understanding of, as permafrost thaws, this awakening of the microorganisms that were alive but not doing a lot as far as cycling of carbon," says Jansson, now a chief scientist at Pacific Northwest National Laboratory. "But as the permafrost thaws, these microorganisms became much more active and were responsible for the release of greenhouse gases, in particular, methane."[3]

Susan Hubbard, a geophysicist who leads the Earth & Environmental Sciences Area at Lawrence Berkeley, says Taş was able to use a permafrost map Hubbard's team had created, then sample the soil to confirm that the microbial assemblages in zones with unique physical properties were indeed different from each other—and that the communities at various depths also differed. "That's pretty groundbreaking to document how the microbial community varies in space," Hubbard says of the study, which is pending publication.

Taş, though, is focused on the many unknowns that remain about the microscopic environmental engineers that shape our world. She says she aims to find out "the rules that they live by—the life strategies they have to function in a given environment—and how they're going to respond to major changes in environmental conditions." ■

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LAWRENCE BERKELEY NATIONAL LABORATORY

The Power of Light

Techniques for label-free cell sorting

BY RACHEL BERKOWITZ

The more biologists learn about disease complexity and the power of personalized treatments, the more important it becomes to develop noninvasive and unbiased methods of sorting, separating, and otherwise gathering information about individual cells.

Traditionally, however, sorting cells has been tricky. Methods for accurately and quickly sorting heterogeneous cell populations—even into just the broad categories of malignant or benign—often rely on the use of fluorescent surface labels or biochemical stains, techniques that frequently alter the cells' properties. And in some applications, researchers simply don't know which surface markers to track. This means that the cells being studied may not be representative of the specific cell subpopulation of interest.

A new wave of label-free methods is offering researchers ways to identify subgroups of cells in live cultures and to home in on the most pertinent populations. Still, many label-free methods rely on only one cell characteristic or are hobbled by their low throughput. To overcome these limitations, researchers are devising tools that rapidly pump high volumes of cells through tiny microfluidic channels etched into a chip and combine the novel use of optics with new image-processing tools.

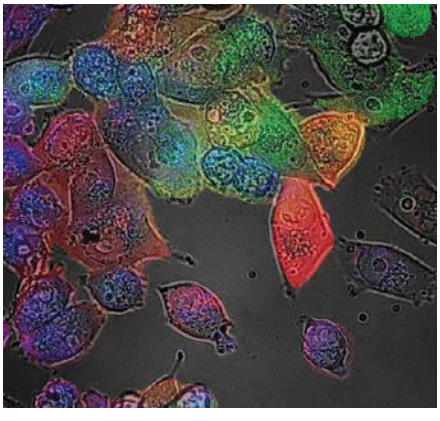
The Scientist explores how these label-free techniques are helping to rapidly and accurately identify and isolate subsets of cells from a larger population.

A HEALTHY COLOR

RESEARCHER: Ewa Goldys, Deputy Director, Centre for Nanoscale BioPhotonics and Professor, Macquarie University, Sydney, Australia

MOTIVATION: Goldys wanted to develop a noninvasive, label-free method for distinguishing between healthy and diseased cells that can be used for medical diagnostics. Inspired by developments in remote sensing for assessing minerals based on soil color, she set out to look for subtle differences in the color of cells. This led her to a novel image-analysis technique that is based on deconstructing the fluorescence patterns intrinsic to a cell sample.

APPROACH: Many cellular components vital to metabolism are autofluorescent. Goldys looks for subtle differences in the fluorescent signals to distinguish healthy from diseased cells. "I can tell whether my daughter is healthy by the color of her face; there's equally as much biological information contained in color differences at the cellular level," she says. Goldys modified a common, wide-field fluorescence microscope by installing 35 spectral channels, chosen to excite the fluorophores in selected wavelength ranges. Repeated snapshots of the same cell sample taken in all the channels generate 35-dimensional vectors, each corresponding to an image pixel. Each image captures a different part of the cell sample and comprises millions of pixels. Then, she uses custom-made software to identify subtle differences in color from a baseline level. Different patterns of color pinpoint the diseased cells in a population, which can then be isolated for further study (*Sci Rep,* 6:23453, 2016).



INTRINSIC COLOR: Hyperspectral image of pancreatic cancer cells, with false colors highlighting spectral differences: control cells appear more green/blue, while cancer cells expressing a mutated protein appear more yellow/red.

