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The Transgender Brain

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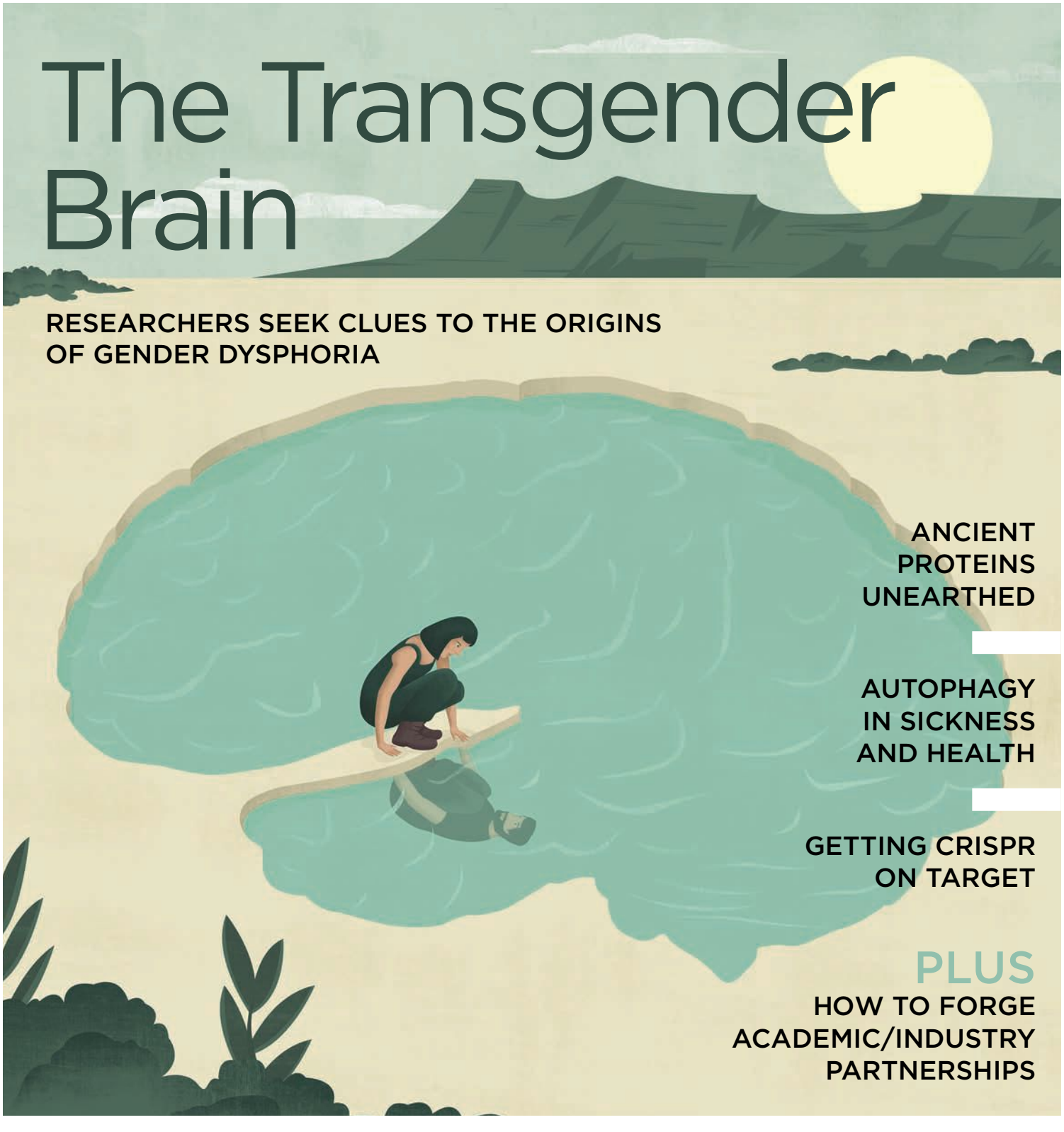

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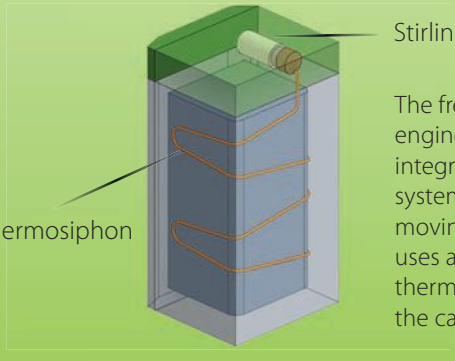

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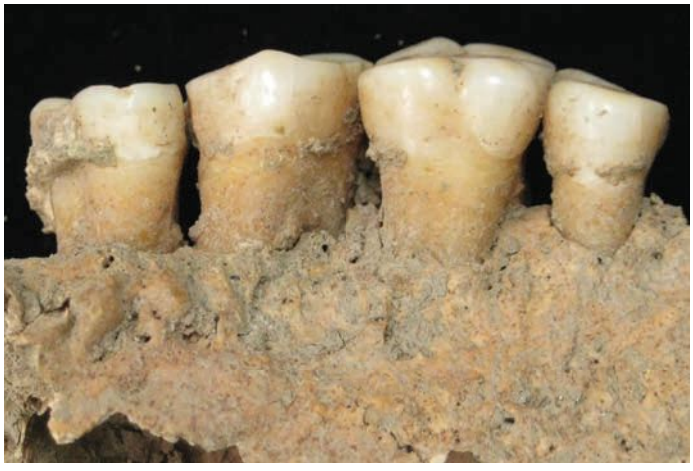

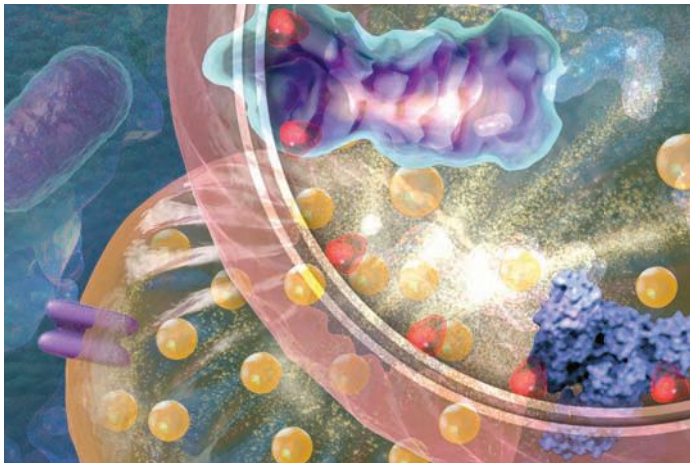

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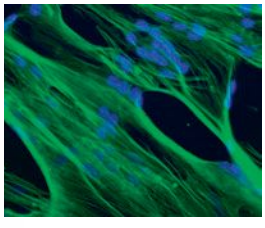

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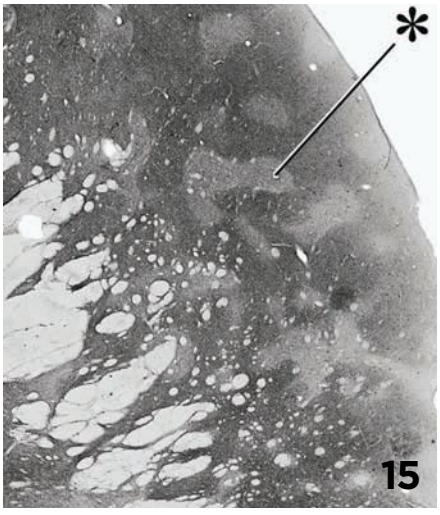

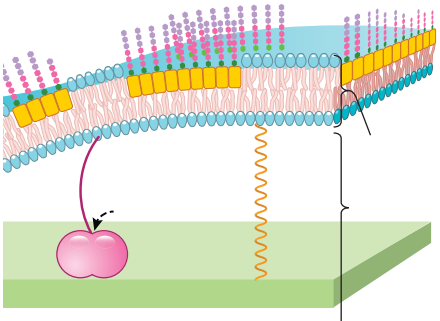

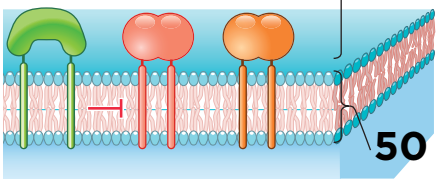

CORRECTIONS:

In "Flies R Us" (*The Scientist*, February 2018) the order of image credits should have read Xiao-Long Lin; Chun-Tang Chen.

In "Robotic Healers" (*The Scientist*, February 2018) MIT's Hugh Herr was incorrectly identified as Conor Walsh's postdoctoral advisor. He was, in fact, Walsh's graduate advisor.

The Scientist regrets the errors.

PUZZLE ON PAGE 12

P	U	M	I	C	E	■	C	A	N	A	L	S
I	■	E	■	A	■	■	T	■	T	■	P	
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A	■	N	■	D	■	L	■	O	■	E	■	O
C	L	O	U	D	■	E	X	T	I	N	C	T
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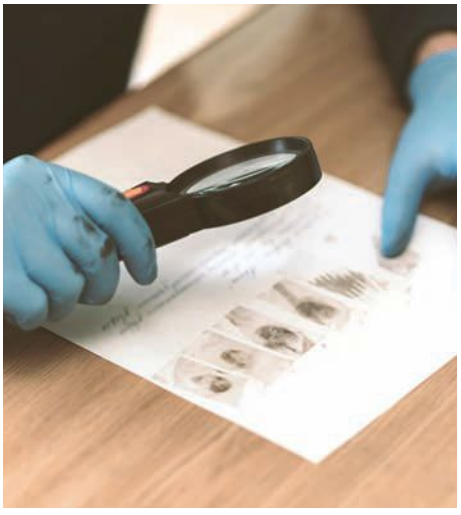

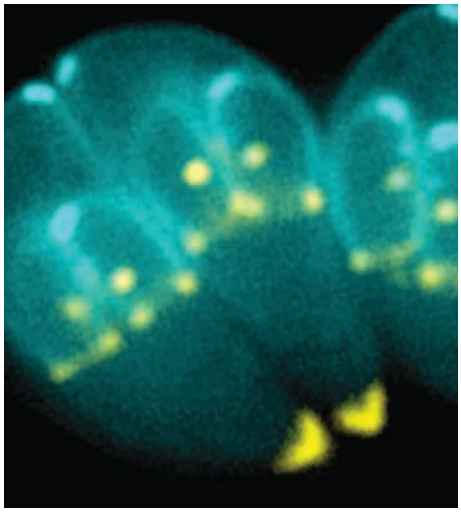

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Relive the Lasker Award speech from University of Basel biologist Michael Hall.

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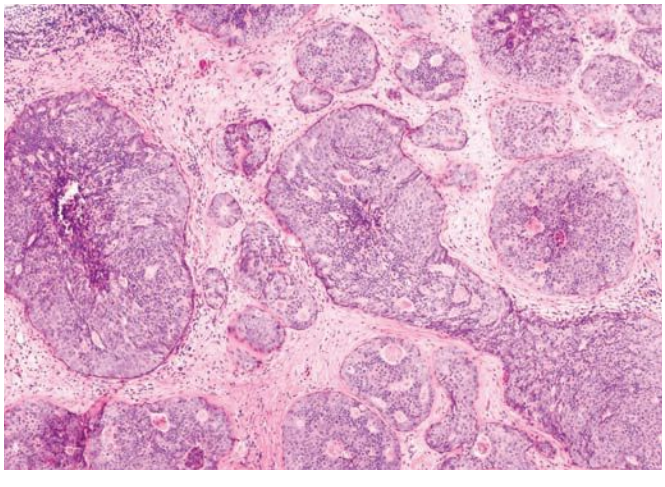
HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- Tumor macrophages, long thought to exacerbate cancer, may one day be recruited to fight it.
- Drug combinations are showing increasing promise in the fight against cancer.
- Meet the neuroscientist who persevered through a brain cancer diagnosis and mental illness.
- Researchers work to resolve the function of delicate structures that surround neuronal cell bodies.

AND MUCH MORE



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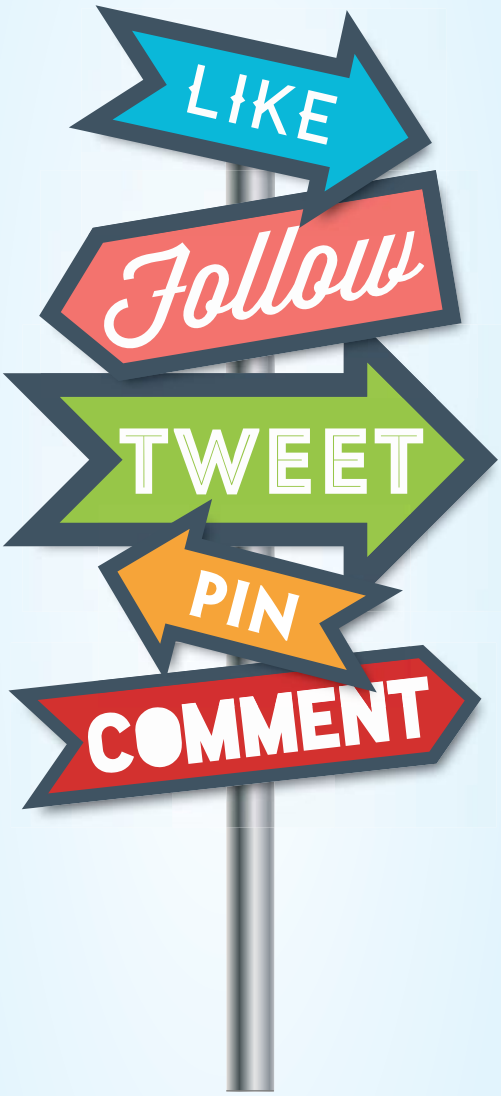

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Biology Illuminated

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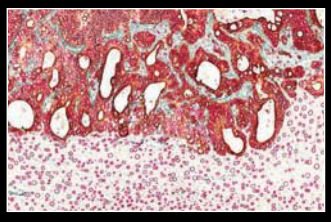

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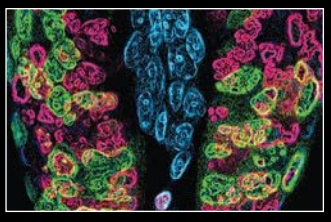

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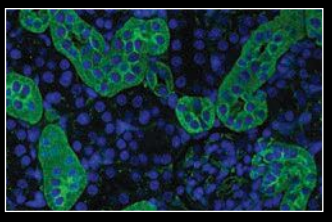

Lectins & Glycobiology

- Lectin Conjugates
- Agarose Lectin Beads
- Anti-Lectin Antibodies
- Blocking Reagents & Sugars

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Top: Image courtesy of Lynn Dong and Jonathan Cheetham, Cornell University
Left: Image courtesy of Steffen Rickelt and Jatin Roper, Massachusetts Institute of Technology
Middle: Image courtesy of Nigel Hammond, The University of Manchester

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Contributors

While **Daniel Klionsky** was an undergraduate at UCLA in the 1980s, introductory biology was one of his favorite courses. A "very enthusiastic" instructor got him interested in marine biology, and Klionsky spent a quarter studying the subject on Catalina Island, one of California's Channel Islands, which lie a short ferry ride from L.A. "We would take these research vessels that were bathtub-shaped and slow," every wave rocking the boats, he recalls. Later, Klionsky used electron microscopy to study cell structure and function as part of a course, and he decided to pursue cell biology. Now running his own lab at the University of Michigan, Klionsky says he's excited by the prospect of translating his research on mutations that affect autophagy in yeast cells into cancer treatments. "We're getting these subtle mutations that allow growth while increasing autophagy."

"I initially wanted to study literature," graduate student **Vikramjit Lahiri** says. "I used to read anything I could get my hands on." Lahiri's favorite author was Khalid Hosseini, who wrote *The Kite Runner*, but he loved the classics as well. "My favorite novel, since childhood, has always been David Copperfield," he says. Lahiri eventually decided to pursue a career in the life sciences because he found research similarly fascinating. He studied biology as an undergraduate, and after completing two post-graduation fellowships in evolutionary biology, he went on to earn a master's degree in molecular biology and biotechnology at Calcutta University. As a PhD candidate in Klionsky's University of Michigan lab, Lahiri studies the process by which autophagy is regulated in yeast cells. He's focused on discovering how each cell orchestrates the contributions of some 40 proteins. Lahiri also enjoys writing. "Working in the lab and doing experiments is rewarding, but writing has its own rewards for me," he says.

Lahiri and Klionsky describe new insights into the process of autophagy on page 42.

Patricia Fara's early interest in mathematics and science led her to study physics at the University of Oxford in 1966. At the time, "it was a very unusual thing to do," she says: she was one of around eight women in her class, which held 220 men. Fara quickly realized that she didn't particularly enjoy the practical side of physics, and was more interested in bigger, philosophical questions. After working for several years setting up a company that produced educational slide programs, Fara decided to return to academia, pursuing a master's degree in history and philosophy and a PhD in the history of science at the University of London. In 1993, she moved to the University of Cambridge as a postdoc, and has been there since, as an affiliate lecturer and director of studies in the university's history and philosophy of science department. Fara is president of the British Society for the History of Science and has authored several books on the topic, including *Science: A Four Thousand Year History*. "For me, it's always been really important not only to write academic articles," she says, but to explain "intellectual ideas to a much wider public." Fara's most recent book, *A Lab Of One's Own: Science and Suffrage in the First World War*, chronicles the change in women's roles in science throughout World War I, and how this paved the way for today's female scientists.

Read Fara's essay about her new book on page 63.

Ashley Yeager knew early on that she wanted to go into writing. Her favorite part of doing science experiments with her parents—both science teachers—wasn't the lab work, it was writing up the results at the end, "because I got to tell a story," she says. Yeager went on to study communication and information at the University of Tennessee as an undergrad. It was only when an advisor there encouraged her to try out a science writing class that she realized that the subject would be a good fit for her. "I fell in love with science writing," Yeager says. A subsequent master's degree in science writing from MIT made her certain she was on the right track. For the next nine years, Yeager dabbled in the field, doing internships at *Science News* and *Nature*, editing academic books for professors, working as a web producer at *Science News*, and serving as an information officer at the W.M. Keck Observatory in Hawaii. For the past year, she has been freelancing for *The Scientist*, and in January she accepted a position as associate editor. Yeager says she looks forward to writing and editing stories for the magazine, but most of all, to "getting to learn something new every day."

LEISA THOMPSON PHOTOGRAPHY; STEPHANIE KING FROM THE LIFE SCIENCES INSTITUTE, UNIVERSITY OF MICHIGAN; DOROTHY LIVINGSTON; PATRICK THORNTON

The Skin We're In

How can science inform the debate on gender?

BY BOB GRANT

Gender has become a hot-button issue. Some politicians and their supporters see gender as a way to divide people, limiting the rights of transgender individuals and therefore encoding a particular moral judgment into law. Across the political spectrum, lawmakers seek to enshrine their own beliefs, with some championing equal rights for all people, regardless of their relationship to their natal sex.

As with any other tangled sociopolitical matter, it's unlikely that an entirely satisfactory solution will ever materialize. When politics, religion, and culture intertwine to complicate such issues, teasing them apart to arrive at some objective truth becomes a tall order. We human beings, if nothing else, are good at clinging to our preconceived biases.

But science, as ever, can serve as an important guiding factor in these types of controversies. And gender is no different.

That is why the research that we highlight in this issue of *The Scientist* is important beyond the quest to understand the biological underpinnings of humans' sense of self. The scientists in our feature story (pg. 26) on how transgender people's brains might (or might not) differ from those of cisgender people strive to disentangle the physiology underlying the mismatches people experience between the gender they perceive themselves to be and that assigned at birth. Several of the researchers interviewed by Associate Editor Shawna Williams express frustration at seeing results from the field frequently interpreted through a political lens; at the same time, they are well aware that seeking objective truths could, and perhaps should, inform the broader debate over transgender rights. Ivanka Savic, a neuroscientist at the Karolinska Institute in Sweden, tells Williams: "This is just part of the biology, the same way as I have black hair and somebody has red hair."

Although scientific fact can be, and too often is, eschewed or distorted in the political arena, I hope that uncovering the roots of gender can serve as a bulwark against unfair, unkind, unwise, and inhumane policy decisions.

A clear picture of the biological contributions to gender dysphoria (or to gender itself, for that matter) has yet to fully emerge, but it heartens me to know that researchers are expending their efforts to increase our dispassionate knowledge of these phenomena.

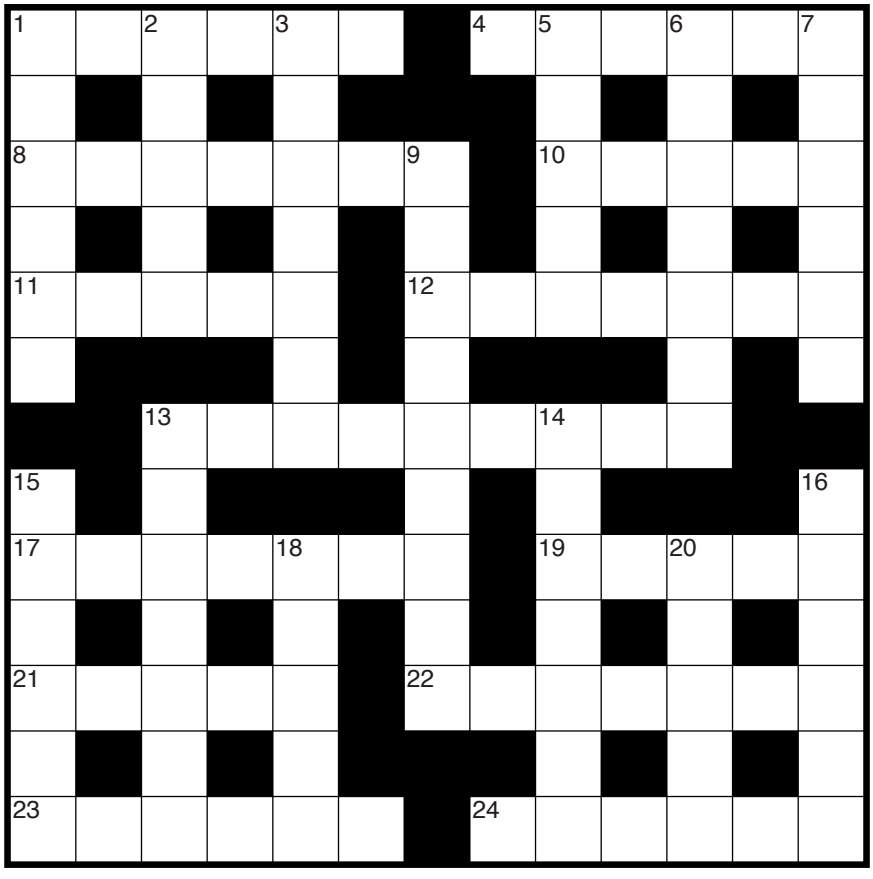
For me, the singular, undeniable truth that underlies this and many other complex societal and cultural upheavals is that people are people, pure and simple. And the mere act of being born *should* ensure that one is treated with respect. Time and again, science has supported this universal right, despite seemingly ceaseless attempts to deny it.