FUNCTIONALITY: Using this technique, Goldys was able to distinguish human pancreatic cancer cells from their healthy counterparts and more recently showed that healthy bovine embryos have different spectral signatures from diseased ones (*Hum Reprod,* 32:2016-25, 2017). Her work also offers potential for future diagnostics in in vitro fertilization systems.

TIPS: Goldys advises researchers to carefully characterize their control cells, typically healthy or normal cells. Variation from this baseline helps to identify diseased cells. Setting up the microscope and adding channels is straightforward.

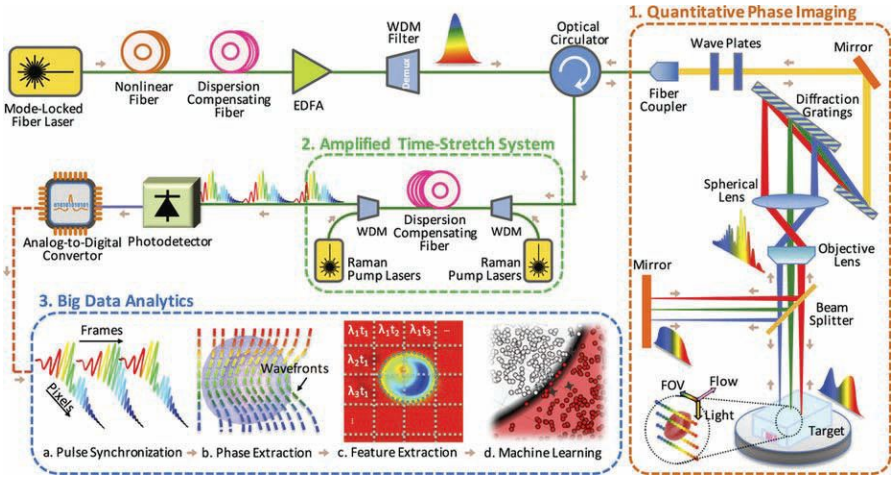
FUTURE PLANS: Goldys's current work aims to identify autofluorescence signatures associated with chronic pain, and to distinguish between the autofluorescence signatures of cancer and neurodegeneration.

A TIME-STRETCH MICROSCOPE

RESEARCHER: Bahram Jalali, Professor, Departments of Electrical Engineering and Biongineering; Postdoc Ata Mahjoubfar; and former PhD student Claire Chen, University of California, Los Angeles

MOTIVATION: Label-free cell assays often rely only on identifying a single feature. Their use is also generally limited to small sample sizes due to their low throughput. Jalali's team developed a machine learning–augmented microscopy technique to rapidly identify multiple biophysical features simultaneously and accurately classify cells in a large population.

APPROACH: "Imagine that you're illuminating a barcode with a line of light with different colors, as in a rainbow. By measuring the colors that are reflected, we can reconstruct the image of the barcode," explains Mahjoubfar. In this case, the barcode target consists of cells pumped at high speed through a tiny microfluidic channel etched into a polymer substrate, where the cells are illuminated by an infrared laser flashed on and off 36 million times per second. The researchers then use a technology they developed, called time-stretch microscopy (which slows down input signals to allow conversion to digital), to measure the spectrum reflected by these individual laser pulses, picking up information about biophysical features such as cellular morphology and opacity. The ultrafast spectroscopy effectively freezes the motion of the cells passing at high speed (100,000 cells/s) in the flow, thereby achieving blur-free imaging of cells' spacial features (*Nat Photonics,* 11:341-51, 2017). These biophysical features are used in a machine-learning algorithm to classify the cells with high accuracy (*Sci Rep,* 6:21471, 2016).



CLEAR AND LABEL-FREE: In time-stretch quantitative phase imaging, laser light is amplified and filtered to generate a spectrum of optical pulses. In Box 1, the flashes of light illuminate and encode spatial features of the cell sample. In Box 2, spatial information is converted to digital. In Box 3, image-processing and machine-learning tools group the cells according to the features that were detected.

FUNCTIONALITY: The UCLA team can distinguish immune cells in the blood from circulating tumor cells that are associated with colon cancer, a step that could lead to earlier diagnosis of metastasis. They've also grouped algal cell strains according to their lipid content—"think of them as fatty algae," says Jalali—an efficient source of biofuel.

TIPS: Experience in optics and microfluidics is required to reconstruct the imaging system. Constructing the system includes fabricating tiny channels that keep the cells aligned and close to the surface of the mirror. Another challenge is getting the classification algorithm to run in real time as images are collected.

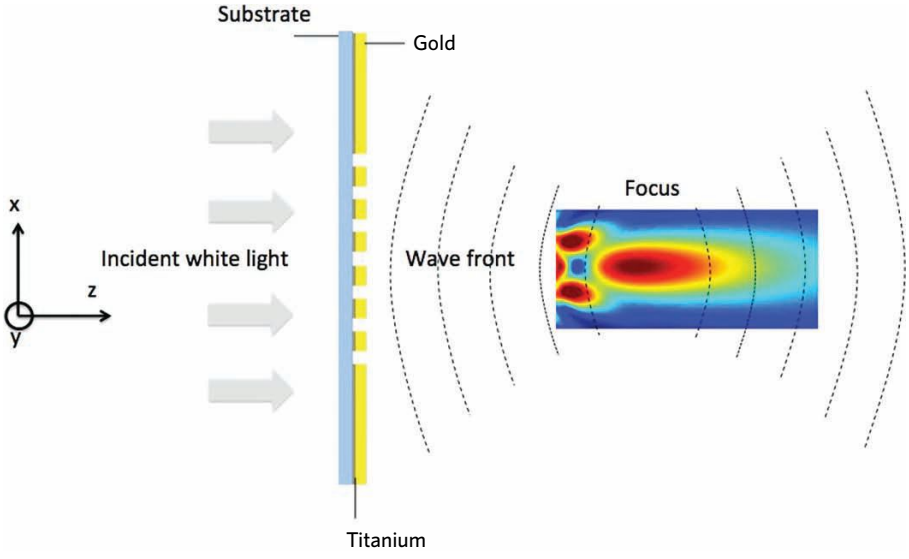
FUTURE PLANS: Jalali's team is continuing to develop and refine the artificial intelligence aspect of their time-stretch microscope, with the aim of improving accuracy and computational efficiency of cancer cell classification.

A PLANAR LENS

RESEARCHER: Ahmet Ali Yanik, Assistant Professor, Department of Electrical Engineering, University of California, Santa Cruz

MOTIVATION: A highly focused laser beam can separate single cells of a specific type from a mixed population. But it's difficult to integrate these so-called optical tweezers with the high-throughput need of cell-sorting applications. "The laser has to be aligned perfectly" with the particles of interest, says Yanik, which is difficult when it is focused through a conventional objective lens some distance from a stream of flowing cells. He solved this problem by developing a planar lens that can focus white light to generate an optical force throughout a microfluidic channel. This force is strong enough to immobilize bioparticles belonging to a cell subset of interest as they move through the channel.

SCI REP, 6:21471, 2016



MAY THE FORCE BE WITH YOU: White light is focused to microscopic dimensions through the tiny holes of a planar optical lens. Using this approach, metal lenses approximately 100 nm thick with a footprint comparable in size to a blood cell can be created on transparent substrates.

APPROACH: The planar lens comprises tiny (5 microns/side) arrays of round subwavelength holes in a thin metal film that forms one side of a microfluidic chip. Light from a standard halogen source is transmitted through these specially engineered holes, which together act as a nanolens. Their arrangement on the film focuses the light as it emerges, thus delivering a well-controlled beam throughout the chip. When the cells are pumped through the channel, the optical force of this beam is countered by the drag force of the flow, thus separating particles with varying size and refractive indices. The balance between optical and fluidic forces can be adjusted via light intensity to selectively sort particles.

FUNCTIONALITY: Yanik has used this technique to isolate bacterial cells of genetically similar species with subtle differences in protein structure, and to separate rare circulating tumor cells (CTCs) from white blood cells based on size. He warns that "you'll still need a conventional [fluorescent or antibody label] marking scheme to identify the specific type of CTCs (*OSA Technical Digest,* doi.org/10.1364/FIO.2015.JTu4A.1, 2015).

TIPS: "Any nanophotonic engineer could make this chip," says Yanik. He notes, though, that the flow channel can clog up with cells that have been trapped by the beam. He advises setting up another cross-channel flow to periodically wash away trapped cells. Also, diluting a blood sample to 25 percent can make it easier to separate particles.

FUTURE PLANS: Yanik is using nanohole lenses to develop point-of-care infection monitoring tools that can detect rare biomarkers in small concentrations. In particular, he wants to identify a circulating glycoprotein shed from infectious bacteria using a blood sample taken by a finger prick.