ANDRZEJ KRAUZE

We are living things, and an intricate interplay of biological components informs all that we are and do. From a scientific perspective, it behooves us *Homo sapiens* to understand that physiological contribution as fully as possible. This obligation seems even stronger when the accumulation of such knowledge might inform debates over how we treat each other. ■

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

Speaking of Science



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

BY EMILY COX AND HENRY RATHVON

ACROSS

1. Volcanic glass full of cavities
4. Otological passages
8. Pisan who championed heliocentrism
10. Grassy South American plain
11. Unit of weight; snow leopard
12. Brand-new brant, perhaps
13. Site of the medulla oblongata (2 words)
17. Type of baleen whale like the humpback or minke
19. Word after *Canis* or *Ursa*
21. Target of a chemical "seeding"
22. Like the dodo, the moa, and *Homo floresiensis*
23. White of the eye
24. Straight-line configuration of three celestial bodies

DOWN

1. Dove's city cousin
2. Fruit in the genus *Cucumis*
3. Volcanologist's depression?
5. Cartology project
6. Reappearance of a lost trait
7. Pore thing?
9. Cellular subunit such as a mitochondrion
13. Rodent hunter on a farm (2 words)
14. Fodder grass that's also a man's name
15. Evidence of a dinosaur's passing
16. Made of small air bubbles, as foam
18. Calf's feeding station
20. Female donkey

Answer key on page 5

The decline in happiness and the rise in depression might be caused by the overuse of screens leaving less time for activities more beneficial for mental health such as seeing friends in person, sports and exercise, and sleeping.

—San Diego State University psychology researcher **Jean Twenge** speaking to *Business Insider* about the emerging phenomenon of tech addiction, especially among young people (February 6)

God only knows what it's doing to our children's brains.

—Facebook founding president **Sean Parker**, on the potential damage wrought by the social network (*The Guardian*, February 5)

I'm getting a second opinion off the internet about your diagnosis of my "screen addiction."

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Notebook

MARCH 2018

DAVID WILLIS, SALMON ENHANCEMENT PROGRAM

HELD BACK: Pacific salmon raised in hatcheries, such as the Big Qualicum Hatchery in British Columbia, are less likely than wild-born fish to survive ocean life.

Something's Fishy

When captive-bred Pacific salmon leave their hatcheries to swim free in the ocean, they soon take on the look of their wild counterparts, with the color of their sides changing from red to silver, the better to mesh with their new saltwater digs. But in important, less visible ways, the fish never shake the legacy of their domestic upbringing: They are less likely than wild-born salmon to survive in rivers and the ocean, and if they live long enough to reproduce, they produce fewer offspring.

Just what's wrong with these hatchery-reared fish? With wild stocks on the decline and aquaculturists stepping in to try and bolster Pacific salmon populations, answering this question—and, if possible, increasing the fitness of hatchery-raised fish—has gained importance.

Some researchers suggest that hatchery rearing selects for alleles for certain traits, such as a tolerance for crowding, that make fish more likely to thrive in confinement, and that these same features work against the animals in the wild. One 2012 study of steelhead trout (*Oncorhynchus mykiss*) seemed to lend support to that theory, finding that the offspring of the most reproductively successful captive fish were the least likely to spawn successfully in the wild (*PNAS*, 109:238-42).

But biologist Louis Bernatchez of Quebec's Laval University wasn't satisfied with this genetic-adaptation explanation. In Canada's Pacific salmon hatcheries, "there's a lot of genetic exchange between hatchery fish and wild fish," because wild-hatched fish sometimes stray into the hatcheries to spawn, and hatchery-reared fish may reproduce in the wild, he says. "It was hard to believe that selection alone could be involved in explaining the performance differences that we see between hatchery and wild."

So Bernatchez and his colleagues set out to search for evidence of a different kind of hatchery adaptation, detectable in the epigenome rather than the genome. They

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NOSEDIVE: Wild populations of coho salmon (*Oncorhynchus kisutch*) have plummeted over the last few decades.

trapped juvenile coho salmon (*Oncorhynchus kisutch*) just before, or a few weeks after, release from hatcheries on two rivers in British Columbia, and wild juveniles from the same area. Then, they collected and sequenced samples of the fish's muscle tissue. When they compared the two populations' genomes, the researchers found no significant genetic differences. But compared to the genomes of their wild counterparts, 100 regions on the genomes of hatchery-reared salmon were differentially methylated, and of those, 89 were hypermethylated (*PNAS*, 114:12964-69, 2017).

"That's a pretty clear-cut and striking result," Bernatchez says, adding that the results imply "that those genes are downregulated, or less expressed, most likely, in the hatchery fish relative to wild fish." Some of the hypermethylated regions contain genes for immunity, the transmission of neural signals to muscles for locomotion, and appetite and feeding behaviors. "Quite a few important biological functions were affected," he adds, indicating that the methylation patterns may play into the fitness differences between wild and hatchery-reared fish.

"I think it's a good first step" toward discovering whether the hatchery environment induces epigenetic change, says Mackenzie Gavery, a postdoc at the Uni-

versity of Washington and NOAA's Northwest Fisheries Science Center who studies epigenetics in aquatic ecosystems and was not involved in the study. But it's not clear whether the epigenetic differences found by the team persist over the years that the fish live in a shared environment, she says, and thus whether they could explain the persistent fitness differences between the two types of salmon.

"I think it's a very important finding," comments Erika Eliason, a comparative physiologist at the University of California, Santa Barbara. But, like Gavery, she thinks the case of the unfit hatchery salmon is far from closed. An important next step will be to look for direct evidence that the epigenetic differences have functional consequences, she says. She'd also like to see the analysis performed on other tissue types, such as the gill—an area crucial for fishes' transition from freshwater to ocean living.

Bernatchez acknowledges that the study doesn't draw a clear causal link between the epigenetic differences and survival. As another step in that direction, his group is now running a similar comparison on adult fish (hatchery-raised salmon have one of their smallest fins clipped before release so that they can be distinguished from wild-born salmon later). Answering the genetic vs. epigenetic question, and determining whether the epigenetic differences seen in hatchery fish are heritable, could be consequential for conservation efforts, he says, because heritable changes "would not disappear or vanish as easily as . . . if those changes were totally environmentally driven and not passed along from one generation to the next." Altering aspects of the hatchery environment such as overcrowding and diet might help mitigate epigenetic changes, he says.

Gavery agrees that with greater understanding, it might be possible to manage hatcheries differently to boost fitness: "If it's an epigenetic change, if you can figure out . . . [how] to reduce that impact, you can in essence make a better hatchery, make more-robust fish."

—Shawna Williams

Forensic Justice

An expert in forensic chemistry, Niamh Nic Daéid is accustomed to presenting evidence in court—often for hours on end. "The longest time I've spent in the witness box is about seven hours," says Nic Daéid, director of research at the University of Dundee's Center for Anatomy and Human Identification (CAHID) in Scotland. Nevertheless, she says, "the thing that is most important is being able to clearly and accurately explain the meaning of the results of whatever tests have been done."

Those results, which can pertain to evidence ranging from the fingerprints at a crime scene to the pattern of burn marks created by an explosive, can help push a trial verdict one way or the other. But ensuring that they are communicated properly can present challenges, even for experienced witnesses. Many judges, not to mention the lawyers and members of the jury in a UK courtroom, do not have a strong background in science, notes CAHID's director Dame Sue Black, a regular expert witness in court. During testimony, therefore, "there is a difference, often, between what is said and what is understood," she explains. "If the judge is in any doubt about the science that's being discussed, then that makes it very challenging for the courtroom."

Such communication difficulties have long been a subject of discussion among the UK judiciary. In particular, Sir John Thomas, a judge who acted as Lord Chief Justice of England and Wales until last year, has advocated for better communication between scientists and the judges presiding over criminal cases, Black notes. After discussing the matter with Thomas, several science organizations came together in the early 2010s to develop explanatory documents, or primers, that would provide a plain-language grounding in the science being brought

ANDRZEJ KRAUZE

into the courtroom, along with an overview of the strengths and weaknesses of various types of scientific evidence. "It seemed a brilliant idea to us," says Dame Jocelyn Bell Burnell, an astrophysicist and president of the Royal Society of Edinburgh, one of the organizations involved. Collaborating with the Royal Society of London and various members of the judiciary, "a joint project was set up," she says. "And it rolled from there."

To get the project going, Black, Nic Daéid, and others set about developing the first two primers a few years ago. They selected two forensic approaches to focus on: DNA analysis and forensic gait analysis, a recently developed science of using aspects of locomotion to identify individuals from, say, security camera footage. These two disparate types of evidence were carefully chosen, explains Black. They "give the judges the opportunity to view the range of what a primer could do, from something that is very detailed, very well supported, and frequent in the courtroom, to something that is less frequent in the courtroom, has a [developing] scientific [basis]," she says. "It was about choosing the ends of a spectrum that allowed us to test whether the primer could cope at both extremes."

There is a difference, often, between what is said and what is understood.

—Dame Sue Black, University of Dundee

Nic Daéid, along with Lady Justice Anne Rafferty, spearheaded the creation of the DNA analysis primer, which builds from biology basics such as "What is DNA?" to a review of the limitations of DNA profiling for mixed samples. "Technology has driven the development of DNA analysis such that we can get an awful lot more information out of a lot less sample," Nic Daéid says. In some cases, this heightened sensitivity can counterintuitively make it more difficult to answer questions about who or where the DNA came from, she adds. "Those questions have always been asked, but now they're being asked in a much more complicated environment because we have multiple DNA profiles in samples."

Heading up the primer on gait analysis, meanwhile, was Black, along with Judge Mark Wall, Queen's Counsel. Their document explains the techniques used to match footage of a person walking or running to a suspect in custody—for example, by comparing knee angles across images. Challenges include a reliance on generally low-quality footage and differences between the crime scene and the environment in which a suspect is later filmed for comparison. "I certainly walk differently whether I'm in flat shoes, flip-flops, or high heels; whether I'm walking on a cobble street or a flat road; whether I have a mobile phone in my hand or a shopping bag," Black says. "There are so many variables to be taken into account . . . we have to be very cautious."

Summarizing these complex issues in plain language was merely the first hurdle the primers had to clear before publication. Once drafted, the documents underwent multiple rounds of high-scrutiny peer review, Black says. All told, she notes, each document was assessed by around 40 scientists and members of the judiciary. "It was very important to us that the science was checked and double-checked," explains Julie Maxton, a British lawyer and executive director of the Royal Society. "Then it went out to the judges to see if it was in a format that they would find useful. They came back with very detailed comments. That process took us probably about 18 months."

This past November, the team published the primers on the Royal Society's website, where they are free to download in full. "The reception has been amazingly positive," says Bell Burnell, who has received feedback from various members of the judiciary. "Everyone that I've spoken to is enormously grateful to have this kind of thing, and think it's a fantastic resource." And while other countries have their own channels for discussion—the U.S., for example, has its Committee on Science, Technology, and the Law, which deals with issues affecting the scientific and legal communities—there's been interest in using the primers abroad, too, Bell Burnell adds.

That's not to say the team's work in the U.K. is done. As Supreme Court Justice Lord Anthony Hughes, chair of the committee overseeing the primers' development, noted in a press release accompanying the primers' publication: "These are the first in a series of primers designed to be working tools for judges." Another two primers are currently in the pipeline—one will cover statistical methods; the other will explain the physics of vehicle collisions. Maxton notes that committee members will meet this year to decide how to arrange annual reviews to keep the documents up-to-date, as well as how to more formally assess the impact of these primers in the courtroom. Although November's launch represented a landmark for the project, "I think for all of us, it's just one step on the road," Maxton says. "What we really want to see is whether they're useful to the judges. So I'm holding the champagne, as it were, until we know that they are." —Catherine Offord

Delaying Death

In the 1990s, pharmacologist Dave Sharp of the University of Texas's Barshop Institute for Longevity and Aging Studies in San Antonio was studying mice with pituitary dwarfism—a condition in which the pituitary gland fails to make enough growth hormone for normal development. The puzzle, Sharp explains, was that research had shown that these hormone-deficient dwarf mice lived longer than normal mice. "I wondered, why is being small connected with longer life?" he says.

Yeast research led by molecular biologist Michael Hall at the University of Basel in Switzerland was to provide Sharp with an unexpected lead. In 1996, a team led by Hall (who would go on to win a Lasker award in 2017 for the work) revealed a new intracellular signaling pathway, mediated by the protein targets of a compound called rapamycin. Using this drug to block the "target of rapamycin" (TOR) proteins in yeast had the same effect as starvation did: treated yeast cells were smaller, but longer-lived than normal cells (*Cell*, 7:25-42, 1996). For Sharp, it

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sparked an idea. "Maybe TOR is a nutrient response system, connecting diet restriction and growth-factor restriction," he recalls thinking. "I proposed that if you fed mice rapamycin, they would live a long time."

Back then, the hypothesis was unconventional. Rapamycin, a compound first identified in the 1970s in a soil sample from Easter Island, has been used for decades to suppress the immune system in transplant patients; it seemed counterintuitive that it could prolong life, Sharp notes. "Nobody would read my proposals," he says. "They'd just laugh. You know, 'An immunosuppressant extending lifespan?'"

But research since then has lent support to Sharp's theory. Studies in the early 2000s showed that the drug could make nematodes and fruit flies live longer, while research by Sharp and others suggested that TOR signaling is downregulated in long-lived dwarf mice. And a collaboration between Sharp and the Barshop Institute's Randy Strong, the principal investigator for the National Institute on Aging's Interventions Testing Program, led to a landmark mouse study that identified rapamycin as the first drug to extend lifespan in mammals (*Nature*, 460:392-95, 2009). By fine-tuning dosage and delivery systems over the next five years, the pair increased longevity in male mice by 23 percent and in females by 26 percent, compared to control animals (*Aging Cell*, 13:468–77, 2014).

Researchers have now expanded the study of rapamycin's lifespan-extending effects to other animal species. For example, an ongoing collaboration between the University of Washington and Texas A&M University College of Veterinary Medicine is studying the effects of rapamycin in companion dogs. Although it's too early to say whether the drug does indeed extend the healthy lifespan, or healthspan, of the animals, "we found significant improvement in cardiac function after just 10 weeks," says Texas A&M's Kate Creevy. As part of her group's efforts to move the drug towards regulatory approval for use as a pet medicine, the researchers initiated a Phase 2 clinical trial with the animals earlier this year. And because companion dogs, like humans, are more genetically diverse than laboratory animals, the studies represent a step toward better understanding how rapamycin performs in people.

Still missing from this research is a mechanistic understanding of how the drug extends lifespan. Part of the problem stems from the fact that the pathway rapamycin acts on is involved in so many biochemical processes. A protein kinase, TOR is widely expressed in the cells of nearly all eukaryotic organisms, and handles a host of cellular functions related to nutrient sensing and growth, including proliferation, transcription, and programmed cell death. "Almost any kind of stress you put on cells is handled by TOR," Sharp explains. In humans and other mammals, "it's involved with the nervous system, the muscles, all your organs." Inhibiting TOR with rapamycin limits cell proliferation, for instance, but also has other, systemic side-effects in humans that are not fully understood, including insulin resistance—though such negative effects can be reduced by tweaking dosing regimens.

> **There's always been a paradox with it.**
>
> —Dave Sharp, Barshop Institute
> University of Texas

One possible explanation for the longevity connection is that, via the TOR pathway, rapamycin helps to prevent age-related disease. In the 2000s, the drug was shown by multiple groups to have antitumor properties in human cell lines and mice. It also seems to reduce some traits associated with later-age cognitive impairment. A few years ago, for example, the Barshop Institute team discovered that rapamycin improved later-life memory and learning, and reduced the development of amyloid plaques—a key feature of Alzheimer's disease—in mouse brains (*PLOS ONE*, 5:e9979, 2010; *Aging Cell*, 11:326-35, 2012).

There are also more-recent hints that some of rapamycin's effects could be mediated by the microbiome, which has multiple effects on immune system function. A couple of years ago, a team at the University of Washington got a clue from mouse droppings. "We noticed the feces of rapamycin-treated mice were a lot smaller than those of control mice," says University of Washington postdoc Alessandro Bitto. "We sent samples to microbiome researchers in Missouri, and they found that gut microorganisms differed significantly between the two groups." The rapamycin-treated mice in that study not only lived longer on average, but performed better on tests of physical skill and endurance (*eLife*, 5:e16351, 2016).

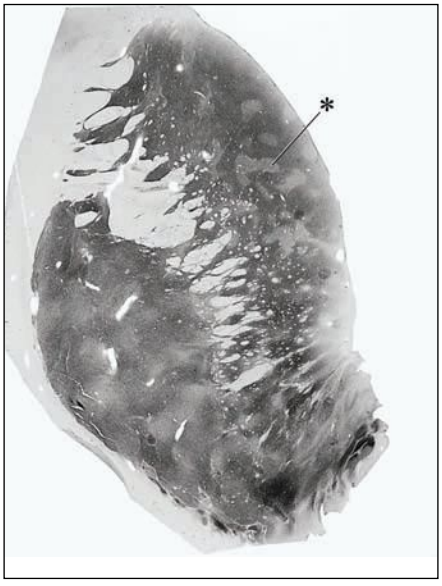
Extending such findings to humans is no easy task: decades-long studies are rarely

attractive to investors, making human clinical trials on longevity difficult to fund. But studies on the health benefits of rapamycin for humans are gaining traction. A 2014 Novartis study suggested that rapamycin counterintuitively boosted the immune response in elderly humans—last year, the company announced a Phase 2 trial to study the drug's impact on diseases affecting older people, and on "age-related decline." And the National Institutes of Health is currently funding a study, led by Dean Kellogg of the Barshop Institute, on rapamycin's effects on muscle strength, cognition, and immune function in healthy seniors. Meanwhile, the last decade has seen the development of several rapamycin derivatives for the treatment of cancer. For example, the FDA approved the derivative everolimus in 2009 for the treatment of renal cell carcinoma and for multiple other cancer types since.

Researchers such as Sharp have come to see such broad—and sometimes apparently conflicting—applications as an inherent feature of rapamycin biology. "Here's a drug that could treat cancer and suppress the immune system, which is supposed to be the system that helps you *not* get cancer," Sharp says. "So there's always been a paradox with it."

Strong sees it differently. "I think there are some gaps in our knowledge," he says. "That's why these things are, to us, paradoxical. When we finally figure it out, they won't be so paradoxical anymore."

—Anne N. Connor



TRICKY SPOT: Neural structures called striosomes, such as the one highlighted by an asterisk in this section of striatum from a human brain, may aid in complex decision making.

Tough Choices

Many of life's trickier decisions share a common denominator: the options all have both pros and cons. This is what psychologists call a "cost-benefit conflict," and it's something that rats and mice in Ann Graybiel's neuroscience laboratory at MIT face on a regular basis.

Graybiel aims to understand how brains evaluate costs and benefits, and why the capacity to do so is sometimes impaired in neurological and neuropsychiatric disorders such as Huntington's disease, anxiety, and depression. Graybiel and her colleagues have pinpointed the specific brain circuit—consisting of prefrontal cortical neurons, neurons in structures known as striosomes, and inhibitory interneurons that suppress the activity of striosomes—that appears to control this type of decision making. In a study published last November, the researchers reported that chronic stress caused rats and mice to make riskier decisions than they normally would, and that the rodents' motivations returned to normal with manipulation of this circuit.

Graybiel has long been fascinated by the striatum, located in the basal ganglia in the deep forebrain. It was assumed to be primitive, "and not mixed up in any kind of terribly interesting behavior," she explains. But that view has since changed. The brain region has many projections into the prefrontal cortex, is innervated by midbrain dopaminergic circuits, and is thought to act as a "relay station" between cognitive tasks and motor-related tasks.

In 1978, Graybiel's lab found small, labyrinthine structures within the striatum, which they called striosomes, or striatal bodies (*PNAS*, 75:5723-26). On further investigation, Graybiel and collaborators discovered that in a normal brain facing a cost-benefit conflict, neurons of the prefrontal cortex stimulate the striatum's inhibitory interneurons, which in turn repress the striosomes' activities. In essence, this striatal circuit controls whether the cortex can talk to the striosomes or not. It's amazing, Graybiel says, that a deep forebrain structure can control what the cortex—considered the master controller of the mammalian brain—activates, giving the striatum "so much influence on affect and motivation, as well as movement."

To test the circuit's importance to cost-benefit conflict decision making, Graybiel's team created a motivational conflict for rats and mice, designing a T maze that mimicked a task used clinically to assess the behavior of patients with depression or anxiety. After getting to know the maze, rats and mice were given two options: to follow a dangerously well-lit pathway (the rodents prefer to avoid bright light) that had deliciously rich chocolate milk at the end, or to choose a dimmer pathway with weaker chocolate milk. Normal rats and mice decided to avoid the riskier pathway, opting for the low-reward, low-risk route, about half the time.