MICROFLUIDICS IN 3-D

RESEARCHER: Lynn Paterson, Lecturer, Institute of Biological Chemistry, Biophysics, and Bioengineering, Heriot-Watt University, Edinburgh

MOTIVATION: Applying optical forces to cells flowing through microfluidic structures is difficult, in part because the laser beam has to be precisely aligned with rapidly flowing particles, as Yanik noted. Paterson addresses this limitation by integrating a waveguide, which directs light into a microfluidic channel. The waveguide behaves like an optical fiber, transmitting light of specific wavelengths in its glass core. The waveguide directs light into a microfluidic channel. Etching the waveguide into the glass results in a 3-D microfluidic device. This combines the optical scattering force of light with the controlled flow of a microfluidic channel to create a high-throughput, passive sorting system.

APPROACH: Paterson uses ultrafast laser inscription followed by selective chemical etching to "write" 3-D channels into fused silica devices. In the same piece of glass, she uses the same ultrafast laser to etch the waveguides. Cells are deflected based on size as they flow past the light emitted from the waveguide and are collected into separate outlet channels. Further imaging is required to identify the deflected cells.

FUNCTIONALITY: Paterson has used 3-D microfluidic devices to separate large mammalian cells from a population of small bacterial cells. But blood cytometry is the holy grail, she says. "Everyone wants to do it faster and cheaper at point of care." Most recently, her team separated 5- and 10-micron synthetic spheres—approximately the size of blood cells—in a 3-D microfluidic structure (*OSA Technical Digest,* doi.org/10.1364/OTA.2017.OtW2E.3, 2017).

ADVANTAGES: The 3-D system allows higher throughput, while exposing the cell to a less-intense beam than, say, the focused infrared beam of optical tweezers. It also opens the possibility of multiple sorting units within the same device: one channel separates one size of cell, while the remainder are passed to a second channel for sorting of another size, and so on.

FUTURE PLANS: The next step is to improve the resolution to allow more-refined size discrimination by optimizing laser and channel parameters. This will increase the utility of the technique in other applications of blood cytometry. ■

OSA TECHNICAL DIGEST, DOI.ORG/10.1364/FIO.2015.JTU4A.1, 2015

Can Philanthropy Save Science?

Private funders are starting to support big research projects, and they're rewriting the playbook on fueling basic science.

BY BOB GRANT

In September 2016, Facebook cofounder and billionaire Mark Zuckerberg and his wife Priscilla Chan announced an exceedingly ambitious plan to "cure, prevent, or manage all diseases by the end of the century." Zuckerberg and Chan pledged $3 billion to be disbursed by the Chan Zuckerberg Initiative (CZI), the charitable foundation they had launched the year before.

With that announcement, the CZI joined the ranks of a handful of other philanthropic mega-donors pumping cash into biomedical research labs. The Bill and Melinda Gates Foundation, for example, has devoted more than $40 billion to research on malaria and other infectious diseases that strike hardest in the developing world, while the Michael J. Fox Foundation has contributed more than $700 million to understanding Parkinson's disease. Others, like the CZI, have much broader goals. But one attribute unites the major players on the philanthropic science-funding scene: they all serve as alternatives to the traditional model of securing federal funding—and could prove especially valuable for life scientists looking to fuel innovative and risky research.

The National Institutes of Health (NIH) and other government agencies demonstrate an almost innate wariness of uncertain outcomes, says Gerald Fischbach, Distinguished Scientist and Fellow at the Simons Foundation, a philanthropic organization that funds basic science. In fact, many government/federal agencies now require that scientists state in their proposals how their research will be "transformative." This push comes from continued fiscal belt-tightening that limits the number of applicants government science agencies, especially the NIH, can fund, Fischbach notes. "When the study sections can give out from two to five grants each cycle out of 150 [applications], there's a real bias against risky research."

Private funders, on the other hand, have the freedom to build longer time lines into the projects they fund, which means returns on investment need not be immediate. As a result, philanthropic money is often essential to getting uncertain projects off the ground, with government dollars coming in at a later stage in the research once a clearer finish line emerges.

"When I was at the NIH as the director of the [National Institute of Neurological Disorders and Stroke], almost every new grant that we funded, they had developed preliminary data from a private source," says Fischbach. "Private foundations have the benefit of not depending on the traditional routes of grant review and they are less risk-averse."

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Perks of private funding

The Human Cell Atlas is one recent example of how philanthropic funders are playing major roles in propelling basic life-science research. Officially launched in late 2016, the project aims to characterize and explore every cell type in the human body—and is expected to take decades to complete and involve many labs scattered across the globe to profile the human body's estimated 37 trillion cells. Given its massive scale, it likely would not have been possible without support from private funding organizations—namely, grants and cooperation from the CZI for 38 pilot projects—says Anthony Philippakis, chief data officer at the Broad Institute of MIT and Harvard University who is heading up the Broad's involvement in the research endeavor. "[It's a] great example of how philanthropy can kick-start ambitious projects and move very quickly," he says.