But disrupting the connection between cortical neurons and the striosomal neurons using optogenetics made the rodents more likely to choose the option with the bigger payoff, and greater risk (*Cell*, 161:1320-33, 2015). "It looks like they no longer care about the cost . . . or they just have to have a lot of [the] good" to choose a given option, Graybiel explains.

In the new study, Graybiel's team chronically stressed rats and mice by either putting them in a cloth bag for a short period, or shocking their feet briefly every day for two weeks, which Graybiel thinks is the rodent equivalent of sitting in a traffic jam every day. The stressed rodents' behavior changed, mirroring decision making in the rats and mice whose circuits had been optogenetically altered. They were significantly more likely to choose the dangerous-looking pathway with the bigger payoff, even if the researchers tried to tempt them by adding extra chocolate to the milk on the dimmer side. This effect lasted for months (*Cell*, 171:1191-1205.e28, 2017).

GRAYBIEL LAB, MIT

"It was stunning," says Graybiel. "We couldn't tell the difference between an animal in which its prefrontal cortex had been disconnected from striosomes . . . and the behavior of a stressed animal to which nothing else had been done."

Graybiel thinks stress disrupted the cortical neurons' ability to suppress the striosomes quickly enough, which in turn became overexcited. To test this, her team optogenetically excited the high-firing interneurons, thereby inhibiting the striosomes, and found that this restored normal behavior in the stressed rodents.

There is some evidence that stress can have a similar effect on human decision making. Mara Mather, a psychology researcher at the University of Southern California, has observed that people placed under stress—in this case, they briefly dipped a hand in ice water—appeared to learn better from positive feedback about a task they were asked to do than when given negative feedback (*Psychol Aging*, 28:35-46, 2013). She suggests that the stressed rats might have been similarly focused more on the reward of the concentrated chocolate milk than on the cost of going down the riskier pathway. This and other studies on stress and decision making "fit with a broader literature [showing] that, under stress, it's much harder for people to avoid their addiction, [such as] if someone is trying to quit smoking or go on a diet."

There's also evidence that the human striatum plays a role in decision making. University of Amsterdam cognitive neuroscientist Birte Forstmann and collaborators performed brain scans on human subjects as participants tried to determine whether the majority of dots on a screen were moving in a coherent direction. The researchers found that under speed stress, the striatum and a motor-associated area in the cortex—which together are thought to be involved in making voluntary action plans—"started to show a lot of activation," she explains (*PNAS*, 105:17538-42, 2008).

In another study, Forstmann and colleagues examined how patients with lesions in their dorsal striatum would handle the same task (*Cortex*, 85:37-45, 2016). These patients were less cautious than controls. Although the results are preliminary and based on only five patients, she notes, they suggest the striatum plays an important role in exercising caution during decision making.

Although it's unclear whether the circuit works the same way in humans as in rodents, Graybiel thinks the neural pathway may be involved in many conditions where decision making is altered, such as addiction, anxiety, depression, and PTSD. "Ultimately, there must be common circuits that are being affected," she says. "And so my hope is that we've found one such circuit and that . . . we can help people. That's the whole point of our research."

—Katarina Zimmer

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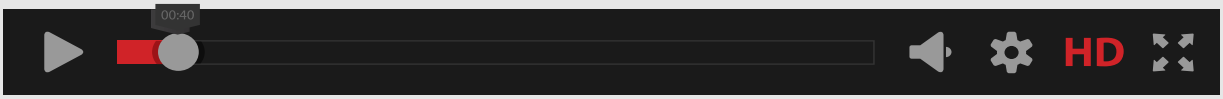

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TheScientist

Precision Optogenetics

A newly engineered, localizable opsin protein enables single-neuron stimulation with high temporal precision.

BY RUTH WILLIAMS

When optogenetics burst onto the scene a little over a decade ago, it added a powerful tool to neuroscientists' arsenal. Instead of merely correlating recorded brain activity with behaviors, researchers could control the cell types of their choosing to produce specific outcomes. Light-sensitive ion channels (opsins) inserted into the cells allow neuronal activity to be controlled by the flick of a switch.

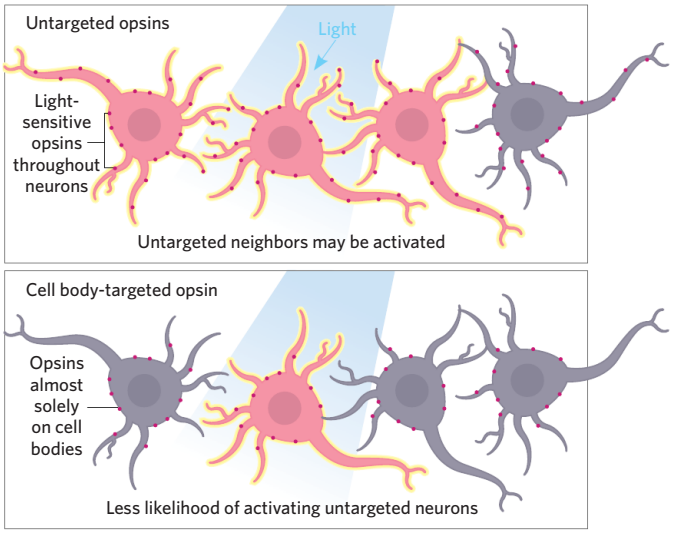
Nevertheless, MIT's Edward Boyden says more precision is needed. Previous approaches achieved temporal resolution in the tens of milliseconds, making them a somewhat blunt instrument for controlling neurons' millisecond-fast firings. In addition, most optogenetics experiments have involved "activation or silencing of a whole set of neurons," he says. "But the problem is the brain doesn't work that way." When a cell is performing a given function—initiating a muscle movement, recalling a memory—"neighboring neurons can be doing completely different things," Boyden explains. "So there is a quest now to do single-cell optogenetics."

Illumination techniques such as two-photon excitation with computer-generated holography (a way to precisely sculpt light in 3D) allow light to be focused tightly enough to hit one cell. But even so, Boyden says, if the targeted cell body lies close to the axons or dendrites of neighboring opsin-expressing cells, those will be activated too.

To avoid this, Boyden's team fused a light-sensitive channelrhodopsin protein to a receptor that localizes to neuronal cell bodies, but not to axons and dendrites. With this approach, the likelihood of hitting just one cell with a targeted laser is far greater. "It eliminates the cross-talk of stimulating nearby cells," says Robert Singer of the Albert Einstein College of Medicine in New York and Janelia Research Campus in Virginia who was not involved with the work.

This opsin localization trick "is not intrinsically new," Singer adds—other researchers have used a similar approach. "What is new is the combination of localization with a novel channelrhodopsin." Boyden's team used a recently discovered protein that requires less-lengthy and less-powerful light stimulation than other commonly used channelrhodopsins to activate neuronal firing. As a result, the firing was more immediate and predictable, increasing the temporal precision to a millisecond.

"Neurons in the brain fire with millisecond precision," Boyden explains. Now there's a single-cell optogenetic technique to match. (*Nat Neurosci*, 20:1796–1806, 2017) ■



ON TARGET: Precision illumination techniques target light to individual neuronal cell bodies, but neighboring cells may be activated if their dendrites or axons lie nearby. Unlike regular opsins, which are distributed throughout the entire neuron, cell body–localized opsins, such as those described by Christopher Baker of the Max Planck Florida Institute for Neuroscience (*eLife*, 5:e14193, 2016) and now Edward Boyden's team (*Nat Neurosci*, 20:1796–1806, 2017), prevent such stray activation. Boyden and his collaborators also use a more responsive channelrhodopsin for high-speed firing.

AT A GLANCE

SINGLE-CELL OPTOGENETICS (REFERENCE)	OPSIN USED	FUSION PROTEIN	TWO-PHOTON STIMULATION	TEMPORAL PRECISION
C.A. Baker et al., *eLife*, 5:e14193, 2016	Human channelrhodopsin 2 (hChR2)	A domain of $K_V2.1$ potassium channel, which localizes mainly to the cell body with a small degree of proximal dendrite localization	180 mW per cell for 150 ms	17 ms
O.A. Shemesh et al., *Nat Neurosci*, 20: 1796–1806, 2017	High-photocurrent channelrhodopsin (CoChR)	N terminus of kainite receptor subunit 2 (KAV2), which localizes almost solely to the cell body of neurons	30 mW per cell for 10 ms to 30 ms	Less than 1 ms

The Transgender Brain

Researchers are probing the neural roots of gender dysphoria.

BY SHAWNA WILLIAMS

Techniques such as functional MRI have begun to yield clues to possible biological underpinnings of gender.

In recent years, US society has seen a sea change in the perception of transgender people, with celebrities such as Caitlyn Jenner and Laverne Cox becoming the recognizable faces of a marginalized population. Transgender rights have also become a mainstream political issue, and the idea that people should be referred to by the names and pronouns they find most fitting—whether or not these designations match those on their birth certificates, or align with the categories of male and female—is gaining acceptance.

Yet a biological understanding of the contrast between the natal sex and the gender identity of transgender people remains elusive. In recent years, techniques such as functional magnetic resonance imaging (fMRI) have begun to yield clues to possible biological underpinnings of the condition known as gender dysphoria. In particular, researchers are identifying similarities and differences between aspects of the structure and function of the brains of trans- and cisgender individuals that could help explain the conviction that one's gender and natal sex don't match.

The results may not have much effect on how gender dysphoria is diagnosed and treated, notes Baudewijntje Kreukels, who studies gender incongruence at VU University Medical Center in Amsterdam. "It's really important that it will not be seen as, 'When you see [gender dysphoria] in the brain, then it's true.'" But the insights from such research could go a long way toward satisfying the desire of some transgender people to understand the roots of their condition, she adds. "In that way, it is good to find out if these differences between them and their sex assigned at birth are reflected by measures in the brain."

Developmental mismatch

One prominent hypothesis on the basis of gender dysphoria is that sexual differentiation of the genitals occurs separately from sexual differentiation of the brain in utero, making it possible that the body can veer in one direction and the mind in another. At the root of this idea is the notion that gender itself—the sense of which category one belongs in, as opposed to biological sex—is determined in the womb for humans. This hasn't always been the scientific consensus. As recently as the 1980s, many researchers argued that social norms in how we raised our children solely dictated the behavioral differences that developed between girls and boys.

Perhaps the most famous proponent of this line of thinking was psychologist John Money, who went so far as to posit that a male baby with a congenital abnormality of the penis, or who had lost his penis in a surgical accident, could successfully

THE TRANSGENDER BRAIN

Since the 1990s, researchers have investigated various features of the brains of transgender people. The results have yielded a mixed picture of the neural mechanisms that may underlie what's known as gender dysphoria. Some studies, for example, have identified aspects of transgender brains that more closely match those of people of the same gender or fall in between typical cisgender women and men, supporting the idea that there is a mismatch between the development of gender in the brain and the body ✹. But others have found features of the brains of transgender individuals that are more similar to those of people who share their sex assigned at birth, or differ from cisgender people of both sexes ❖.

❖ THALAMUS AND PUTAMEN
Relevant to: Self-perception
Findings: In one study, these areas were smaller in transgender women than in cisgender people of either sex. However, other comparisons of putamen size have yielded inconsistent results.

✹ RIGHT SUPERIOR FRONTAL GYRUS
Relevant to: Higher cognitive functions, including working memory
Findings: While discerning male from female voices, trans and cis women had similar levels of activation in this area, while cisgender men showed higher activity.

✹ BED NUCLEUS OF THE STRIA TERMINALIS (BNST)
Relevant to: Sexual behavior
Findings: Size and neuron numbers are comparable in trans- and cisgender women, and different from trans- and cisgender men, whose numbers are similar.

✹ HYPOTHALAMUS
Relevant to: Hormone release, sexual orientation
Findings: In adolescents, neural activation was in line with the experienced gender when subjects smelled the male chemical signal androstadienone.

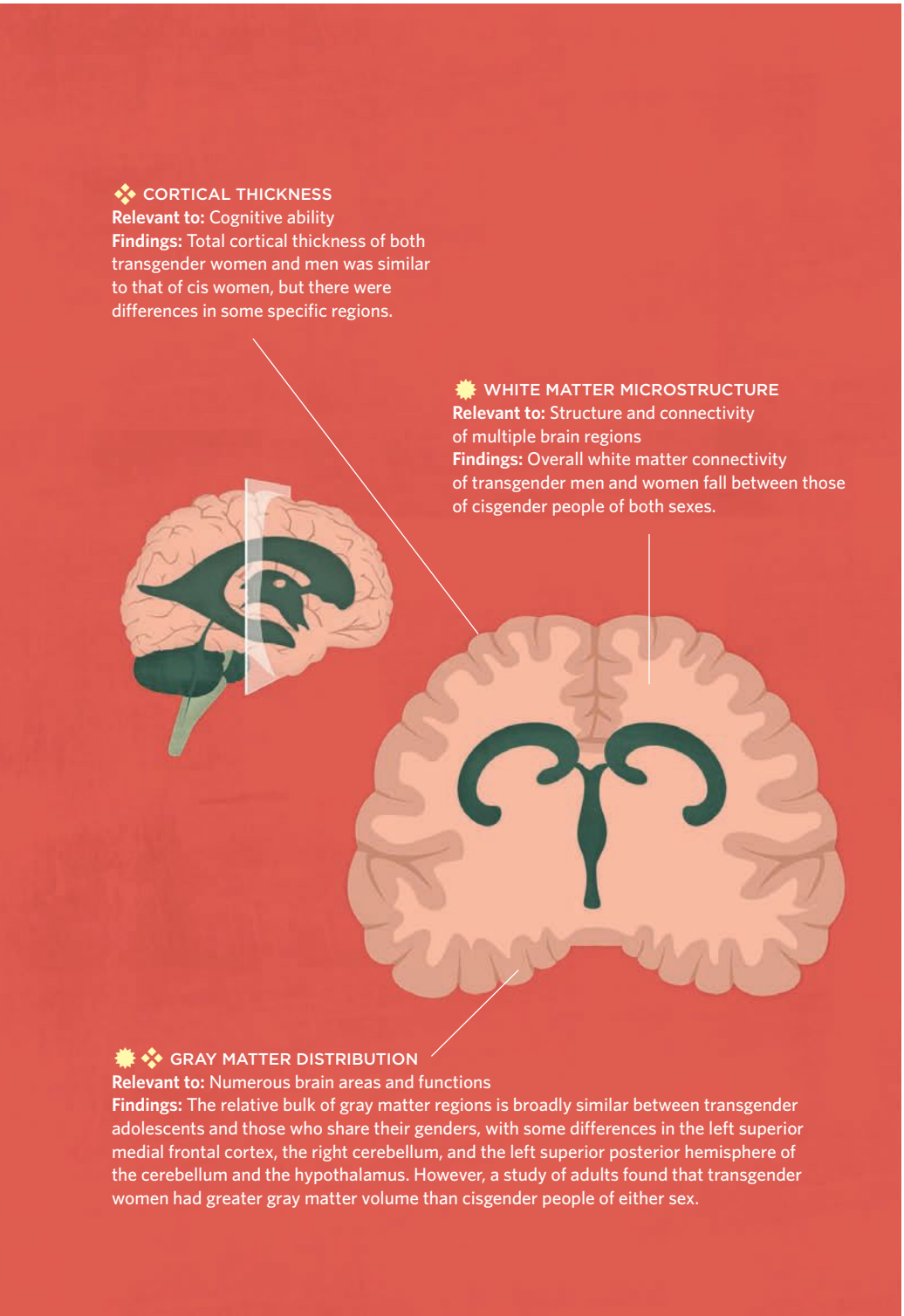
✹ INAH3 SUBNUCLEUS (PART OF HYPOTHALAMUS)
Relevant to: Sexual orientation
Findings: Volume is similar in trans- and cisgender women.

be raised as a female following treatment with surgery and hormones. In at least one of Money's cases, however, this course of action backfired dramatically: the subject reverted to living as a man during his teen years, and later committed suicide. Sex differences in the brain are now well documented, although the extent to which these arise from biological versus social factors is still hotly debated.

The developmental mismatch idea draws support from two sets of findings. Animal studies demonstrated that the genitals and the brain acquire masculine or feminine traits at different stages of development in utero, setting up the potential for hormone fluctuations or other factors to put those organs on different tracks. (See "Sex Differences in the Brain," *The Scientist,* October 2015.) And human studies have found that, in several regions, the brains of trans people bear a greater resemblance to those of cis people who share the trans subjects' gender than to those of the same natal sex.

Dick Swaab of the Netherlands Institute for Neuroscience is a pioneer in the neuroscience underlying gender identity. In the mid-1990s, his group examined the postmortem brains of six transgender women and reported that the size of the central subdivision of the bed nucleus of the stria terminalis (BSTc or BNSTc), a sexually dimorphic area in the forebrain known to be important to sexual behavior, was closer to that of cisgender women than cisgender men.[2] A follow-up study of autopsied brains also found similarities in the number of a certain class of neurons in the BSTc between transgender women and their cisgender counterparts—and between a transgender man and cisgender men.[3] These differences did not appear to be attributable to the influence of endogenous sex hormone fluctuations or hormone treatment in adulthood. In another study published in 2008, Swaab and a coauthor examined the postmortem volume of the INAH3 subnucleus, an area of the hypothalamus previously linked to sexual orientation. The researchers found that this region was about twice as big in cisgender men as in women, whether trans- or cisgender.[4]

And it's not just brain structure that appears to link transgender individuals more closely to people of their experienced gender than those of their natal sex. Functional similarities between transgender people and their cisgender counterparts were apparent in a study led by Julie Bakker of VU University Medical Center and the Netherlands Institute for Neuroscience in Amsterdam that examined neural activity during a spatial-reasoning task. Previous studies had indicated that the exercise engaged different brain areas in men and women. Bakker and colleagues found that trans boys (who had not been



Some studies have pinpointed characteristics of the transgender brain that fall in between what is typical for either sex.

exposed to testosterone, but had had female pubertal hormones suppressed) as well as cisgender boys, displayed less activation than cisgender girls in frontal brain areas when they performed the task.[5]

Other studies have pinpointed characteristics of the transgender brain that fall in between what is typical for either sex—results that proponents of the developmental mismatch hypothesis generally see as support for their idea. In 2014, for example, Georg Kranz, a neuroscientist at the Medical University of Vienna, used diffusion MRI data to investigate differences in white matter microstructure among trans- and cisgender subjects. Cisgender women had the highest levels of a measure of a neural property known as mean diffusivity, cisgender men the lowest, and both transgender men and women fell in between—though it's not fully understood what mean diffusivity may represent physiologically.[6] "It seems that these transgender groups were at an intermediate stage," Kranz says. Controlling for individuals' hormone levels did not alter the differences between groups, leading the authors to suggest that white matter microstructure had instead been shaped by the hormonal environment before and soon after birth—though the possibility that later life experiences also play a role cannot be ruled out, he adds.

"All available evidence points towards a biologically determined identity," Kranz says. "In [transgender] people you would say there was a mismatch in the testosterone milieu during the development of the body and then during development of the brain, so that the body was masculinized and the brain was feminized, or the other way around."

Mixed results

It's unlikely that gender identity has such a straightforward biological explanation, however, and some studies have identified features of the transgender brain that appear closer to the natal sex, casting doubt on the developmental mismatch hypothesis. In a 2015 study from the Netherlands Institute for Neuroscience, a comparison of the distribution of gray matter in 55 female-to-male and 38 male-to-female transgender adolescents with cisgender controls in the same age group found broad similarities in the hypothalami and the cerebellums of the transgender subjects and cisgender participants of the same natal sex.[7] There were, however, some differences in specific subregions.

A 2013 study that focused on cortical thickness, which tends to be slightly greater in women than in men, also yielded mixed results. Led by Antonio Guillamon, a neuroscientist at the National Distance Education University in Spain, researchers analyzed the MRI scans of 94 subjects and found that the total cortical thickness of both transgender women and men was more similar to that of cis women than that of cis men. But this finding did not hold true across the entire brain: in a structure in the forebrain known as the right putamen, which is involved in motor tasks and learning, cortical thickness in transgender men was more similar to that in cisgender men, and transgender women showed no significant differences from either cisgender control group.[8]

"What we found is that, in several regions, cis women, male-to-female trans, and female-to-male trans have thicker cortex than cis males, but not in the same regions," says Guillamon, who hypothesized in a 2016 review article that the brains of cisgender women, transgender women, transgender men, and cisgender men may each have a distinct phenotype.[9] "The cortex is vital for gender."

In another study that yielded mixed results with regard to the developmental mismatch hypothesis, researchers at RWTH Aachen University in Germany tested how cisgender people and transgender women discriminate between men's and women's voices. The team found that in some respects, such as the level of activation of a brain area called the right superior frontal gyrus, trans and cis women were similar, while cisgender men showed higher activity, possibly reflecting greater cognitive effort on the task.[10] Despite similar levels of activation between trans and cis women, however, the transgender women were equally good at identifying male and female voices, while both cisgender groups found it easier to identify voices of the opposite sex.

"Overall, we see in some measures that [transgender people] actually do show these similarities with people [who] share their gender identity, but not for all measures," says Kreukels. Researchers are "still trying to unravel" those similarities and differences in the brain, she says.

A complex phenomenon

Even if the prenatal environment can nudge the body and the brain in different directions, that's probably only one facet of the forces underlying gender dysphoria, says Kreukels. The full picture, she explains, is likely to be "a combination between biological, psychological, and social factors—because we really think it's a complex interplay between all these factors, and thus far research has not given a solution for that."

Ivanka Savic, a neuroscientist at the Karolinska Institute in Sweden, also doubts the explanatory power of the developmental mismatch hypothesis. "It is not that simple that transgenderism is due to this disparity between the sex of the brain and the sex of the body," she says. In 2011, for example, Savic and a colleague found that two brain regions, the thalamus and putamen, were smaller in transgender women than in cisgender controls, but overall gray matter volume was greater.[11] These brain regions had been shown in previous studies to "mediate perception of the body," Savic notes—for example, in fMRI studies where people were shown photographs of themselves and others. "The dysphoria is being unhappy with [one's] own body, feeling every morning that 'This body is mine, but it's not me,'" she says.