The support of the CZI is especially important given the international nature of the project, Philippakis notes, as non-US scientists typically face a much steeper hill to climb than their American counterparts in tapping into NIH funding. CZI's president of science Cori Bargmann agrees that coordinated private support made it easier to encourage the international collaboration and long-term vision that serve as key ingredients of the Human Cell Atlas's sweeping goal. "If you really want to have an atlas of the whole human body, you want people to be using some common frameworks and putting their data into a common platform so that you can compare data that people are getting from different

FOUNDATIONAL FUNDING

A sampling of the philanthropic organizations supporting scientific enterprise

Organization	Areas Funded	Total 2016 Research Support
Bill & Melinda Gates Foundation	Global development, global health, US education, global policy and advocacy	$4.6 billion
Howard Hughes Medical Institute	Basic biomedical research, science education	$663 million
Chan Zuckerberg Initiative	Science, education, affordable housing (with a focus in the San Francisco Bay Area), criminal justice reform	$600 million
Simons Foundation	Mathematics and physical sciences, life sciences, autism research, outreach and education	$231.7 million
Gordon and Betty Moore Foundation	Environmental conservation, science, patient care, especially in the San Francisco Bay Area	$288.4 million
Alfred P. Sloan Foundation	Research and education related to science, technology, engineering, mathematics, and economics	$74.3 million
The Wellcome Trust	Science, culture and society, innovations, strategy	£502.7 million ($663.6 million)

places together," she says. "That was something that didn't seem like it was going to happen on its own."

Bargmann says the Human Cell Atlas grew from a wide web of labs that were independently seeking to characterize different cell types using money from a hodgepodge of funders, both public and private. Pulling all of those loose threads together into a cohesive whole and generating tools and techniques that could be shared between all those labs was the perfect fit for a philanthropic funder with a broad mandate such as CZI. "We stepped in because we saw an area that was exciting, because we saw a field that good leaders and great scientists were starting to get interested in that could use support to put it together," she says.

In mapping the cellular makeup of the human body, Bargmann adds, the CZI hopes that the Human Cell Atlas will also lay the technological and methodological groundwork for future life-science research projects. "Building tools is a way of accelerating everyone's research," she says. "The Human Cell Atlas is an example of a tool that we think can have a great effect in making a lot of different research in a lot of different diseases move more quickly."

Jeremy Freeman, CZI's director of computational biology, agrees, emphasizing that tools for data management and analysis will be especially important and also likely to be widely applicable to future research projects. And CZI is uniquely positioned to oversee the development of such tools, given the experience of the funding body's cofounder, he adds. "So this might be, for example, infrastructure for lots of labs to take the data that they're generating and share it and make it broadly and openly available with the rest of the scientific community."

Tapping all sources

The CZI is not the sole funder of the Human Cell Atlas; the project also involves a host of other private sources of money, as well as governmental support. As many people involved in the private funding of science are quick to point out, the goal is not to replace or supplant the crucial role that government dollars play in the research enterprise. "When you think about the role of how Chan Zuckerberg fits with other funding bodies, especially NIH or its equivalents in other countries, it's really synergistic," says Philippakis.

The bulk of research dollars still comes from the government. According to statistics from the Science Philanthropy Alliance, an advocacy group that seeks to increase philanthropic support for basic research, private sources gave about $2.3 billion to basic science in 2016, while federal science agencies contributed approximately $40 billion. "There's no way that philanthropic funding can compete with federal funding," says Marc Kastner, president of the alliance.

But at the institutional level, philanthropic funding can fill gaps left by flagging budgets at federal funders, says Rick McCullough, vice provost for research at Harvard University. "We've seen, like every university, declines in federal funding over the past five years or so—anywhere from 1 to 2 to 3 percent. Other universities I've heard from have seen swings as large as 12 percent drops in their federal funding. We've been working really hard here . . . at trying to make that up through nonfederal resources." Funding from private foundations, for example, has increased at Harvard by about 112 percent since 2007 for research across all disciplines, he says. "We've been able, then, to hold our research funding essentially flat over the last five years by aggressively going after these kinds of sponsors."

While philanthropic dollars can add value to basic science, such funding mechanisms also diverge from the goals and protocols employed by major public funders. "One of the major differences is that when it's a federally funded grant, there's a very formal process of peer review and scoring and feedback," says Philippakis. "For a lot of philanthropic organizations, the process is often a little bit more lightweight and doesn't have quite the same level of rigidity."

This, Philippakis adds, can free up scientists to alter plans, change course, or tweak protocols as data emerge from their research.

For this and other reasons, the types of research funded through these different channels also vary, with private organizations often supporting more projects with no immediate return on investment, says Kastner. "Philanthropists are able to . . . take a longer-term view. That sounds at first sight ironic that the federal government has a shorter-term view, but it's again this issue of reporting to the Congress about the efficacy of their investing. In many agencies, the grants are three years, and that means that after the first year, you have to start preparing the next proposal."

Hand in hand with wanting to see more-immediate returns on investment, federal agencies are less likely to fund high-risk research. "That's what we are sorely missing—a chance for people almost to do the play side of science, of taking that idea that has very high risks, but has the potential to really unveil something very important," says David Scadden, the director of Massachusetts General Hospital's Center for Regenerative Medicine. "And that's where I think these foundations can get in."

Setting an example

Private granting sources are likely to only become more important as basic research continues to face a rocky federal funding environment. The plateauing budgets and stagnant granting success rates seen at US federal science organizations over the past several years have been thrown into even starker relief by the Trump administration's seeming lack of enthusiasm for funding basic research. This summer, in a somewhat cryptic memo on science spending priorities for fiscal year 2019, the administration noted that federal science agencies should fund research that "can result in the development of transformative commercial products and services."

As institutions and researchers weather what Harvard's McCullough calls "a period of 'capital-U' uncertainty," funding for foundational and basic research becomes a rising concern. "I worry that the downturn in federal research support will likely winnow out to some degree other very high-quality research institutions because they may not be able to make up that difference," McCullough says.

While philanthropic dollars are extremely unlikely to ever take over the primary funding role of federal budget allocations to US science agencies, Kastner says he is hopeful that private funders might demonstrate some of the benefits of a new model for supporting science. "I think philanthropy is more important than ever," he says. "It's most important for setting an example for the Congress, for showing the Congress that it's important to take risks and to take a long-term view when you're talking about science, and not to look [only] for short-term applications." ■

The Polyvagal Perspective

How our minds, brains, and bodies respond to threat and safety.

BY WENDY JONES

In Jane Austen's *Sense and Sensibility,* Elinor Dashwood is talking to a new acquaintance, Lucy Steele. Based on their previous encounters, Elinor doesn't think much of Lucy's character. But Lucy seems determined to befriend Elinor and to make her a confidante. Elinor discovers Lucy's true motives when the latter reveals that she is secretly engaged to Edward Ferrars, the man Elinor loves. Elinor is speechless: "Her astonishment at what she heard was at first too great for words."

Elinor isn't the only one to experience this kind of shutdown and its accompanying frustration. When we're angry, or upset, or fearful—in the grip of any strong emotion—most of us find it difficult to think clearly. This has to do with the inverse relationship between our sympathetic and parasympathetic nervous systems, which manage (respectively) the degree to which we're excited or calm.

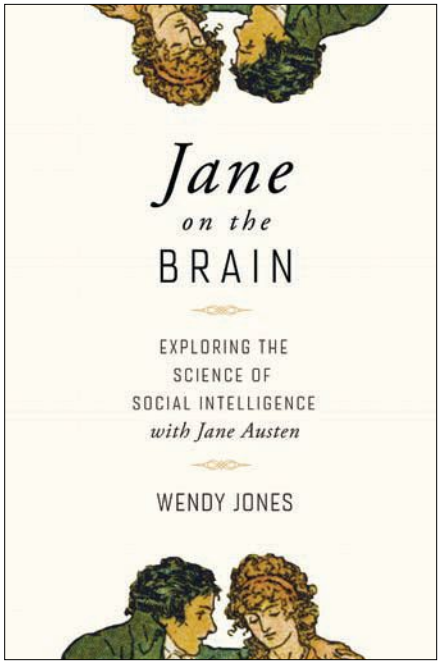
Neuroscientist Stephen Porges has suggested that the thermostat for adjusting sympathetic and parasympathetic input can be found within these systems themselves. He has highlighted the operations involved from a "polyvagal perspective," which considers our neurophysiological functioning in the context of safety, whether our environments are threatening or benign.