In follow-up work, Savic's group began exploring the brain's neural networks, as revealed by fMRI, and found that "the connections between the networks mediating self and the networks mediating own body—my body—were weaker in transgender people," she explains. Specifically, compared with cisgender individuals of both sexes, transgender men showed less connectivity among regions known as the anterior cingulate, posterior cingulate, and precuneus when they viewed images of themselves. But when the images were morphed to appear more male, connectivity between the anterior cingulate and the other two regions increased.[12]

One difficulty in interpreting the differences observed among groups is that it remains unclear when or why those differences developed, says Sven Müller, a psychologist at Ghent University in Belgium; and reported correlations may not reflect causal relationships. "I think the judgment is still out" about the extent to which gender incongruence has a biological cause, he says. "The brain is extremely plastic in adulthood," he notes, so differences iden-

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HOMING IN ON HORMONES

In order to avoid confounding effects, many studies comparing the brains of trans- and cisgender people only include transgender subjects who have not yet begun treatments to bring levels of key sex hormones in line with those of their experienced genders. But some groups are specifically exploring the effects that these treatments might have on the brain. "There is an ongoing debate over whether hormonal administration in adult individuals changes the brain or not," says Sven Müller, a psychologist at Ghent University in Belgium. If cross-sex hormone treatment can shape the adult brain, he notes, it's important to find out "what happens to the brain, and what are the implications for certain cognitive functions."

Only a handful of studies have addressed the question of how these hormone treatments affect the brain. In one led by Antonio Guillamon of National Distance Education University in Madrid, researchers found that testosterone thickened the cortex of transgender men, while six months or more of estrogen and antiandrogen treatment led to a thinning of the cortex in transgender women (*J Sex Med,* 11:1248-61, 2014). A Dutch study similarly concluded that the overall brain volumes of transgender women dropped as a result of treatment, while those of transgender men increased, particularly in the hypothalamus (*Eur J Endocrinol,* 155:S107-14, 2006). And last year, Karolinska Institute neuroscientist Ivanka Savic found that the brains of transgender men taking testosterone showed several changes, including increases in connectivity between the temporoparietal junction (involved in own-body perception) and other brain areas (*Cereb Cortex,* doi:10.1093/cercor/bhx054, 2017).

In another study published last year, of 18 transgender men and 17 transgender women who'd undergone at least two years of hormone therapy, and 57 cisgender controls of both sexes, Müller and colleagues found indications that such hormone treatments might even affect regions the brain that are not commonly considered to be among those sensitive to sex steroids—specifically, the fusiform gyrus, involved in the recognition of faces and bodies, and the cerebellum, known in part for its role in motor control (*Neuroendocrinology,* 105:123-30, 2017). Moreover, he notes, the changes in the cerebellum were linked to treatment duration. "People might need to broaden the scope as to where in the brain they are looking for effects [of hormone treatments]."

In addition to shedding light on the brain networks controlling gender perception and dysphoria, the results of these studies will add to what's known about the effects of hormone treatment on transgender individuals, says Savic. "If we potentially provide treatment with sex hormones, which we should do for persons who need that, it is very important to know what sex hormones do to the brain."

tified between transgender and cisgender people may or may not have been present from birth.

Additionally, logistical challenges confront scientists searching for a biological understanding of gender dysphoria. It is typically difficult to recruit enough transgender subjects to conduct studies with high statistical power. But some researchers are working to remedy that problem. In 2017, for example, the ENIGMA Consortium, which promotes networking and information-sharing among researchers working to detect modest gene effects on brain structure and function, launched a new, transgender-focused working group. And geneticist Lea Davis of Vanderbilt University is organizing a yet-to-be-funded effort to sequence and analyze the genomes of thousands of trans- and cisgender people in search of variations linked to gender dysphoria.

Apart from the big mystery regarding the roots of gender identity, researchers in the field have a number of lingering questions. For example, for people who transition to identifying as a binary gender different from that assigned at birth, "we still also don't know whether male-to-female and female-to-male transsexualism is actually the same phenomenon, or . . . [whether] you have an analogous outcome in both sexes but you have different mechanisms behind it," says Elke Smith, a graduate student at RWTH Aachen University in Germany and author of a review on the transgender brain.[13] Other outstanding questions include what, if any, differences there are in the brains of transgender people with different sexual orientations, and between those whose gender dysphoria manifests very early in life and those who begin to feel dysphoric during adolescence or adulthood, says Kreukels.

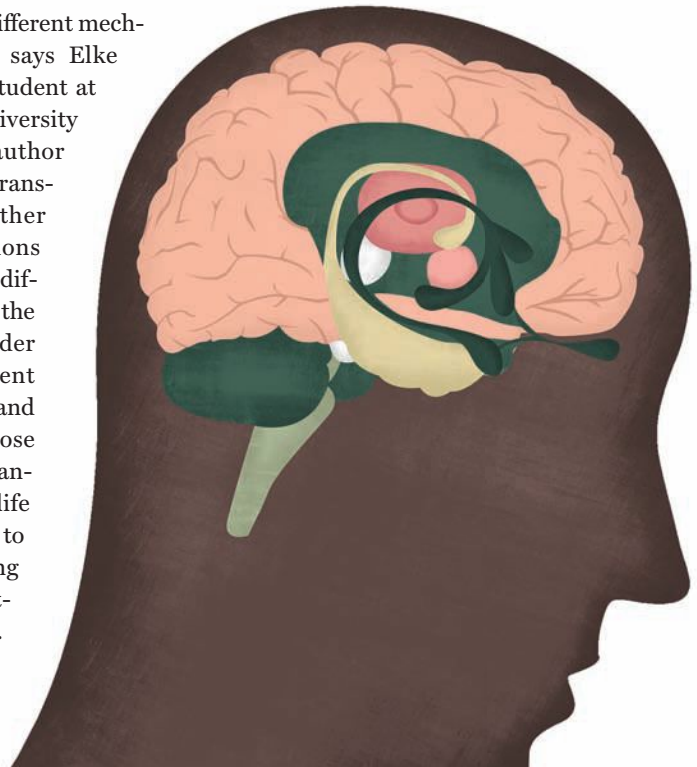
Also still to be determined, adds Savic, is whether the brain differences that have been identified between cis and trans people persist after hormone treatment. (See "Homing in on Hormones" on page 31.)

More research could further clarify the basis not just of gender dysphoria, but also of gender itself, Guillamon suggests—with implications far beyond the pronouns with which we identify. "Phylogenetically, and with respect to evolution . . . it is important to know whether one is a male or a female," and with whom to copulate, he says. "It is one of the pivotal points in biology, and the biology of humans."

Savic says she hopes the results of studies on transgender people will help make gender identity a less-charged issue. "This is just part of the biology, the same way as I have black hair and somebody has red hair."

For now, as is the case for many aspects of human experience, the neural mechanisms underlying gender remain largely mysterious. While researchers have documented some differences between cis- and transgender people's brains, a definitive neural signature of gender has yet to be found—and perhaps it never will be. But with the availability of an increasingly powerful arsenal of neuroimaging, genomic, and other tools, researchers are bound to gain more insight into this fundamental facet of identity. ■

> It is one of the pivotal points in biology, and the biology of humans.
>
> —Antonio Guillamon, National Distance Education University, Spain



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The Ghosts of Proteins Past

The field of paleoproteomics is gathering steam in its quest to use ancient peptides to explore the history of life on Earth.

BY CATHERINE OFFORD

Elena Schroeter is accustomed to being economical with her samples. A postdoctoral researcher at North Carolina State University, Schroeter analyzes pieces of ancient bone that have been preserved in the ground for millions of years—and in doing so, destroys them. So her collaborators rarely give her more than a gram or two of material to work with. "People don't want you to grind up their dinosaurs," she explains. "You have to learn how to do a lot with a little."

But even just a pinch of dinosaur bone dust could help reveal the ancient animal's secrets. In one recent project, for example, Schroeter and her advisor Mary Schweitzer extracted and analyzed collagen peptides from just 200 mg of an 80-million-year-old fossil of a Cretaceous-era herbivore, *Brachylophosaurus canadensis*, excavated in Montana. The amino acid sequences of those peptides, published last year, placed the dinosaur on a branch of the phylogenetic tree between crocodiles and basal birds such as ostriches.[1] What's more, the team's collection of analyzable peptides from the ancient specimen suggests that there might be other fossils out there with similar molecular information hidden in them.

Although the findings were controversial—some researchers still doubt that proteins can resist degradation for tens of millions of years—Schroeter is one of a small but growing number of researchers specializing in the analysis of ancient proteins, or paleoproteomics, to learn about the biology of organisms past. It's been a goal of scientists for some time now; in the 1950s, several researchers were already discussing the possibility of studying peptides preserved in fossils. But only in the last two decades have advances in techniques for protein analysis, such as mass spectrometry, made the feat practical.

The potential for learning about ancient life from paleoproteomics is substantial. Via their amino acid sequences, peptides offer many of the same insights as DNA about genomic makeup—information that can support new or existing phylogenetic trees, inform research on past migrations, and assist with species identifications, even amidst a jumble of ancient remains. (See "What's Old Is New Again," *The Scientist*, June 2015.) But proteins tend to last longer in the geological record than nucleic acids, thanks to both greater volumes at deposition and more-degradation-proof molecular structures. "Both DNA and proteins are chains of building blocks," explains Enrico Cappellini, a paleoproteomics researcher at the Natural History Museum of Denmark. "But the bonds connecting those blocks are more stable in proteins than in DNA." The oldest confirmed DNA samples, extracted from ice cores taken in southern Greenland, are less than 800,000 years old, while the oldest

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SOMETHING TO CHEW ON: Proteins extracted from dinosaur fossils could offer unprecedented insight into these animals' biology. Their ability to survive the test of time is hotly debated.

protein, even by conservative estimates, dates back several million years.

In some respects, peptides offer even more biological insight than their genetic precursors. "Proteins are the functional representation of the genome," says Tim Cleland, a physical scientist at the Smithsonian Institution and previously Schweitzer's PhD student. Protein levels vary between tissues and change as an organism ages. And posttranslational modifications to the molecules could potentially offer information about an organism's physiology or biochemistry that DNA sequences alone can't provide.

To explore these possibilities, researchers are now prospecting for proteins in various ancient materials, from the dental plaque of medieval skeletons to the bones of dinosaurs that walked the Earth hundreds of millions of years ago. And although the field is grappling with its fair share of debates—over where to look for proteins and how to confirm their identity, for instance—the results of recent efforts are providing an unprecedented view of ancient life on Earth. "For me, it's huge," says Cappellini, whose team published 126 protein sequences from a 43,000-year-old woolly mammoth bone in 2011.[2] The study of ancient proteins "opens a new chapter in paleontology."

Panning for peptides

Modern proteins are generally identified with the aid of a mass spectrometer, a machine that analyzes the mass and charge of fragments of a molecule to infer the compound's makeup. Using mass spectrometry, researchers can reconstruct a protein's amino acid sequence, and even potentially its posttranslational modifications. The technique has allowed rapid advances in the field of proteomics. Notably, the international Human Proteome Project is reportedly close to reaching its goal of mapping all human proteins. Yet there are several wrinkles to be ironed out in the method's application to ancient peptides.

For a start, the abundance of intact proteins in samples retrieved from ancient remains tends to be low due to degradation. And extracting those proteins becomes messier the older the material gets. "When you extract proteins from fossils, a lot of other gunky stuff from the fossil—humic acid and other kinds of organics—coextracts with them," says Schroeter. "Part of the struggle in trying to get protein out of fossils is trying to concentrate it, but at the same time clean it in a way that you just don't have to do with modern tissue." Researchers work with various compounds, including acids to remove organics, and resins that can draw peptides out of a mixture, but the field has yet to agree on the best approach, Schroeter says.

There's also an inherent risk that specimens will contain proteins from a contaminating source, a problem that has always plagued studies of ancient tissues. "It's extremely easy for proteins to get into archaeological or ancient artifacts," says Matthew Collins, a biomolecular archaeologist at the University of York in the U.K. Peptides from people handling the specimens, or from animals that have been near the dig site, may not be easily distinguishable from proteins endogenous to the specimens, particularly in the case of peptides that are well-conserved across the animal kingdom, such as collagen, he adds. "How can you tell something is definitely old?"

To try to avoid such complications, paleoproteomic researchers take extreme caution with their samples, notes Schweitzer. In addition to using different workspaces and equipment for ancient and modern material, labs collect controls at every step in the procedure to try to keep track of any contamination sources. "We've got the bone we're interested in, and then we've got sediment the bones were embedded in, which shouldn't have [the same] proteins," Schweitzer says. Researchers also analyze blank samples to detect any rogue peptides, she adds.

WHEN YOU EXTRACT PROTEINS FROM FOSSILS, A LOT OF OTHER GUNKY STUFF FROM THE FOSSIL—HUMIC ACID AND OTHER KINDS OF ORGANICS—COEXTRACTS WITH THEM.

—Elena Schroeter, North Carolina State University

KENNETH LACOVARA

DIGGING FOR BONES: North Carolina State University paleontologist Mary Schweitzer excavates part of a hadrosaur skeleton from the Judith River Formation in Montana.

"Everything that we could possibly control is controlled for."

Nevertheless, the specter of contamination hovers over much of the research in the field, particularly where surprising claims are involved. In fact, few of the researchers who spoke to *The Scientist* had not faced contamination challenges in the past. Collins highlights one of his own papers as an example: a 1992 study reporting the presence of the bone protein osteocalcin in the fossils of two Cretaceous dinosaurs, each more than 70 million years old.[3] "Altogether, that paper was quite convincing. We did a pretty careful study," he says. "I'm convinced that it was contamination now."

CHRISTINA WARINNER

NICE GNASHERS: Proteins preserved in the hardened dental plaque of 1,000-year-old human teeth provide a sneak peak into our ancestors' oral microbiome.

A trip through recent history

In spite of the technical challenges, scientists continue to apply the tools of paleoproteomics to the study of hominins and other animals that have walked the Earth over the past few hundred thousand years. In 2014, a research group that included Collins, Cappellini, and paleogeneticist Christina Warinner of the Max Planck Institute for the Science of Human History published an analysis of proteins extracted from the hardened dental plaque, or calculus, of several 1,000-year-old human skeletons that had been buried in a medieval German monastic site and excavated in 1990. The team characterized 239 bacterial proteins, shedding light on the medieval oral microbiome, along with 43 human proteins, more than half of which are involved in the innate immune system and one-third of which are common to modern calculus samples.[4]

Later that year, the same researchers reported finding the whey protein β-lactoglobulin in the plaque of human teeth dating back to the Bronze Age, around 3,000 BCE.[5] The sequences provided the first ever direct evidence of milk consumption during the period. Using such approaches, "you can begin to explore who is consuming what" throughout human history, says Collins. "It's a really interesting area."

Several research groups are also using proteins for species identification thanks to a tool known as collagen fingerprinting, or zooarcheology by mass spectrometry (ZooMS). Developed in 2009 by the University of Manchester's Michael Buckley, the method uses enzymes to break proteins into fragments that can be analyzed using mass spectrometry and compared with libraries of collagen sequences. "[It's] a simple and cheap means to obtain species identification," Buckley, previously a PhD student with Collins, writes in an email to *The Scientist*. Just last summer, his group used collagen fingerprinting to identify the 50,000-year-old remains of extinct kangaroos retrieved from caves in Tasmania.[6]

The technique has become a mainstay in Collins's lab. He and Frido Welker, a postdoc at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, recently used ZooMS and other paleoproteomic analyses as part of a project to identify a collection of 40,000-year-old hominin remains pulled from a cave in France.[7] Well-known for the array of jewelry, tools, and other artifacts discovered there, the Grotte du Renne site was assumed by many researchers to have been constructed by *Homo sapiens*. But an analysis of proteins in bone fragments found at the site indicated that the remains were in fact from Neanderthals—a finding that some anthropologists took as evidence that these archaic hominins were capable of greater creative expression than previously thought.

The same researchers have used a similar approach to solve non-hominin mysteries, too. A few years ago, Welker, Collins, and colleagues extracted collagen from the roughly 12,000-year-old fossils of two South American ungulates, *Toxodon* and *Macrauchenia*. The remains have puzzled evolutionary biologists since Darwin's era because each genus shares traits with multiple extant mammalian lineages. *Macrauchenia*, for example, resembled a humpless camel with a trunk. Thanks to their collagen sequences, however, the pair has now been placed in a sister group to Peris-

FROM SEDIMENTS TO SEQUENCES

Proteins residing in fossils are rarely ready to analyze immediately after paleontological digs. Instead, researchers have to put samples through a series of steps designed to break down surrounding material and to solubilize and separate out the proteins. Only then can the peptides be sequenced, a process generally carried out using mass spectrometry. To minimize the risk of contamination of samples with external peptides—a particular concern for ancient samples given that endogenous proteins are usually present in low abundance—researchers prepare controls at every step of the analysis.

1. COLLECTION

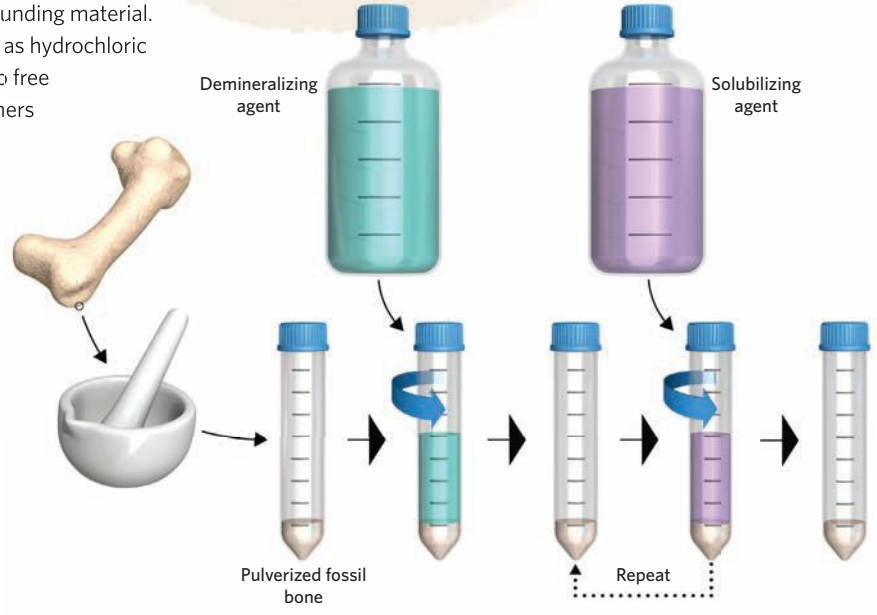
The starting point for many projects in paleoproteomics is a paleontological site. In the right conditions, proteins can survive for hundreds of thousands, potentially millions, of years, before completely succumbing to the ravages of degradation. Fossilized bones that have been preserved in dry ground, for example, may harbor structural proteins such as collagen and hold a wealth of information about the biology of ancient organisms.

Contamination control: Researchers take samples of the soil or sediments that surround fossils in order to eliminate any proteins that are not endogenous to the ancient material being studied from subsequent analyses.

2. PREPARATION

The fossil is ground into a powder and then treated with various chemicals to separate the proteins from the surrounding material. First, researchers add demineralizing agents such as hydrochloric acid or ethylenediaminetetraacetic acid (EDTA) to free proteins from minerals in fossils. Second, researchers add solubilizing agents such as ammonium bicarbonate or guanidine hydrochloride. The resulting solution is then put through multiple rounds of centrifugation and resolubilization before the proteins are analyzed by mass spectrometry.

Contamination control: Researchers simultaneously perform the above steps with a buffer solution. Unexpected proteins discovered in these controls are likely to be from human investigators—keratin from hair or clothes, for example—and can be flagged in later analyses.



sodactyla, the animal order containing horses and rhinos.[8]

In general, these findings have been relatively uncontroversial, having involved specimens that are less than 100,000 years old. When it comes to more-ancient inquiries, there's less agreement about the legitimacy of the results.

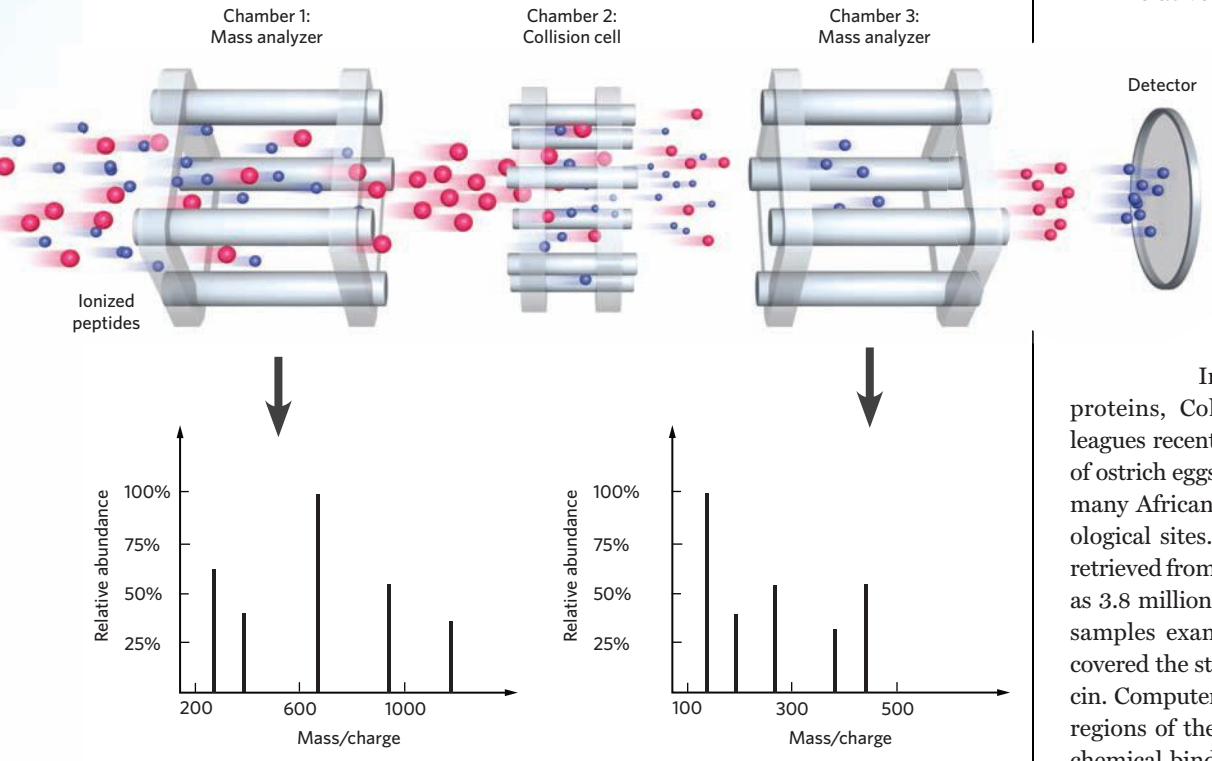
Dinosaur dilemma

In search of more-ancient proteins, Collins, Cappellini, and colleagues recently analyzed fossilized pieces of ostrich eggshell, which are found across many African paleontological and archeological sites. The oldest shell fragment, retrieved from a dig in Tanzania, registered as 3.8 million years old. In this and other samples examined, the researchers discovered the structural protein struthiocalcin. Computer simulations suggested that regions of the protein with the strongest chemical binding to the surface of calcite crystals in the shell had survived in the best condition, indicating that minerals could play a key role in protein preservation.[9]

Other researchers claim to have plucked analyzable proteins from much older biological remains. In 2007, Schweitzer and her colleagues published a paper in *Science* that described soft tissue from a 68-million-year-old *Tyrannosaurus rex* fossil. Mass spectrometry, as well as immunological assays using antibodies, indicated the presence of collagen, the authors wrote.[10] In 2009, Schweitzer published similar findings in *B. canadensis*, using the same fossil that she and Schroeter would go on to study in more detail over the next decade. This paper extended the age limit of ancient proteins to around 80 million years, and suggested that preserved peptides might regularly be found in Cretaceous dinosaurs.

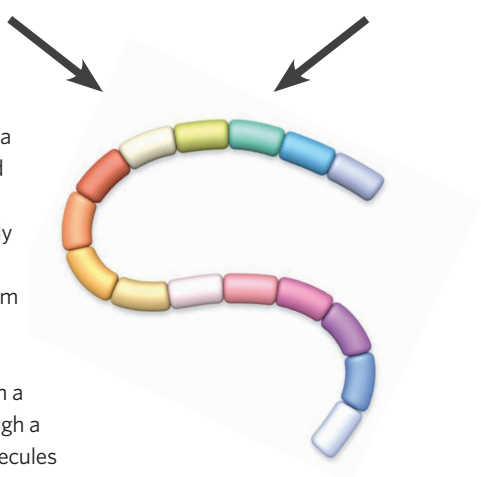
Recently, a team led by University of Toronto researcher Robert Reisz pushed that limit even further back. In 2017, the group made headlines when it reported

3. ANALYSIS



Most research groups analyze ancient proteins using mass spectrometry—an approach that determines the molecular makeup of a compound by analyzing the mass and charge of its particles.

For identification, proteins are typically digested into smaller peptides by chemicals or enzymes. Inside a tandem mass spectrometer, one of several types of mass spectrometer, these peptides are then ionized to give them a positive charge and accelerated through a specialized chamber, with lighter molecules accelerating more quickly than heavier ones. The peptides are measured by a mass analyzer before being broken into even smaller fragments and measured by a second mass analyzer. These measurements produce two sets of mass spectra that can then be compared against a database for protein identification or used to help reveal the sequence of amino acids making up the original peptide.



Contamination control: Researchers analyze control samples to test for the possibility that some protein fragments have been left inside the mass spectrometer from a previous experiment. Some groups also use separate machines for old and new samples to reduce the possibility of accidental detection of modern proteins in ancient material.

I TEND TO TAKE THE STANDPOINT THAT THERE ARE BASIC LAWS OF CHEMISTRY AND PHYSICS WHICH ARE LIMITING THE LONG-TERM PERSISTENCE OF THESE MOLECULES.

—Matthew Collins, University of York

finding collagen in a rib bone from the Jurassic plant eater *Lufengosaurus*. In this case, the researchers used a method that avoided destruction of their 195-million-year-old fossil: Fourier transform infrared (FTIR) spectroscopy, which measures how a sample absorbs radiation in order to infer the types of chemical bonds present. Although this technique cannot reliably determine the sequences of amino acids in a sample, Reisz and colleagues detected bonds typical of collagen.

This finding could stretch the age limit for ancient proteins, albeit probably in a very degraded form, into the realm of hundreds of millions of years. For now, "we're not able to do anything more than identify the presence of collagen," Reisz says. "But technology is advancing at a very rapid rate. What was not possible even 10 years ago is now possible. . . . With further work and further materials, who knows what we'll be able to find."

Claims of proteins this old have met skepticism, with other researchers in the field arguing that the results don't jibe with theories about protein degradation. For some researchers, including Collins and many of his collaborators, the ostrich eggshell struthiocalcin, not peptides from the older dinosaur remains, currently represents the oldest analyzed protein on record. "I tend to take the standpoint that there are basic laws of chemistry and physics which are limiting the long-term persistence of these molecules," Collins says. He suggests that there's currently a split in the paleoproteomics community between researchers who, like him, operate under the assumption that proteins can't survive more than a few million years (and so focus their efforts on specimens that fall within that limit), and those who search for proteins in more-degraded samples, some of which are tens of millions of years old. "It's the difference between people who wade into deep water from the shore, and people who jump from a high place into deep water," he says. "They're different ways of approaching the problem."

OILS AND PIGMENTS

Proteins and DNA aren't the only biomolecules to survive degradation in ancient material. Lipids, for example, are more resistant to degradation than peptides and nucleic acids. Late last year, researchers set a new record for these molecules' survival in vertebrate remains, extracting and characterizing lipid residues in a 48-million-year old fossilized bird (*Proc R Soc B*, 284:20171050, 2017). The small, hoopoe-like creature was found to contain an array of hydrocarbons in its uropygial gland, an organ at the base of the tail that secretes oil for waterproofing feathers in the majority of bird species. The compounds were likely derived from fatty acids and alcohols and may have contributed to the gland's "exceptional preservation," the authors write.

Some pigments, such as melanin, a polymer derived from the amino acid tyrosine, also survive relatively well in the fossil record and can provide clues about the physical appearance of ancient organisms. In 2015, a team at Lund University in Sweden reported the discovery of pigment in a 150-million-year-old fossilized specimen of *Anchiornis huxleyi*, a small, four-winged Jurassic dinosaur (*Sci Rep*, 5:13520, 2015). Mass spectrometry revealed that the animal's fossilized feathers contained eumelanin, the pigment responsible for brownish-black colorations. And just last August, researchers in California and Germany reported the presence of bluish-green pigment molecules in fossilized eggshells from a Cretaceous dinosaur, a possible indication of egg camouflage, the team suggests (*PeerJ*, 5:e3706, 2017).

COLORFUL CREATURE: A recent study of this fossilized specimen of Jurassic dinosaur *Anchiornis huxleyi* revealed the presence of melanin pigment.

Although ancient biomolecules such as these are unable to offer the degree of evolutionary insight afforded by amino acid or nucleic acid sequences, such discoveries exemplify "the growing field of molecular paleontology," Montana State University paleontologist David Varricchio told *National Geographic* last September following the eggshell paper's publication. "With new machines and new techniques, it's very exciting what can potentially be found in fossils."

More-specific criticisms have been leveled at the dinosaur studies, too, including suggestions that methods other than mass spectrometry are unlikely to yield reliable information about degraded proteins, and that cross-contamination cannot be definitively ruled out. Following Schroeter's recent analysis of *B. canadensis*, for example, Buckley's group compared the published dino peptide sequences with those of modern animals. The researchers concluded that the sequences in both the 2007 and the 2009 studies could be matched to mass spectrometry data from ostriches, while the most recent sequences from *B. canadensis* matched those of alligators; they point out that both animals were sources of protein in the labs where the analyses of the fossils were conducted.[13] Schroeter and Schweitzer note that, given the team's cautious protocols, such double contamination is highly unlikely.

Cappellini, who told *Science* at the time of the North Carolina team's 2017 publication that he was "convinced beyond reasonable doubt" by the most recent analysis of *B. canadensis*, has agreed to work with Schweitzer's group to try to replicate the findings. "I don't have a predefined position," he tells *The Scientist*. "If they or, together with them, we will find that dinosaur proteins are there and can be retrieved, I will be super happy about that. It'll be proof that we can go that far back in time in genetic reconstruction."

The future of ancient proteins

In addition to stimulating debate on the limits of protein preservation, disagreement over the validity of multiple reports of very ancient proteins has highlighted a lack of consensus in the paleoproteomics community about which methods to use when. From relatively indirect techniques, such as FTIR, to a suite of varying mass spec protocols, multiple approaches are reported in the literature. And not all necessarily produce the same results. "[Paleoproteomics needs] a standard methodology that's tested and used by everyone," says Schweitzer. "The field is not going to get as strong as it could get if we're all using different methods that say different things."

To help facilitate the discussion, Collins is organizing an ancient protein conference for later this year. "I'd like people to meet together more, share ideas," he says, adding that currently, researchers in paleoproteomics tend to be spread out at various conferences depending on the age of their samples. "The people who work on old things tend to go to paleontology conferences; the ones who work on young things go to archaeology conferences," he says. The most recent meeting specifically built around an ancient protein theme was 20 years ago, he adds. "I think the time is ripe for the next one, to try to get all these different groups together."

Meanwhile, efforts are underway to open up the field to a wider community of scientists. Schroeter and Cleland have put together a review paper, published earlier this year, that explains mass spectrometry and its application to studying ancient proteins for a nonexpert audience.[14] "I think there's a gap in the community of paleo at large in our understanding of how these techniques work," says Schroeter. A broader appreciation could both help resolve discussions about controversial findings, and lead to collaborations for people working on all sorts of ancient samples, she adds. "We need to be more accessible with our methods. Because the way to get more people involved in this work is to give them a grounding in that information, [and] make them see the value of it."

The range of samples may soon be expanding too. Part of Cleland's work at the Smithsonian involves developing methods to study samples kept in museum collections, rather than those obtained from expensive and time-consuming fieldwork. He and his colleagues recently showed that one specimen—a 12,000-year-old skull of an extinct giant beaver that has been kept in the New York State Museum since 1845—contains "quite a bit of protein," Cleland says.[15] "We have these wonderful museums around the world that have these huge collections of material. It opens up the possibility of looking at a large number of things." ■

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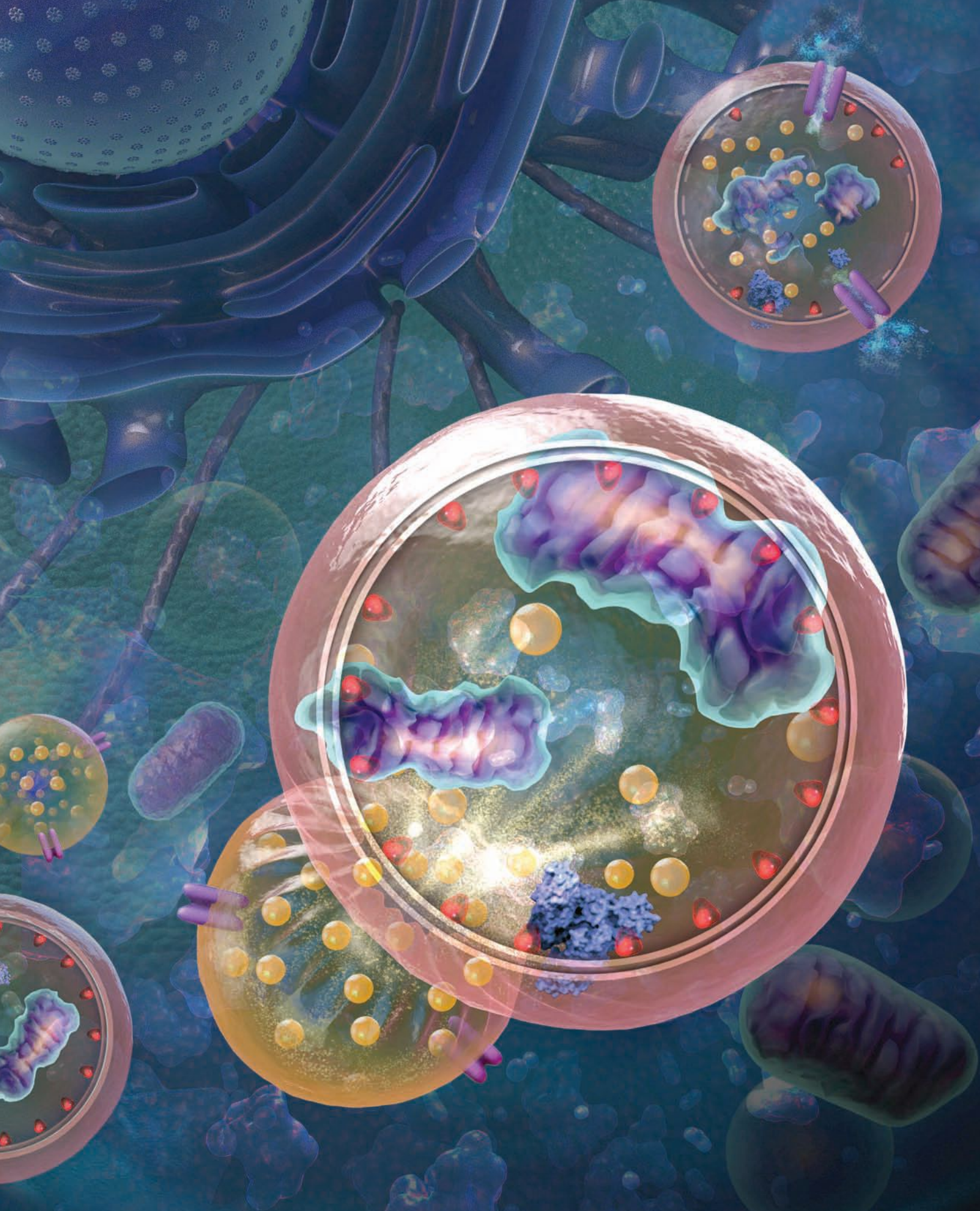


Eating Yourself to Live

As researchers uncover new molecular details of the process of autophagy, potential therapeutic targets emerge.

BY VIKRAMJIT LAHIRI AND DANIEL J. KLIONSKY

In the mid-1950s, Sam Clark Jr. of the School of Medicine at Washington University in St. Louis looked through his electron microscope at newborn-mouse kidneys and spotted something he'd never seen before. As he later described it, there appeared to be membrane-bound structures within the cytoplasm of the kidney cells. Intriguingly, these structures seemed to contain altered mitochondria.[1]

Soon after Clark published his observations, several independent researchers supported his findings. These included the Albert Einstein College of Medicine's Alex Novikoff, who used the term cytolysome for the structures. "Within these cytolysomes remarkable events are in progress . . ." he and his colleague Edward Essner wrote in 1962.[2] "Cytoplasm has somehow found its way inside the droplets and is apparently in the process of digestion."

These were the first descriptions of what is known today as macroautophagy (hereafter referred to as autophagy). The term is derived from the Greek "auto," meaning self, and "phagein," meaning to eat. During autophagy, the cell consumes parts of itself in a regulated manner.

The hallmark of autophagy is the formation of a transient double-membrane structure termed a phagophore. In contrast to secretory transport vesicles, which bud off from an organelle with cargo already enclosed within, the phagophore acquires cargo during its assembly. It may form de novo in the cytoplasm as a free-standing structure, or it may be in contact with an organelle such as the endoplasmic reticulum. The phagophore expands sequentially, providing tremendous flexibility with regard to cargo capacity. As it expands, it sequesters cytoplasmic components, including proteins, lipids, and even entire organelles. Once its payload is secured, the phagophore closes and matures into an autophagosome, with the sequestered cargo now enclosed in the lumen of this compartment. The autophagosome then delivers the cargo, via membrane fusion, to the lytic compartments—vacuoles in fungi and plants and lysosomes in metazoans—for degradation and recycling. It was the autophagosome that captured the attention of Clark and Novikoff more than half a century ago.

Today, autophagy is recognized as a critical process for maintaining cellular homeostasis, as well as for responding to stressors, such as nutrient deficiency, which may potentially compromise cell survival. When a cell is exposed to such stressors, autophagy, which occurs constitutively at low levels to balance the constant synthesis of biomolecules, is strongly upregulated. This upregulation increases

sequestration and degradation of portions of the cell, releasing macromolecules back into the cytosol to power essential metabolic reactions and generate energy.

The contribution of autophagy to cellular health under both normal and stress conditions implies important physiological and pathological roles for this tightly regulated and precisely orchestrated process. Indeed, autophagy has been found to be instrumental during the course of mammalian development. Additionally, recent research has discovered that autophagy is a critical modulator of a wide range of diseases and disorders. Probing the involvement of autophagy in development and disease is crucial for a more complete understanding of the pathway's roles, and could have implications for maintaining health or treating disease. While we partially understand its overall morphology and function, information about several steps in this intricate pathway are still emerging.

TYPES OF AUTOPHAGY

Autophagy can be divided into two broad categories, selective and nonselective, based on the nature of what's being eaten. The most extensively studied and characterized form of autophagy is macroautophagy, which involves the delivery of cellular components to the lysosome (or vacuole in fungi and plants) via a double membrane–bound structure. Two other forms, beyond the scope of this article, are microautophagy and chaperone-mediated autophagy. During microautophagy, the lysosomal membrane invaginates and sequesters nearby cellular materials for degradation and recycling. Chaperone-mediated autophagy, in contrast, is a specialized protein-degradation process involving dedicated lysosomal transporters.

Mechanisms of autophagy

Several catabolic pathways in the cell break down large molecules. Notably, the conjugation of a small protein called ubiquitin to another cellular protein—often followed by the sequential addition of ubiquitin molecules to generate a polyubiquitin chain—can tag that protein for degradation by the proteasome, resulting in the release of amino acids. (See "The Proteasome: A Powerful Target for Manipulating Protein Levels," *The Scientist*, May 2017.) Similar degradation mechanisms exist for other biological polymers such as carbohydrates and lipids.

So what makes autophagy unique? The answer lies in the flexibility of autophagosome size and cargo selection. Autophagy can promote degradation en masse for a large number and variety of substrates, enabling cells to quickly and efficiently generate recycled basic building materials in the face of a wide range of nutritional deficiencies. Additionally, autophagy is the only pathway that is capable of degrading entire organelles, either randomly or in a targeted fashion—a critical process for maintaining homeostasis in the complex landscape of the eukaryotic cell.

Autophagy is tightly regulated to ensure that it is ramped up only when required, and then in a timely manner. The central metabolic sensor of the cell, the TOR complex 1 (TORC1, or MTORC1 in mammals), is sensitive to the availability of amino acids and growth factors, and inhibits autophagy induction when these components are abundant. When cells are starved of these molecules, TORC1/MTORC1 is inactivated, promoting an increase in autophagy. Meanwhile, other molecular regulators monitor cells for the status of various nutrients, such as glucose, or for energy in the form of ATP, and trigger autophagy when such nutrients or metabolites reach critically low levels.

Once autophagy is initiated, several autophagy-related (Atg) proteins act together to coordinate the formation of the phagophore and the subsequent steps of autophagy. Yeast *ATG* genes were discovered in the 1990s, transforming autophagy research, which had been largely descriptive, to being strongly mechanistic at the molecular level. Experiments using the genetically tractable budding yeast *Saccharomyces cerevisiae* played a major role in helping scientists decipher the basic mechanism of autophagy. Research in other organisms followed shortly afterward, revealing a remarkable evolutionary conservation in the nature and function of the autophagy machinery from yeast to human. (See illustration on page 45.)