I explore these and other neurosocial phenomena through the lens of the immensely popular novels of Jane Austen in my new book, *Jane on the Brain: Exploring the Science of Social Intelligence.*

The sympathetic and parasympathetic systems form the two main branches of the autonomic nervous system, the "UR-system" that controls our automatic bodily functions. When we're safe and in a business-as-usual mode, the parasympathetic system dominates. This drives ongoing bodily functions such as digestion and growth, and allows for clear thinking and social engagement. But when we encounter danger, the sympathetic system activates, and those normative processes are decreased or suspended.

The polyvagal perspective considers the different branches of the vagus nerve, really a cluster of nerves, that originate in the brain stem (right above the spinal cord), as having distinct functions. The first branch is the ventral vagus, so called because its neurons run closer to the ventral side of the brain, toward the front of the body; it's also called the "smart vagus." The ventral vagus connects to and controls the heart's pacemaker, a small, specialized muscle called the sinoatrial node. Porges calls the action of this section of the ventral vagus the "vagal brake." If we perceive something dangerous in the environment, the vagal brake is lifted, and the heart beats faster, which causes the sympathetic nervous system to activate. Increased heart rate is therefore the catalyst, and not just the indicator, of excitement. The automotive metaphor of a (vagal) brake makes sense if you think about driving downhill rather than on a level surface. You need to press the brake to keep driving at a moderate speed.

If activation of the sympathetic system is sufficiently strong—that is, the heart beats very quickly—and we also know we are in danger, our stress responses kick in; these reactions are the second focus of the polyvagal perspective. Stress responses involve the release of excitatory hormones and glucose into the blood, which give us the energy to engage the fight-or-flight response. The third focus of the polyvagal perspective is the dorsal vagus, a branch whose circuits run closer to the back of the brain. If a situation is so threatening that fighting or fleeing is useless, we freeze, a response induced by the dorsal vagus, which is responsible for deactivating responses such as fainting.



Pegasus Books, December 2017

In addition to inducing states of emergency and calm by controlling the vagal brake, the ventral vagus generates all the states of mind and body that we experience between these extremes. With the vagal brake on, you return to coasting at a more even speed rather than racing downhill, and resources are available for both thinking clearly and fully exercising social skills. It is only as Elinor calms down to some extent that she is able to maintain polite conversation with Lucy, "forcing herself to speak, and to speak cautiously." Possessing a capable smart vagus, Elinor quickly recovers her self-possession, and so ultimately deprives Lucy of her triumph. ■

Wendy Jones is a practicing psychotherapist and former English professor known for her work on the connection between literature and the mind-brain sciences. Read an excerpt of Jane on the Brain *at the-scientist.com.*

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Meet the Press, 1967

BY KERRY GRENS

Arthur Kornberg's discovery of DNA polymerase in the 1950s was one of the most fundamental contributions to the newly born field of molecular biology, one that allowed him to make strings of nucleotides identical to a template and to show, essentially, how life itself is assembled.

The finding garnered Kornberg a Nobel Prize, shared with Severo Ochoa, in 1959. Yet, as he wrote in a 1989 memoir in *The Scientist*, there was still a piece missing from the scientific story. "For more than 10 years, I had to find excuses at the end of every seminar to explain why the DNA product had no biologic activity. If the template had been copied accurately, why were we unsuccessful in all our attempts to multiply the transforming factor activity of DNA from *Pneumococcus*, *Hemophilus*, and *Bacillus* species?"

The missing ingredient, it turned out, was another enzyme: a DNA ligase. In 1967, 37-year-old Mehran Goulian had been experimenting with ligases, which had been recently discovered by other groups, and DNA polymerase in Kornberg's lab at Stanford University. Using the two enzymes, Goulian found he could convert the single-stranded, circular genome of a bacteriophage, ΦX174, into the double-stranded form, as happens within an infected bacterium, where the phage commandeers its host's enzymes. Then, with the help of Robert Sinsheimer at Caltech, Goulian and Kornberg showed that the newly synthesized genome could infect *E. coli* and behave just like the natural virus.

Goulian, Kornberg, and Sinsheimer published their work in *PNAS* that year, and Kornberg, with Goulian by his side, held a press conference on December 14 to announce their achievement. "He was responding to a widespread concern at that time about the availability of funds for scientific research, especially basic research," Goulian, now a professor emeritus at the University of California, San Diego, tells *The Scientist* in an email. "Kornberg hoped that a press conference about this research would increase the level of discussion and appreciation by the American public of accomplishments in government-funded research."

HOLDING COURT: On December 14, 1967, Mehran Goulian and Arthur Kornberg held a press conference at Stanford University to discuss their assembly of a functional, 5,000-nucleotide-long bacteriophage genome. Goulian recalls little of the event, and says modestly, "I assume that I said little or nothing, and I am certain that I was happy for Kornberg to be doing the talking."