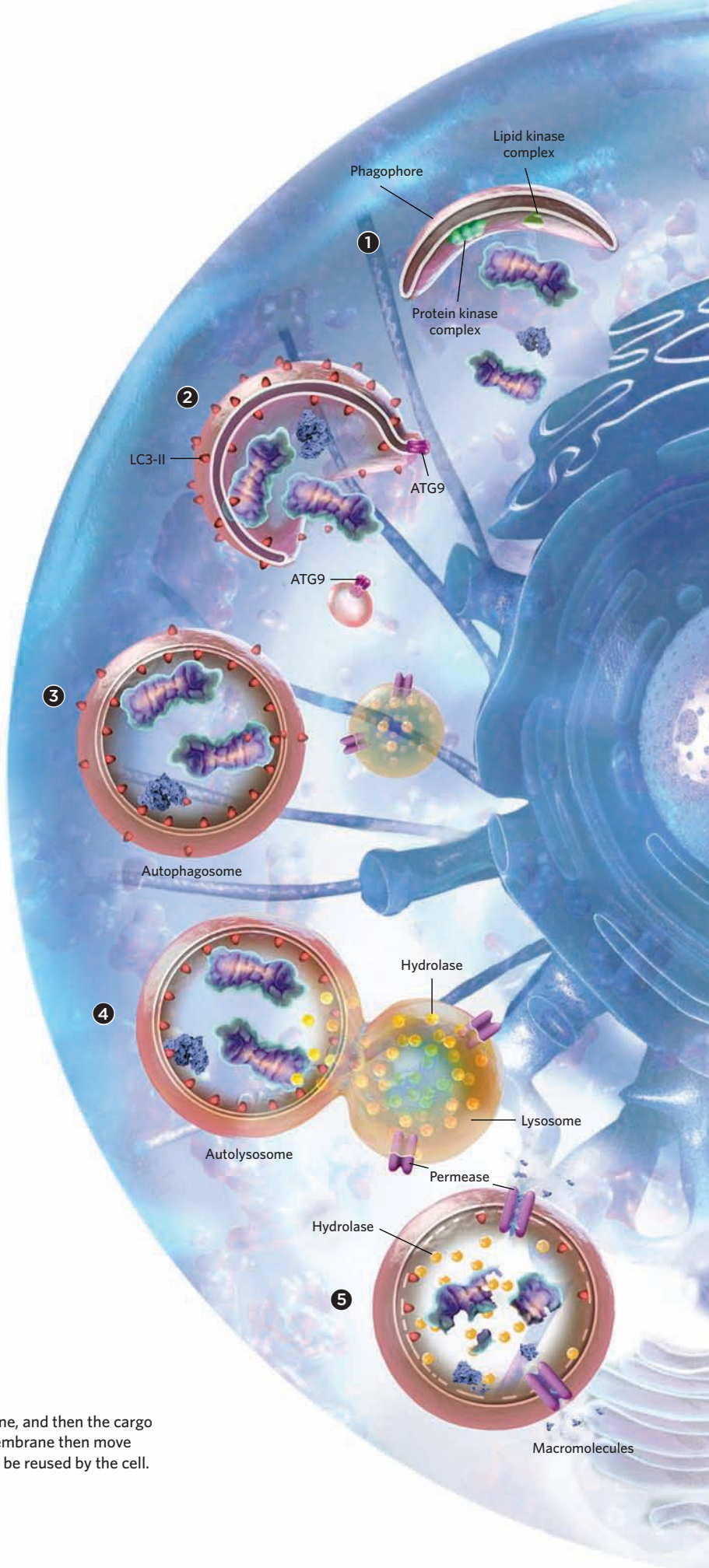
While the overall process of autophagy is now somewhat clear to scientists, the field is still hard at work trying to find and fit in many missing pieces of the puzzle. The donor of the membrane for the autophagosome, for example, has not been concretely established. (See "The Enigmatic Membrane," *The Scientist*, February 2012.) Similarly, we do not fully understand how phagophore expansion is regulated, or what dictates the frequency of autophagosome generation. Even more questions arise when we consider that many types of autophagy are highly selective, and the initiation and regulation of these processes remain mysterious. Further understanding of these selective pathways is crucial because they are intimately linked to embryonic development, healthy growth, and human disease.

Autophagy in development

In the late 1970s, Richard Lockshin of St. John's University in New York and colleagues demonstrated that autophagy occurs during insect metamorphosis.[3] A decade and a half later, researchers showed that mutant yeast cells defective in autophagy do not sporulate.[4] These were the first hints that autophagy may play a role in organismal development, but only relatively recently have researchers begun to elucidate the process's contributions to cellular differentiation and development in metazoans.

SELF DIGESTION

Autophagy—the process by which a cell digests and recycles various molecules and organelles in its cytoplasm—is critical for maintaining homeostasis and for helping cells survive low-nutrient conditions. In a series of steps, a vesicle precursor, known as the phagophore, is formed, and cellular contents accumulate as it matures into an autophagosome. Then, after fusion with a lysosome, the inner vesicle of the autophagosome is digested along with the cargo, and the products are released into the cytoplasm. Over the past 25 years, researchers have detailed the molecular regulators of this process, with recent insights shedding light on autophagy's link to both health and disease.



1 PHAGOPHORE NUCLEATION
A protein kinase complex and a lipid kinase complex coordinate the recruitment of a piece of membrane that will form the basis of the phagophore. The origin of the donor membrane is unknown, but may include the endoplasmic reticulum (ER), the Golgi apparatus, or the plasma membrane.

2 PHAGOPHORE EXPANSION
With the help of several autophagy-related (ATG) proteins, a small, ubiquitin-like protein called LC3 (LC3-II when bound) binds the nascent phagophore to direct its expansion around cytoplasmic components to be degraded—which may be randomly selected or may include specific cargo such as damaged organelles or misfolded proteins, depending on the nature of the stress. ATG9 is a transmembrane protein that shuttles between the site of phagophore formation and peripheral membrane sites and is thought to recruit more membrane for the expanding phagophore.

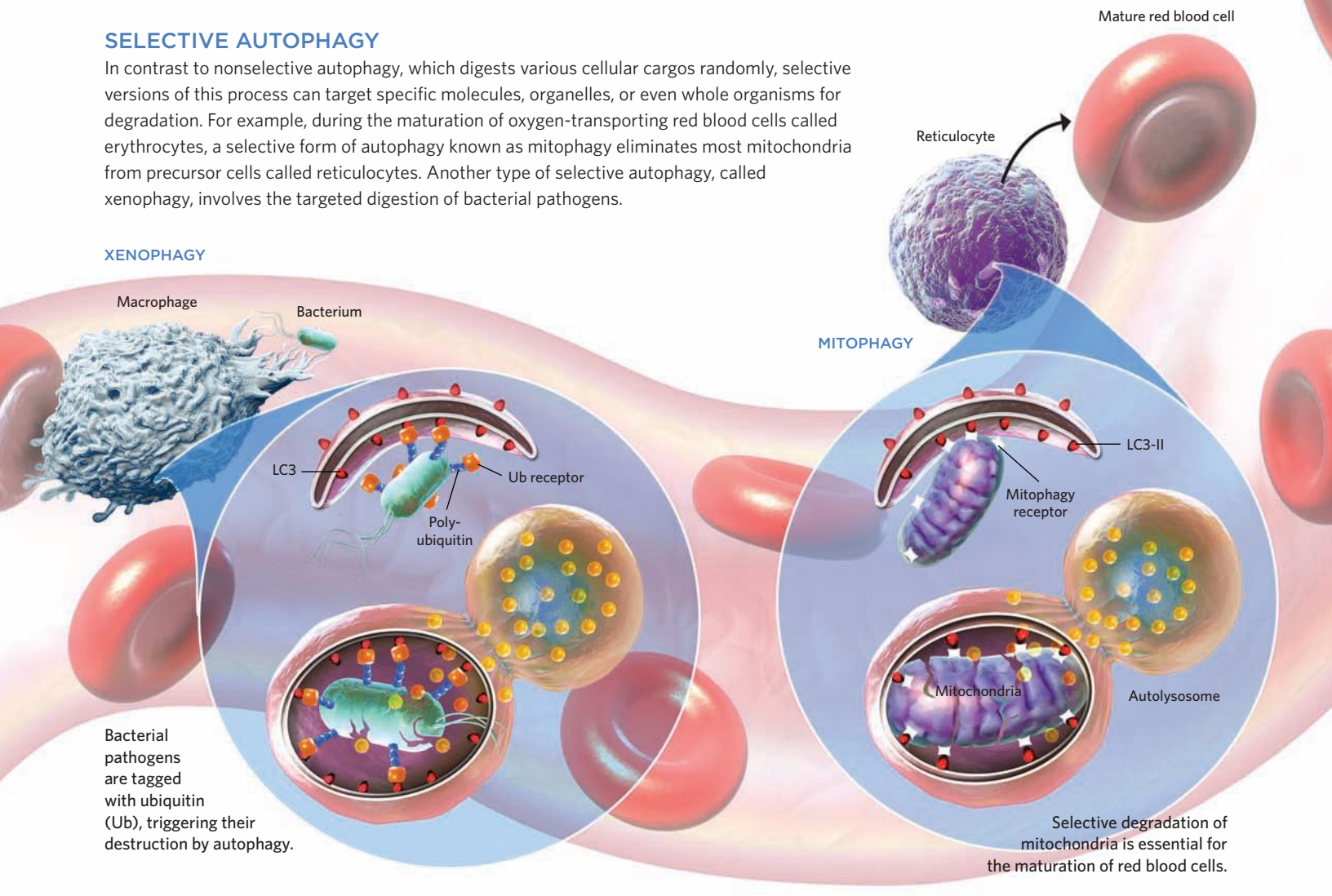
3 AUTOPHAGOSOME FORMATION
The elongating phagophore closes to form the double-membrane autophagosome, enclosing cytoplasmic cargo targeted for degradation.

4 AUTOPHAGOSOME-LYSOSOME FUSION
The outer membrane of the autophagosome fuses with the membrane of the lysosome, leading to the exposure of the cargo-enclosing inner autophagosomal membrane to the hydrolases present in the lysosomal lumen. This lysosome is now called an autolysosome.

5 DEGRADATION AND EFFLUX
Lysosomal hydrolases degrade the inner autophagosomal membrane, and then the cargo within. Transporters known as permeases on the autolysosome membrane then move the macromolecules generated into the cytoplasm, where they can be reused by the cell.

SELECTIVE AUTOPHAGY

In contrast to nonselective autophagy, which digests various cellular cargos randomly, selective versions of this process can target specific molecules, organelles, or even whole organisms for degradation. For example, during the maturation of oxygen-transporting red blood cells called erythrocytes, a selective form of autophagy known as mitophagy eliminates most mitochondria from precursor cells called reticulocytes. Another type of selective autophagy, called xenophagy, involves the targeted digestion of bacterial pathogens.



Over the past 15 years, researchers have demonstrated that nonselective autophagy, which is primarily a starvation response, is also required during early mammalian embryogenesis. Autophagy is likely critical for oocyte reprogramming post-fertilization. Oocytes come loaded with maternally derived mRNAs and proteins that are degraded after an egg fuses with a sperm and the zygote divides into two cells, when the embryo's own genome takes over. (See "New Techniques Detail Embryos' First Hours and Days," *The Scientist*, December 2017.) This transition from maternal to embryonic control likely depends, at least partially, on autophagy. One piece of evidence for this hypothesis is that homozygous *Atg5*-knockout mouse embryos derived from oocytes that have been depleted of *Atg5* transcripts die somewhere in the four- to eight-cell stage.[5]

Autophagy is likely also necessary for the fetal-to-neonatal transition that occurs at birth. Homozygous *Atg5*-null mouse embryos created from unmanipulated oocytes of heterozygous *Atg5* mothers survive gestation, probably due to the presence of maternally derived ATG5 in the oocyte, but die within one day of delivery.[6] Indeed, a large increase in autophagy is observed in certain tissues in mice, including in the heart, shortly after birth. The cause of this increase might be the temporary but severe starvation induced by the termination of placental nourishment; autophagy helps release the amino acids and energy sources necessary for survival during this period of need.

Selective autophagy, too, plays an important role in post-embryonic development. The oxygen-transporting function of red blood cells depends on the presence of large quantities of the oxygen-binding protein hemoglobin. To meet this demand, precursor reticulocytes must eliminate most organelles as they mature into erythrocytes. Reticulocytes lose mitochondria through mitophagy, a type of selective autophagic degradation. Targeted mitochondrial degradation also occurs during zygotic development in several metazoan species, enabling the elimination of paternal mitochondria so that only maternal mitochondria are inherited.[7] In 2017, Beth Levine of the University of Texas Southwestern Medical Center and colleagues identified an inner mitochondrial mem-

brane protein that functions as a novel mitophagy receptor, and showed that this protein plays an essential role in clearing paternal mitochondria in *C. elegans*.[8]

These are but a few of autophagy's suspected roles in normal development. And as organisms mature, this recycling pathway continues to maintain a healthy balance of functional organelles and basic biomacromolecules in the cellular environment. Further elucidating autophagy's roles, not only at the mechanistic level but also in terms of physiological outcomes, will be important for understanding how this dynamic process regulates metabolic homeostasis in organisms of all ages.

Autophagy gone awry

Given the role of autophagy in physiology, it's not surprising that dysregulation of the process is tied to a number of pathologies, ranging from infectious diseases to neurodegenerative disorders to cancer. Understanding the relationship between autophagy and disease could be critical to designing effective therapeutic interventions.

The first report of autophagy induction in response to infection was published in 1984, when Yasuko Rikihisa, then at Virginia Tech's College of Veterinary Medicine, reported that incubating mammalian cells with rickettsiae, bacteria that cause a variety of tick-borne diseases, triggered the formation of autophagosomes.[9] However, researchers have only recently begun to understand how the eukaryotic cell uses a type of selective autophagy called xenophagy to destroy invading pathogens. Xenophagy is initiated by the ubiquitination of bacterial surface proteins, which are then recognized by ubiquitin-binding autophagy receptors that promote the sequestration of pathogens by phagophores. (See illustration on page 46.)

In this context, autophagy works in concert with components of the innate immune system, such as toll-like receptors (TLRs). TLRs mediate the initial recognition of pathogens, and subsequent TLR signaling promotes inflammation and may stimulate autophagy, at least in part by modifying the autophagy receptors.[10] While inflammation is a necessary step in the recruitment of immune cells to the site of infection, an extended inflammatory response can be detrimental to the tissue. Research from the lab of Shizuo Akira at Osaka University in Japan has revealed that loss of the essential autophagy protein ATG16L1 leads to increased production of proinflammatory cytokines in mice.[11] This finding suggests that, during the course of the immune response, autophagy not only mediates pathogen elimination but may also limit inflammation to prevent unnecessary tissue damage.

AUTOPHAGY HAS BEEN FOUND TO BE INSTRUMENTAL DURING THE COURSE OF MAMMALIAN DEVELOPMENT.

More recently, work from J. Magarian Blander's laboratory at Weill Cornell Medical College has revealed that autophagy of the endoplasmic reticulum is an essential component of a cellular stress response pathway initiated upon infection with live, Gram-positive bacteria. In this cascade, autophagy plays an instrumental role in mobilizing an efficient innate immune response following a stress response from infected cells, in part by causing the relocalization of the sensor protein TMEM173/STING.[12]

However, modulating the immune system by autophagy may not always be beneficial. A recent study from Jan Lünemann's laboratory at the University of Zurich suggested that components of the autophagy machinery might play a role in aggravating multiple sclerosis, an autoimmune disease affecting the central nervous system (CNS). A class of immune cells called dendritic cells use ATG-dependent phagocytosis to present peptides derived from the degradation of myelinated CNS-resident cells as antigens. These self antigens promote the activation of autoreactive effector T cells, leading to an autoimmune attack and progressive nerve degeneration.[13]

Autophagy's predominant role, however, is cytoprotective, as is highlighted by its protective function in several neurodegenerative disorders, such as Huntington's disease (HD) and Parkinson's disease (PD). David Rubinsztein of the Cambridge Institute for Medical Research and colleagues were among the first to show that autophagy promotes the degradation of aggregation-prone proteins such as mutant HTT (huntingtin), one of the primary culprits in HD.[14] They later reported that the activation of autophagy rescues neurodegenerative phenotypes in fly and mouse models of HD.[15]

In PD, the loss of mitophagy has been identified as a key factor in pathogenesis. Although most PD cases are sporadic, mutations in the mitophagy-related genes *PINK1* and *PRKN* are commonly associated with familial PD. And in 2016, research from the Stanford University School of Medicine laboratory of Xinnan Wang indicated that sporadic cases of PD may also be intimately connected to mitophagy deficiencies.[16] This is not unexpected, because neurons heavily depend on mitochondrial ATP synthesis for carrying out energy-intensive processes such as generation and transmission of action potentials, neurotransmitter trafficking, and synaptic signaling. Therefore, mitochondrial homeostasis is critical for proper neuronal function. Neurons use mitophagy to selectively eliminate damaged mitochondria that are not only inefficient at producing ATP, but also generate high levels of reactive oxygen species (ROS), which promote protein and lipid oxidation and DNA damage, causing further cellular dysfunction. The ROS-induced death of mutant mitophagy-deficient neurons is likely a major factor in PD pathogenesis.

Autophagy has also been linked to cancer. In 1999, the Levine group made the landmark discovery that mice with only one functional copy of the autophagy-related

gene *Becn1* exhibit increased tumorigenesis.[17] In contrast to the apparent role of autophagy in blocking tumor initiation, however, there is evidence that autophagy can also promote malignancy in established tumors. The duality of autophagy's role in cancer is partially explained by the factors involved in tumor generation versus progression. Oncogenic transformation is promoted by stress factors such as genomic instability, metabolic disruption, and mitochondrial dysfunction; autophagy actively suppresses these stresses and thereby inhibits tumor initiation. However, once a tumor has formed, the playing field radically changes. Cells within the tumor have increased metabolic demands due to faster growth and proliferation rates, but these cancerous cells are not well supplied with nutrients by the vasculature. These cells meet their metabolic needs in part by upregulating autophagy.

Recent research has revealed that the role of autophagy in promoting tumor progression is not limited to cellular effects in the cancer itself, but extends to modulation of the tumor microenvironment. In 2017, Tor Erik Rusten's group from the University of Oslo demonstrated that in *Drosophila melanogaster*, increased autophagy within the tumor microenvironment promotes tumor growth and invasion.[18] Similarly, work from Alec Kimmelman and colleagues at NYU Langone Health's Perlmutter Cancer Center has elucidated the interaction between tumor cells of pancreatic ductal adenocarcinoma and stroma-associated pancreatic stellate cells (PSCs). This research suggests that the tumor cells promote autophagy in PSCs, stimulating the production of metabolites and basic biomolecules by these cells. The tumor cells then capture and use these metabolites for their survival and growth under otherwise nutrient-poor conditions.[19]

Because cancer cells rely on autophagy for survival, inhibiting the process is an intriguing strategy for antitumor therapeutics; however, limiting autophagy can increase susceptibility to infection and neurodegeneration.[20] Therapeutically targeting a pathway as critical as autophagy is not straightforward.

MUTATIONS IN THE MITOPHAGY-RELATED GENES *PINK1* AND *PRKN* ARE COMMONLY ASSOCIATED WITH FAMILIAL PD.

Outlook

As a scientific field, the study of autophagy is at a very exciting juncture. Although researchers have partially uncovered some of the mechanisms related to autophagy initiation, progression, and regulation, our understanding of the role of autophagy in development and disease is at an early stage, and there are still more questions than answers concerning the functions of the autophagy-related proteins. Further realization of the role of the process in pathophysiology promises to reveal as yet unanticipated avenues for therapeutic intervention. We already knew that we must eat to live, and it's now clear that this can sometimes extend to eating ourselves—at least on a cellular level. ■

Vikramjit Lahiri is a second-year PhD candidate in Professor Daniel J. Klionsky's laboratory at the University of Michigan in Ann Arbor, where they study the molecular details of autophagy in the budding yeast Saccharomyces cerevisiae.

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The Literature

MICROBIOLOGY

Mind the Gap

THE PAPER

A. T. Asmar et al., "Communication across the bacterial cell envelope depends on the size of the periplasm," *PLOS Biol*, 15:e2004303, 2017.

The cell envelope of a gram-negative bacterium protects it from its surroundings and aids survival in another key way: relaying stress signals. "A bacterium like *E. coli* has several systems that are used to sense stress in the cell envelope," says Jean-François Collet, a microbiologist at the de Duve Institute in Belgium. One of these, the regulator of capsule synthesis (Rcs) system, depends on the width of the periplasm—the space between the envelope's inner and outer layers—to function, according to a new study.

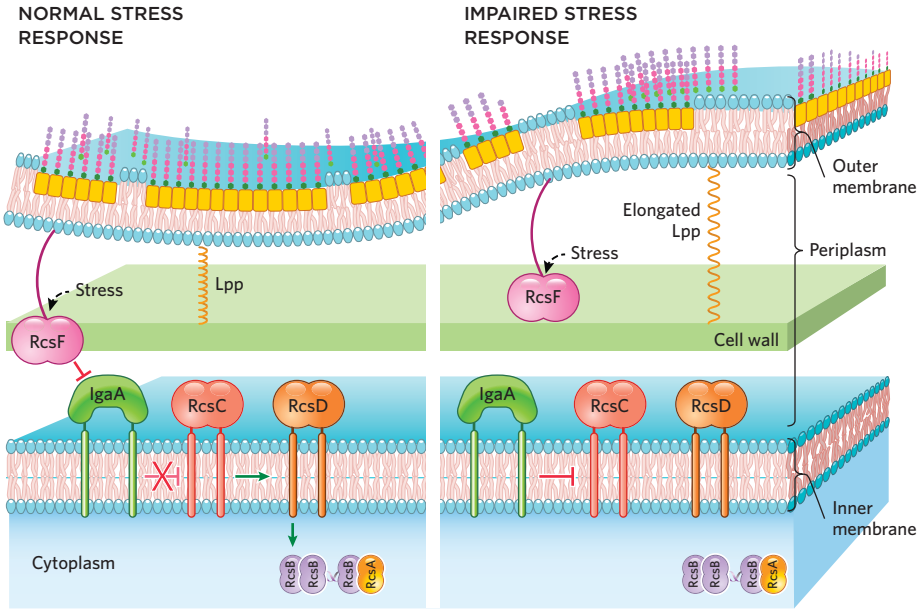
The Rcs system senses damage to the cell envelope and responds by modifying the expression of genes involved in functions such as motility and biofilm formation. The system consists of multiple molecular components that work together to activate the stress response, including the stress-sensing outer membrane lipoprotein RcsF and the inner membrane protein IgaA.

Collet's team previously found that under normal conditions, RcsF is exported to the cell surface. However, when the organism encounters a stressor, such as a chemical that damages the envelope, the protein remains inside the periplasm, allowing it to interact with and activate the inner-membrane components of the stress-response pathway. The Rcs system and several other important protein complexes span the periplasm, so Collet and his colleagues asked: "What happens if we increase [that] distance?"

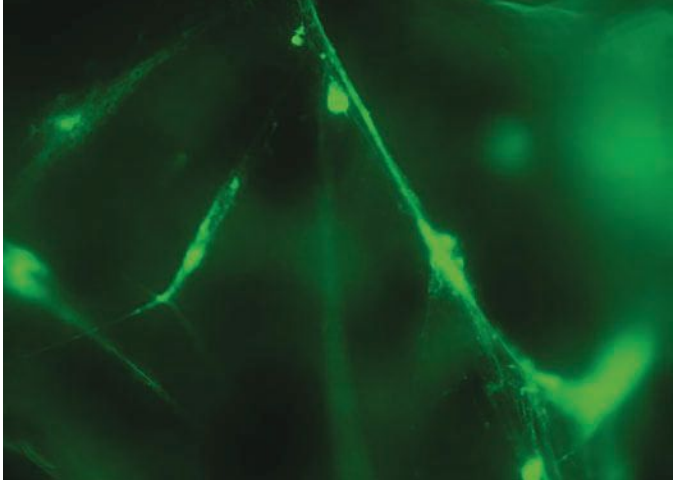
To investigate this question, his team increased the length of Braun's lipoprotein (Lpp), which anchors the outer membrane to the cell wall, the layer lying within the periplasm. They discovered that when the periplasmic space was increased, the Rcs pathway did not respond to stressors such as A22 and mecillinam, drugs that target complexes involved in cell wall genesis. To see whether this was truly due to the larger gap, the team increased the length of RcsF to correspond with the longer Lpp and found that the stress response was restored. The experiment "seemed so simple . . . [that] we didn't think that it would work," Collet says. "But it worked beautifully."

These findings suggest that the width of the periplasmic space dictates the optimal size for the Rcs system to work, says Anna Konovalova, a microbiologist at the University of Texas Health Science Center in Houston who was not involved in the work. However, she adds, there are multiple ways to induce the Rcs stress response, and it's important to investigate whether this holds true with other stressors, such as polymyxin B, an antibiotic that targets the outer membrane.

The paper's authors suggest that their findings could pave the way for new antibiotics that weaken bacteria by altering the width of the periplasmic space. "They've identified one system—and we may now identify other systems that require, or at least rely on, signaling based on the length of the periplasmic space," says Nicholas Noinaj, a Purdue University structural biologist who was not involved in this work. "But whether that's something that we can target or not remains to be determined." —Diana Kwon



SIZE MATTERS: When a bacterium encounters a stressor, RcsF inhibits IgaA, lifting its blockage on the activity of downstream components of the Rcs network. But if the periplasmic distance widens, RcsF is unable to reach IgaA, preventing the system from initiating a stress response.



SPIDEY SENSE: Lymphocytes ejected weblike, fluorescing strands of mitochondrial DNA (green) when exposed to certain oligonucleotides.

IMMUNOLOGY

A New Line Of Defense

THE PAPER

B. Ingelsson et al., "Lymphocytes eject interferogenic mitochondrial DNA webs in response to CpG and non-CpG oligodeoxynucleotides of class C," *PNAS*, 115:E478-87, 2018.

MOLECULAR BATTLEGROUND

Beyond acting as a genetic blueprint, DNA can play a direct role in the immune system. For instance, neutrophils cast webs of DNA and antibacterial proteins into the bloodstream to trap pathogens. When a team of Swedish researchers observed that B lymphocytes also appear to eject DNA, they decided to investigate further.

IMMUNE ARTILLERY

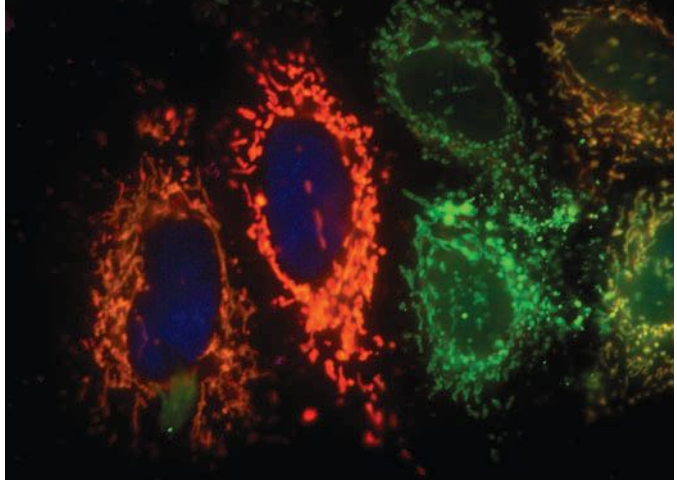
The researchers isolated several types of lymphocytes—B cells, T cells, and natural killer cells—from healthy blood donors and leukemia patients. They exposed them to a variety of triggering molecules, such as ionomycin from *Streptomyces conglobatus*, together with a fluorescent DNA-binding substance in vitro. Only when exposed to a specific type of oligonucleotide that resembled pathogenic microbial DNA did the cells rapidly eject weblike, fluorescing strands of mitochondrial DNA (mtDNA). The lymphocytes remained intact and healthy.

DANGER SIGNAL

To see whether the lymphocyte mtDNA elicited an immune response, the researchers tested it on another type of white blood cell, peripheral blood mononuclear cells (PBMCs). An immunosorbent assay revealed that mtDNA induced the PBMCs to release interferon type 1, which can trigger an immune response. "It's like a new warning system," says Anders Rosén, a cell biologist at Linköping University and senior author of the paper.

UNTANGLING THE WEB

Dana Crawford, an immunologist at Albany Medical College who was not involved in the study, is surprised and puzzled by the finding. "It almost seems to be redundant that these cells are being exposed to a type of DNA, and . . . in response they're releasing DNA that triggers a response," he notes. The advantage of having a system set up in this way is yet to be understood. **—Katarina Zimmer**



UNEARTHED TREASURE: Confocal microscopy image of a previously unannotated mitochondrial protein, altMiD51 (green), alongside mitochondria (red)

GENETICS & GENOMICS

Undocumented Proteins

THE PAPER

S. Samandi et al., "Deep transcriptome annotation enables the discovery and functional characterization of cryptic small proteins," *eLife*, 6:e27860, 2017.

HIDDEN GEMS

For many years, scientists believed that each eukaryotic gene encoded just one protein and its isoforms, and researchers annotated genomes accordingly. But recent research has shown that individual genes can encode multiple different proteins, and that plenty of proteins arise from regions of the genome that are considered noncoding. Xavier Roucou, a biochemist at the University of Sherbrooke in Quebec, Canada, decided to take a systematic approach to annotating these undocumented proteins.

TREASURE HUNT

To detect regions of the genome that might encode these proteins—so-called "alternative open reading frames" (altORFs)—Roucou and colleagues scanned nine eukaryotic genomes, including the human genome, for transcription initiation sites and stop codons. They then translated these in silico to predict the corresponding proteins, ending up with 183,191 possible unannotated proteins in the human transcriptome alone. Many of these had orthologs in the genomes of other species examined, and appeared to have functional domains.

ELUSIVE PROTEINS

To estimate how many of the putative alternative proteins are expressed in humans, the researchers searched in proteomics data collected from human samples in other studies, and detected nearly 5,000 of them. For Roucou, the results suggest that the genome harbors many overlooked proteins. "We cannot ignore them anymore," he says.

JUST THE BEGINNING

Judith Steen, a neurologist at Harvard Medical School, finds the results intriguing. However, she notes that it's still unknown how many of the predicted proteins are actively translated in vivo, under what circumstances, and what role they play. "From my perspective, a lot of work needs to be done," she says. "These are baby steps." **—Katarina Zimmer**

PNAS/ANNIE ROY

PROFILE

Parasitologist, Reprogrammed

After discovering a novel organelle in protozoan parasites, David Roos created a widely used eukaryotic pathogen database.

BY ANNA AZVOLINSKY

David Roos had been studying nucleated parasites such as *Toxoplasma* and *Plasmodium* (malaria) for several years when he decided to ask a simple question: How do antibiotics such as clindamycin work in treating both malaria and toxoplasmosis? The answer turned out to be a discovery that simultaneously solved three biological mysteries, rewrote biology textbooks, and helped to launch the field of evolutionary cell biology.

Clindamycin and related drugs kill bacteria by inhibiting the ability of bacterial ribosomes to synthesize proteins, but don't affect ribosomes in eukaryotic cells, including those of humans. Yet both malaria and toxoplasmosis are caused by eukaryotic unicellular parasites, which clindamycin also treats. In 1996, Roos, a professor of biology at the University of Pennsylvania, and then-graduate student Maria Fichera tested three possible hypotheses: clindamycin does indeed inhibit protein synthesis in *Toxoplasma* enough to prevent disease but somehow fails to kill the parasite; the drug kills *Toxoplasma* by some other mechanism; or the antibiotic targets the parasite's mitochondrial but not cytoplasmic ribosomes. After several months of experiments, none of these models could explain the antibiotic's parasite-fighting abilities.

I want to create tools that allow other users to ask their own questions, rather than my being the 'big computer scientist' who does the analysis and then tells others an answer.

A fourth, "crazier" idea, according to Roos, was that the parasites that cause toxoplasmosis and malaria harbor yet another type of undiscovered ribosome targeted by the antibiotic. The idea, says Roos, was inspired by prior reports of ribosomal genes on small circular DNA that was mistakenly assumed to have come from the parasites' mitochondria. The experiments to test this wild hypothesis ended up resolving three mysteries in parasite biology: why some antibiotics are effective against protozoan parasites; the source and function of the circular DNA originally observed in the 1970s in *Toxoplasma* and other eukaryotic parasites; and the identity of an uncharacterized organelle-like structure with multiple membranes.

In two back-to-back papers in *Science* and *Nature* in 1997, Roos, then-postoc Sabine Köhler, and Fichera established that clindamycin and a few other antibiotics target ribosomes encoded by these novel DNAs found in the parasites and that these sequences are associated with a distinctive organelle surrounded by four membranes. They dubbed the newly discovered organelle the apicoplast. The organelle was acquired, evolutionarily speaking, when an ancestral parasite "ate" a eukaryotic alga that already harbored a plastid through the endosymbiosis of a free-living cyanobacterium. Unlike plant and algal plastids, the team showed, the apicoplast had lost the ability to photosynthesize, but the organelle was essential for the parasite, including for protein synthesis.

The acquisition of apicoplasts by certain parasites studied by the team was the first proven example of secondary endosymbiosis—a "nesting dolls" phenomenon in which a eukaryotic cell swallows another eukaryote, which itself had a prokaryotic cell already residing in it.

For Roos, the story highlights the "remarkable cell biological and evolutionary insights that can be gleaned only by studying eukaryotic diversity."

AN EARLY COMPUTER PROGRAMMING START

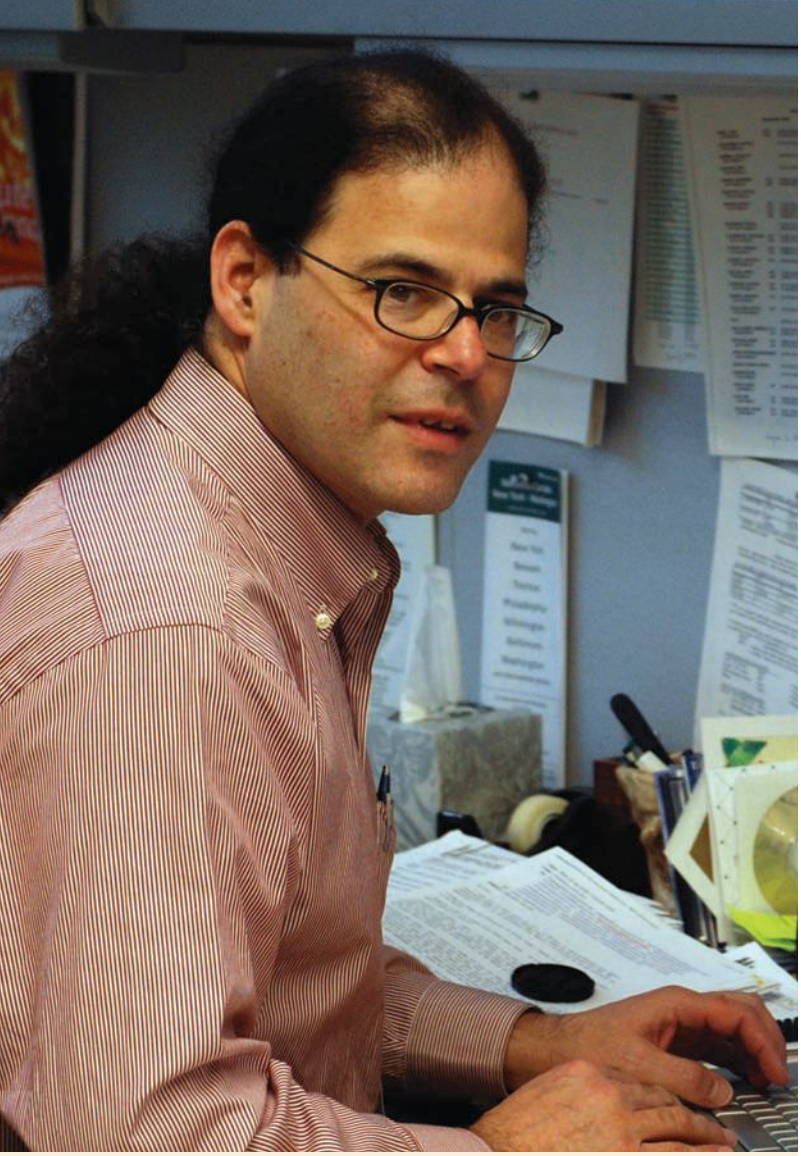
Although Roos made a name for himself when the discovery of the apicoplast made it into textbooks, biology was not his initial career ambition. He enjoyed the natural world growing up—hiking, skiing, and exploring the mountains and forests around his hometown—but his interest in computers also had an early start.

Born in 1956 in Boston, Massachusetts, Roos was raised in Hanover, New Hampshire, his father a biology professor at Dartmouth College. Roos's elementary school participated in an innovative pilot program initiated by Dartmouth's John Kemeny, a computer scientist who co-invented BASIC, one of the most widely used computer languages worldwide. Kemeny had a vision that computers should be accessible tools for everyone, and Roos, along with his second-grade classmates, was taught how to program.

"This was visionary in the 1960s when computers were seen as tools for computer scientists that would benefit the public indirectly," says Roos. "Most people didn't think that computers would be something everyone would use in their daily lives."

Roos continued to program through high school but also had many other interests, including art and math. During that time, he took college math classes at Dartmouth, and when he applied for college his senior year in 1973, Roos was "shocked to discover the backwards computer science technology at both MIT and Stanford" compared to what he was used to at Dartmouth. "I visited MIT, and they told us with great pride how they could have

COURTESY OF DAVID ROOS



DAVID ROOS

E. Otis Kendall Professor of Biology, University of Pennsylvania
Director, Penn Genomics Institute, 2001 to 2006

Greatest Hits

- Isolated and biochemically characterized the lipid membranes of mutant mammalian cells altered in their membrane fusion properties
- Created assays to determine drug targets and resistance mechanisms in mammalian cells and protozoan parasites
- Developed molecular genetic and cell biological tools, including selectable markers for drug resistance, enabling the functional dissection of *Toxoplasma* and *Plasmodium* parasites and helping to launch the field of evolutionary cell biology
- With lab members Sabine Köhler and Maria Fichera, uncovered a novel organelle unique to parasites belonging to the phylum Apicomplexa, including *Toxoplasma gondii* and malaria-causing *Plasmodium*
- Developed the Eukaryotic Pathogen Genomics Database (EuPathDB.org), a data-mining resource facilitating the interrogation of multi-omic datasets relating to hundreds of microbial species, hosts, and diseases

twenty users time-sharing their computers, when at Dartmouth, we had ten times that many computer users at the same time."

Dismayed, Roos decided to enter the workforce instead, becoming a computer programmer for the computer company Honeywell. After a year and half on the job, he applied to colleges, entering Harvard University in 1975.

FIRST ART, THEN SCIENCE

Initially, Roos was an art major, taking both studio art classes and art history. "It's a pet peeve of mine that some find a contradiction between interest in art and science. I find that science is a very aesthetically pleasing and artistically driven discipline in many respects," he says.

Eventually, though, biology won out. Roos was singled out by a biology teaching assistant who suggested he approach Morris Karnovsky, a cell biologist and morphologist, about working in his lab. Roos accepted the nudge, and in Karnovsky's lab he researched how cell membranes change during polyethylene glycol (PEG)–induced cell fusion. He also characterized the membranes of mammalian cell lines he had isolated that could resist PEG-induced fusion in Richard Davidson's lab, then at Children's Hospital Medical Center in Boston. These hands-on experiences made Roos switch his major to biology and also apply to graduate school.

STARTING FROM SCRATCH

Initially, Roos applied to MD/PhD programs, but the admission committees gave him a hard time, he recalls, questioning his commitment to the field based on his prior meanderings in computer science and art. Even though he thought he was only interested in MD/PhD programs, on a whim Roos accepted an invitation from Rockefeller University in New York City to interview for the bioscience PhD program and began his graduate studies in Purnell Choppin's lab in 1979.

There, Roos found that the fusion-resistant mouse cell lines he had generated at Harvard had membranes with a distinct mix of lipids that directly correlated with their reduced ability to fuse with other cells, suggesting that the types of lipids present in the cell membrane control whether cells could merge with each other. He also found that cell fusion was not guided by changes in the fluidity of the cell membrane, which was previously hypothesized to be the way membrane fusion occurs.

In 1985, Roos joined Robert Schimke's lab at Stanford University as a postdoc, to get the molecular biology training he

was lacking. He studied the action of antifolate drugs—a class of chemotherapy agents that block the activity of folic acid—and developed an assay to test sensitivity and resistance to these compounds in cultured human cells.

Roos applied for a Markey Trust fellowship, which provides funding for newly minted professors to start their own laboratories, but initially had no idea what research questions he wanted to address. He settled on parasitology for the "not very good reason that the research was relevant to parts of the world where I wanted to travel," says Roos. After perusing the literature, Roos concluded that what was missing from malaria and other parasitology investigations were molecular genetic tools.

He came across the work of Dartmouth's Elmer Pfefferkorn, who had developed a plaque assay in which *Toxoplasma* would infect and lyse mammalian cells, in turn infecting their neighbors and generating plaques—pockets of lysed cells. The assay, Pfefferkorn had shown, demonstrated that generating *Toxoplasma* mutants was possible and could be used to do genetic crosses and identify biochemical pathways. "I proposed to develop an in vitro *Toxoplasma* culturing and transfection system to be able to add genetic markers in order to do molecular genetic experiments," says Roos.

Roos's funding proposal failed to convince the reviewers, but his interest in parasitology nevertheless became invaluable to his career, establishing the research direction he wanted to pursue in his own laboratory.

A BOLD MOVE LEADS TO BIOMARKER SUCCESS

Researchers had previously tried to create genetic tools to manipulate *Toxoplasma* and *Plasmodium* by using the same type of antibiotic-resistance biomarkers that were successful in yeast. But these attempts failed, and molecular biology experiments had stalled in the field of parasitology. So Roos took a different genetic marker tack, taking advantage of what he knew about antifolates as a tack to clone the organisms' genes for dihydrofolate reductase, an enzyme required for the synthesis of the purines adenine and guanine and of some amino acids, and a target for certain chemotherapy drugs and antimicrobials. Roos began the genetic marker project while still in Schimke's lab, but was only successful after he started his own laboratory at the University of Pennsylvania in 1989.

He exposed parasites to antifolate drugs and selected cells that were resistant to the treatment and therefore must have had a mutation in their dihydrofolate reductase genes. In 1993, he cloned the gene for dihydrofolate reductase–thymidylate synthase (DHFR). With postdoc Robert Donald, Roos created a plasmid with a mutated version of the gene, which, when introduced into the parasite, results in antifolate resistance and can be used as a selectable marker. In 1998, using this genetic tool, Roos and postdoc Mary Reynolds uncovered how mutations in certain amino acids within the gene for DHFR alter interactions between the DHFR protein and antifolates, resulting in drug resistance.

Looking back, Roos sees his younger self as overly bold. "If I had had any sense, I would have done a second postdoc and learned how to do this work with Pfefferkorn," says Roos. "It was a really terrible idea to do something you've never done before when starting your own lab. You can't train people in something you have never done before!"

Roos also learned an important lesson from his Penn colleague Lewis Tilney. Roos initially wanted to have his postdoc advisor, Schimke, listed as an author on the dihydrofolate reductase paper. "The first draft listed Schimke because I started the work in his lab, but then Lou read the paper and said, 'You're the one that did all of the work. If you don't call Schimke and tell him that he doesn't need to be an author on the paper, then I will.' So I called and of course Bob was very gracious and said that this was always my project. Lou had given me good advice, and I try to maintain that if I am not directly involved in the work, even if it's done in my lab, then I am not an author on a paper."

LAUNCHING A BIOINFORMATICS CAREER

Even with his roots in laboratory biology, since 1998 Roos's work has become subsumed by data management and integration. These days, with just a few postdocs and graduate students, his lab has "dwindled almost to vanishing," he says.

Since 2000, much of Roos's time has been spent leading a team that is responsible for supporting EuPathDB, the Eukaryotic Pathogen Genomics Database, a catalog of parasites and other pathogens. He helps grow and manage the database as new omics data are generated by researchers around the world. A full-time staff of almost 50 people now maintains and updates the collection, funded mostly by the National Institute of Allergy and Infectious Diseases.

The database began as a research question. After discovering the apicoplast in 1997, Roos's lab wanted to understand the functions of the organelle in the context of other endosymbiotic organelles, and turned to evolutionary biology. Roos's team identified some of the key apicoplast and nuclear genes necessary for the function of the organelle.

In 1999, as the human genome was being assembled, the full genomic sequences of *Plasmodium* and *Toxoplasma* were still incomplete. Roos recognized that the parasite sequences that were emerging needed to be compiled, but that manually comparing sequences was not feasible. He also realized that the small computer programs his lab members had written to analyze their sequences of interest could be modified and used by others to ask their own scientific questions. That was the origin of the initial *Plasmodium falciparum* Genome Database (PlasmoDB) that Roos, along with then-postdoc Jessica Kissinger, launched in 2001. "Its success has come to dominate my life ever since," he adds. The database continued to grow and now includes genomic and other omics data on 285 organisms, from fungi to pathogenic parasites.