Indeed, the results garnered publicity, but not in the way Kornberg had anticipated. According to Errol Friedberg's biography of Kornberg, *Emperor of Enzymes*, he had wanted to squelch any suggestion that he had created life. Yet, at an event at the Smithsonian Institution that day, President Lyndon Johnson, who had been briefed about the study, made an off-the-cuff remark about it to his audience. "That evening the Kornberg/Goulian experiments were the lead story on the televised news," Friedberg writes, "which featured the President extemporaneously stating: 'Some geniuses at Stanford University have created life in the test tube!'"

Questions about manipulating and creating life have not abated in the decades since Goulian and Kornberg synthesized the bacteriophage genome. And their work provided the foundation for much of modern genomic tinkering.

"After so many years of trying, we had finally done it," Kornberg wrote in *The Scientist*. "The way was open to create novel DNA and genes by manipulating the building blocks and their templates." ■

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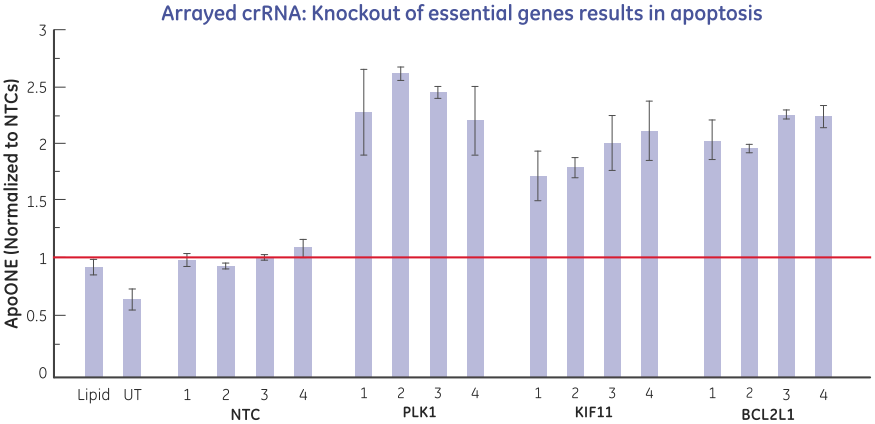
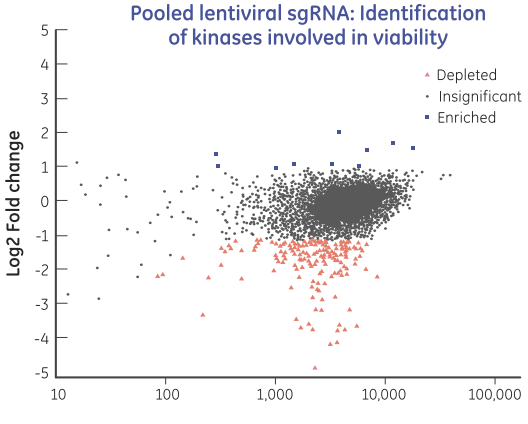
Arrayed crRNA: Knockout of essential genes results in apoptosis



U2OS-(Ubi)EGFP-Cas9 stable cell seeded at 10,000 cells/well in 96-well format were transfected the following day with four different crRNA:tracrRNA complexes at 25 nM concentration targeting PLK1, KIF11 or BCL2L1 or four non-targeting crRNA controls (NTC). Wells transfected with lipid alone (Lipid) or left untreated (UT) were also included as controls. The effects on apoptosis were assayed using Casp3/9 homogeneous assay (ApoONE, Promega) at 48 hours post-transfection. Data normalized to average of NTC (non-targeting) crRNA controls.

Pooled lentiviral sgRNA: Identification of kinases involved in viability



Hits are identified by how strongly and significantly the experimental constructs are depleted at T1 compared to the constructs in the reference sample (T0). ≥ 1.5-fold depletion | ≤ 0.05 padj value

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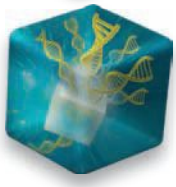

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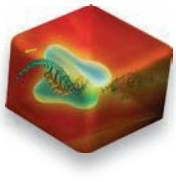

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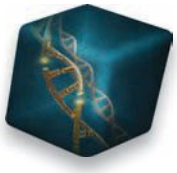

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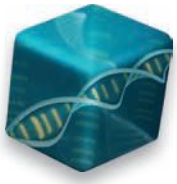

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