Roos's initial interest in computer science has come full circle. "I want to create tools that allow other users to ask their own questions, rather than my being the 'big computer scientist' who does the analysis and then tells others an answer," he says. "That philosophy is in line with what John Kemeny had in mind when he wanted anyone to have access to computers as a resource and to use them the way they want." ■

Jermaine Jones: Untangling Addiction

Assistant Professor, Department of Psychiatry, Columbia University. Age: 37

BY KATARINA ZIMMER

Jermaine Jones's first memory of being a "bit of a scientist" was discovering that toilet water is actually pretty clean. While conducting a science fair project during his junior year of high school in Virginia, he learned that "you get much more varied bacteria from the toilet seat as opposed to the water," he explains.

With encouragement from his aunt, who was a biologist, Jones chose to pursue a degree in science at the University of Virginia. Over the course of several undergraduate internships, he got a taste of different fields of research, from probing decision making in mice to examining the analgesic effects of rainforest plant extracts in Brazil. By the time Jones had earned his master's degree from Old Dominion University, he was certain that investigating drug abuse would be a good fit for his two main interests, pharmacology and psychology.

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For his PhD, Jones moved to Washington, D.C., where he worked on elucidating the neurobiological mechanisms of cocaine's aversive effects in rodent models with Anthony Riley, a behavioral pharmacologist at American University. At the same time, Jones examined the effects of knocking out genes encoding the neurotransmitter transporters that the drug acts upon in mice with neuroscientists George Uhl and Scott Hall at the National Institute on Drug Abuse. For his dissertation, "he was able to show, pretty unequivocally, the role of neurotransmitter systems in the aversive effects of cocaine in these mice," Riley recalls.[1]

Riley describes Jones as a meticulous, dedicated, and focused researcher. "What I liked best about Jermaine," he adds, "was that his long-term goal was [to do] translational work with humans with drug addiction."

In 2008, Jones transitioned to clinical research, starting a postdoc in the Columbia University lab of neurobiologist Sandra Comer, who focuses on testing novel medications for treating opioid use disorder. Jones's compassionate nature helped him make the challenging jump from preclinical research, Comer says. "He's good at making a research subject feel comfortable."

In Comer's lab, Jones worked on a range of studies, including an inpatient investigation demonstrating the efficacy of a buprenorphine/naloxone treatment for managing chronic pain[2] and an analysis that quantified how brief educational training could help heroin users accurately identify an opioid overdose.[3] He also cowrote a review on the effects of combined opioid and benzodiazepine use.[4] "He really took the lead on that," Comer says.

Jones became an assistant professor at Columbia in 2011, and his next goal is to explore the role of genetics in substance use disorders and the medications to treat them. He is currently examining how genetic variance alters the effectiveness of naltrexone in treating methamphetamine abuse.

Beyond teaching at the college level, Jones also participates in a program that encourages minority graduate and medical students to pursue research careers. "The students that he's worked with now have done really, really well in the program," Comer says.

Looking back, Jones is pleased that he made the move into clinical research. "I've learned so much just from sitting and [talking] with individuals, because oftentimes they'll tell you you're asking the wrong question," he says. "That really shapes you as an investigator." ■

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Getting On Target

New techniques for detecting when CRISPR is off the mark

BY SANDEEP RAVINDRAN

Winston Yan's graduate school project involved using a variant of the CRISPR-Cas9 genome editing system to knock down a gene that regulates cholesterol in mice. "The real goal was to eventually pave the way for therapeutic uses," says Yan, who recently completed his graduate work in Feng Zhang's laboratory at MIT. That's when he encountered, firsthand, the problem of CRISPR's off-target effects.

CRISPR allows researchers to quickly and efficiently make targeted cuts to genomes. Its specificity and ease of use gives the gene-editing tool great potential for removing defective genes and treating genetic diseases or cancer, or for editing the genome of crop plants to increase their yield or disease resistance.

To wield this power, however, researchers will first have to overcome one of CRISPR's main limitations—its propensity to cut not just at its target site, but also at unintended sites with similar sequences. These off-target cuts can occur across the genome and can lead to harmful mutations that impair a cell's function or kill it outright.

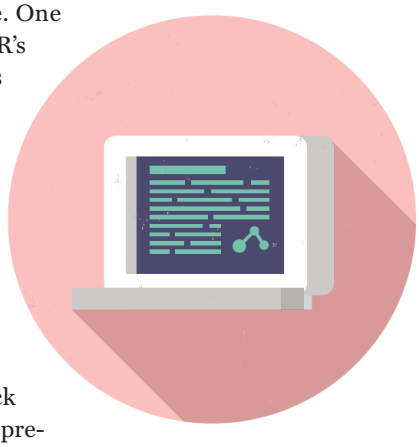
Developing methods to detect CRISPR off-target mutations has been a challenge, but over the past few years, researchers have come up with a variety of new approaches. Here, *The Scientist* leads a guided tour of when and how to use them.

IN SILICO PREDICTION VS. UNBIASED DETECTION

The specificity of CRISPR editing can vary widely. A key driver of CRISPR's precision is guide RNA, an RNA sequence that guides the Cas9 nuclease to cut at a specific location on the genome. "Even in the same organism, choosing one guide RNA might let you get away with no off-target mutations at all, while a different guide RNA might give you up to 150 off-targets," says Julia Jansing, a PhD student in Luisa Bortesi's lab at RWTH Aachen University in Germany.

Researchers have also found or engineered other nucleases that are more specific than the commonly-used version of Cas9 and cut at fewer off-target sites. These include the Cpf1 nuclease and a high-fidelity version of Cas9.

But CRISPR, even with optimized guide RNA or nucleases, can still cause inadvertent changes to the genome. One way to predict CRISPR's off-target activity is with computational algorithms that identify likely off-target sites based on the sequence of the guide RNA. Researchers can then use targeted sequencing after they attempt a genomic cut to check for mutations at those predicted off-target sites. Each algorithm has its own secret sauce, and their results don't always align. "Depending on the quality of your prediction tool, you get a varying degree of reliability," says Jansing. In silico prediction tools have not been systematically compared, so researchers could choose one based on their preferred user interface or whether it supports the genome of their species of interest—researchers working on mice or humans have more tools to pick from than those working on tomatoes, for example.



For basic research applications, such as making a mutant cell line, in silico prediction may be the most practical option. It is relatively quick, easy, and cheap, while still generally being accurate enough to ensure that off-target mutations aren't too numerous and don't confound the interpretation of experimental results.

But such prognostication is far from foolproof. "We're putting this intrinsic bias on where we're looking because we assume we know how Cas9 cuts," says Yan. "But from many different studies, we know that this isn't the case." In addition, computational predictions can be overly broad. "You're never going to do a PCR on thousands of sites to look for a minor amount of off-targets," he adds.

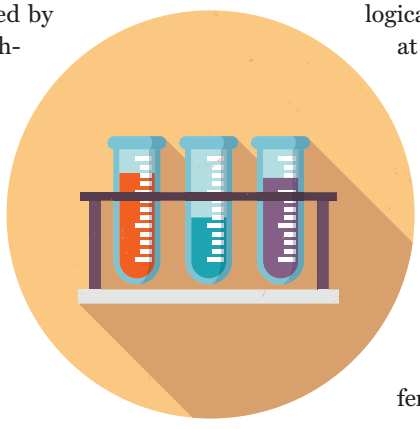
To overcome the current limitations of in silico prediction, researchers have developed multiple in vitro and cell-based techniques to detect CRISPR off-target mutations in an unbiased, genome-wide fashion. Such approaches are crucial when developing therapeutics, or even in preclinical studies, because these methods can detect rare and unforeseen off-target edits that could have potentially harmful effects on a patient, for instance by activating an oncogene. "The regulators are probably going to ask for some kind of genome-wide, off-target analysis," says Keith Joung, a pathologist at Massachusetts General Hospital and professor of pathology at Harvard Medical School.

IN VITRO GENOME-WIDE ASSAYS

When Cas9 and similar nucleases cut the genome, they create double-stranded breaks. Most in vitro assays that pinpoint off-target effects use Cas9 or other nucleases to cleave cell-free genomic DNA, then use software to detect double-stranded breaks in sequencing data.

These assays are generally extremely sensitive and can detect off-target sites that are mutated at frequencies lower than 0.1 percent. They can be used to run large-scale screens for off-target effects, or be adapted to a clinical setting by extracting genomic DNA from patients. But because they use cell-free genomic DNA, they can't predict mutations that occur inside cells.

Digested genome sequencing, or Digenome-seq, is an in vitro assay that has become increasingly popular since its introduction in 2015. It has a simple two-step protocol: in vitro Cas9 cleavage followed by next-gen sequencing. Two newer methods, CIRCLE-Seq and SITE-Seq, are slightly more complex but are also more sensitive, as they enrich for nuclease-cleaved genomic DNA before sequencing. Researchers are continuing to improve the accuracy and throughput of these methods. "I think in the long term, in vitro is going to be the way to go, but that's an evolving space right now," says Joung.



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CELL-BASED GENOME-WIDE ASSAYS

Cell-based assays for off-target detection use different techniques to identify where Cas9 or other nucleases cleave genomic DNA in cells and create double-stranded breaks. One advantage of this approach over in vitro methods is that it can identify off-target sites in a specific cell type and under particular experimental conditions.

Cell-based assays detect double-stranded breaks that occur endogenously in addition to those created by the CRISPR nuclease. Their sensitivity can vary depending on the characteristics of the cells involved, including how easy it is to culture and transfect them and how efficiently they repair double-stranded breaks.

Genome-wide Unbiased Identification of Double-stranded breaks Enabled by Sequencing (GUIDE-Seq) is a widely-used and highly sensitive cell-based assay that can detect off-target sites that occur at a frequency of 0.1 percent in a cell population. Small, double-stranded oligonucleotides are used to tag double-stranded breaks created by Cas9 or other nucleases. The tagged genomic sites are then PCR amplified and sequenced to map the double-stranded breaks. One drawback of GUIDE-Seq is that some primary cells can be difficult to transfect with the oligonucleotides.

Linear amplification–mediated high-throughput genome-wide translocation sequencing (LAM-HTGTS) detects more than just breaks; it identifies genomic rearrangements resulting from these breaks. Most double-stranded breaks cleaved by the Cas9 nuclease actively repair themselves, but some fuse to ends created by other double-stranded breaks, resulting in a chromosomal translocation of off-target and on-target breaks. By detecting these translocations, LAM-HTGTS can provide a sense of the collateral damage from accumulating off-target edits, including their effect on genome instability.

"With this assay, you're measuring the end events from a biological process," says Richard Frock, a molecular geneticist at Stanford University who helped develop this method as a postdoc in the laboratory of Frederick Alt at Harvard Medical School.

Breaks Labeling In Situ and Sequencing (BLISS), developed by Magda Bienko and Nicola Crosetto of the Karolinska Institute in Sweden in collaboration with Zhang and Yan at MIT, profiles off-target edits by biochemically labeling double-stranded breaks in fixed cells, thus directly capturing the number of breaks in primary cells. The sensitivity of this assay can depend on when the cells are harvested, and different tissues might have different optimal harvest points.

"I think our assay has a lot of potential for use in the clinic because it interrogates breaks in the natural environment," says Crosetto. "I'm very optimistic that we will see useful translational applications."

DECIDING ON AN ASSAY

There's still no gold standard among these techniques, and for now researchers just have to pick the one that makes the most sense for their research. "It's a question of which method you have the equipment and the know-how for," says Jansing. Those who want to be extra thorough could use a combination of methods.

None of these methods require special reagents for their pre-sequencing steps and all generally take only a few days to a week to finish. Assays can cost from a few hundred to a few thousand dollars depending on the number of samples, and the cost of the different assays is broadly comparable, says Crosetto.

But researchers will need access to a next-gen sequencing service or machine. Sequencing costs can vary widely depending on the number of samples and the sequencing depth, and costs are going down. But sequencing is still the most expensive part of these assays. "It's definitely in the thousands of dollars" to perform the sequencing involved in one of these assays, says Jansing. "It's not something you do for fun."

Researchers employing these assays will also need bioinformatics expertise to analyze the next-gen sequencing data. "The biggest challenge is the bioinformatics analysis, because there is no off-the-shelf, commercial software package to do the analysis," says Joung. He and his collaborators wrote their own code to analyze GUIDE-Seq and CIRCLE-Seq results. Similarly, Frock's lab wrote custom Perl scripts for their LAM-HTGTS pipeline. "Each assay is a little bit different in its approach, so coming up with a universal pipeline to analyze these things is going to be a little bit challenging," he says.

Meanwhile, Joung and his colleagues are working on a commercial solution. They set up a company, Beacon Genomics—now called Monitor Biotechnologies—that plans to offer GUIDE-Seq and CIRCLE-Seq on a fee-for-service basis. Joung says he hopes that the company can make these assays easier to use by allowing researchers to outsource the next-gen sequencing and bioinformatics analyses steps.

Such commercialized assays could promote the widespread adoption of unbiased, off-target detection—paving the way for improving CRISPR and making it safer for medical and biotech applications. "Having a tool for researchers just out of the box, used within a couple of hours just like a miniprep kit or something," says Yan, "that would be fantastic." ■

HOW THE ASSAYS STACK UP

Selected in silico prediction assays	Resources
Cas-OFFinder	http://www.rgenome.net/cas-offinder/
CRISPR Design Tool	http://crispr.mit.edu/
CasFinder	http://arep.med.harvard.edu/CasFinder/
E-CRISP	http://www.e-crisp.org/E-CRISP/
Breaking-cas	http://bioinfogp.cnb.csic.es/tools/breakingcas/
CRISPOR	http://crispor.tefor.net
CHOPCHOP	http://chopchop.cbu.uib.no

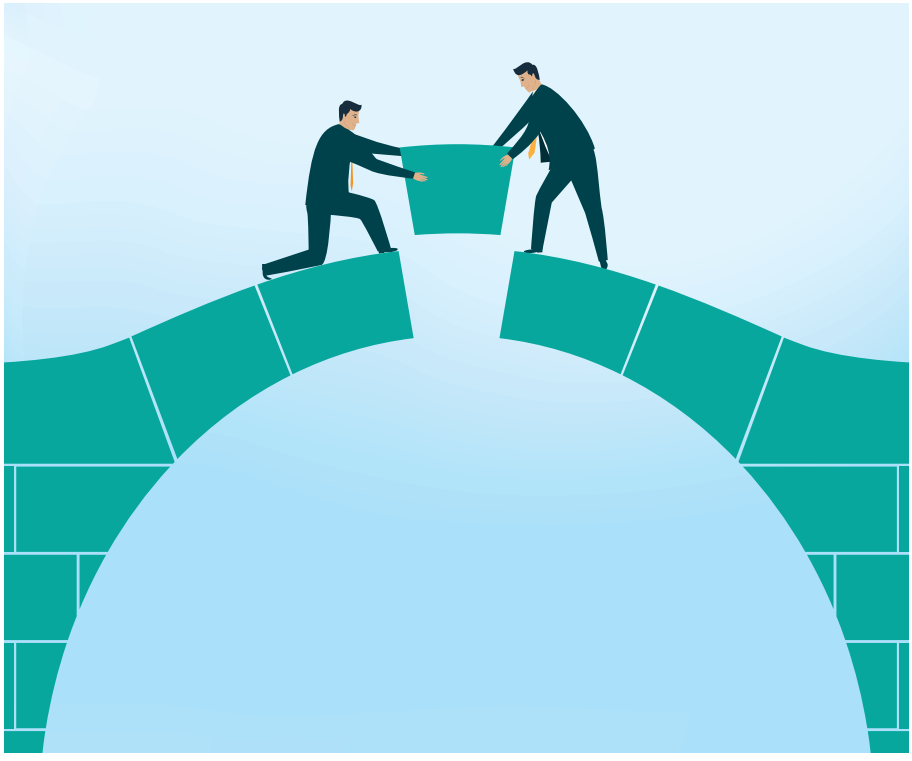
Selected in vitro genome-wide assays	Description	Resources
Digenome-seq	Purified genomic DNA is digested with a nuclease and subjected to whole genome sequencing. Off-targets are computationally identified.	*Nat Methods*, 12:237-43, 2015 **Web tool:** http://www.rgenome.net/digenome-js/#! **Code:** https://github.com/chizksh/digenome-toolkit2
CIRCLE-Seq	Purified genomic DNA is sheared and circularized, and residual linear DNA is degraded. The Cas9 nuclease is used to linearize circular DNA containing a Cas9 cleavage site, and the cleaved ends are PCR-amplified and sequenced to identify off-targets.	*Nat Methods*, 14:607-14, 2017 **Code:** https://github.com/tsailabSJ/circleseq
SITE-Seq	Purified genomic DNA is cleaved using Cas9, and Cas9 cleavage sites are biochemically tagged and enriched. Next-gen sequencing and bioinformatics analysis is then used to identify off-target cleavage sites.	*Nat Methods*, 14:600-606, 2017 **Protocol:** *Protocol Exchange*, doi:10.1038/protex.2017.043

Selected cell-based genome-wide assays	Description	Resources
GUIDE-Seq	Double-stranded breaks created by the Cas9 nuclease are tagged using small double-stranded oligonucleotides, PCR amplified, and sequenced to map the double-stranded breaks.	*Nat Biotechnol*, 33:187-97, 2015 **Code:** https://github.com/aryeelab/guideseq
LAM-HTGTS	Chromosomal translocations of off-target and on-target breaks are PCR amplified and analyzed by next-gen sequencing.	*Nat Biotechnol*, 33:179-86, 2015 **Protocol:** *Nat Protoc*, 11:853-71, 2016 **Code:** http://robinmeyers.github.io/transloc_pipeline/index.html
BLISS	Double-stranded breaks are biochemically labeled, and their downstream sequences are amplified using in vitro transcription and analyzed using next-gen sequencing.	*Nat Commun*, 8:15058, 2017

Don't Wait, Collaborate

Academic and industry researchers have come up with various strategies to help their collaborations succeed.

BY ASHLEY P. TAYLOR



Back in the '90s, immunologist James Allison wasn't trying to develop a cancer drug. "I was doing just really fundamental research trying to understand T-cell regulation," he says. But in the course of that work, performed at the University of California, Berkeley, Allison discovered that a protein receptor called CTLA-4 negatively regulated T-cell responses to antigens, and that inhibiting that receptor with an antibody enhanced T-cell activity.

The clinical applications were obvious. "I had the idea that you might be able to exploit that to get immunological responses, T-cell responses, to tumor cells," says Allison, now chair of immunology and director of immunotherapy at the University of Texas's MD Anderson Cancer Center. In a 1996 *Science* paper, he and his colleagues reported that, in mice, this approach worked: rodents treated with an anti-CTLA-4 antibody rejected tumors. "I thought this was pretty cool. We patented it," says Allison. "I thought everybody would jump at it." But everybody—in particular pharmaceutical companies—did not.

For more than two years, Allison says, every company he approached turned him down. Then, the first company to express interest in licensing Allison's patent and attempting to develop an anti-CTLA-4 drug floundered in its translational efforts. That firm, NeXstar Pharmaceuticals, tried in the late '90s to develop an RNA aptamer to bind and inhibit CTLA-4, but, as Allison describes it, they "just couldn't get it."

Eventually, through a friend, Allison connected with the pharmaceutical company Medarex (later acquired by Bristol-Myers Squibb), which took on the project, and, working with Allison, developed an anti-CTLA-4 antibody into a drug, ipilimumab (Yervoy). After years of clinical trials, in 2011 the FDA approved ipilimumab for treatment of late-stage melanoma—about 15 years after the therapy's conception—and in 2015, Allison won the Lasker-DeBakey Clinical Medical Research Award in honor of the work.

Allison's wish to translate his research findings into drugs or other technologies is an increasingly common one. According to *Nature Index Science Inc. 2017*, the number of academic-industry collaborations more than doubled from 12,672 in 2012 to 25,962 in 2016, and half of those 2016 collaborations were in the life sciences. Most labs do not have the resources to take a discovery from the bench to the clinic, and industry offers funding, personnel, and experience in manufacturing products and bringing them to market.

"I think it's been borne out that really to get treatments to patients . . . it needs to be a partnership," says Joan W. Miller, chair of ophthalmology at Harvard Medical School, who has worked with industry partners to develop drugs for macular degeneration and other eye diseases.

But, as Allison found out, the success of those partnerships is far from guaranteed. New therapies and technologies can be delayed, or even blocked entirely, on the road to the clinic. Although most universities have technology transfer offices to help researchers file for patents, form start-ups, and collaborate with industry, researchers across the academic-industry divide have to contend with differences in institutional cultures, on top of the scientific challenges of translating academic results into clinical treatments.

For instance, while academic researchers generally receive grants to pursue given projects over several years, industry labs tend to expect faster results and are quicker to abandon projects that don't show immediate promise. Academic culture encourages openness, with researchers hurrying to publish their results. Industry, meanwhile, has a tendency to want to safeguard, rather than publicize, results from which a company might be able to profit.

But where there's a will there's a way, and collaborators have developed a variety of strategies to overcome such differences and facilitate academic-industry partnerships, from forging relationships as soon as possible, to placing emphasis on trust. As Isaac Kohlberg, chief technology development officer of Harvard's technology transfer office, says, "It has been our experience that if there is the intent that these partnerships should work, they in the end work."

Get a head start

One of the major problems in translating research from academia to industry is a lack of reproducibility. According to studies by pharmaceutical companies Bayer and Amgen, when industry labs try to reproduce academic results they are unsuccessful approximately 80 percent of the time, as McGill University ophthalmologist Leonard Levin and coauthor Francine Behar-Cohen note in a 2017 *Trends in Pharmacological Sciences* editorial.

There are likely various reasons for this reproducibility problem, from insufficiently detailed methods in published studies to the use of different animal models and statistical approaches in academic vs. industry labs. The result is that academic results get "lost in translation" when industry labs try to reproduce them, Levin tells *The Scientist*—"something that is well recognized by industry, but is not so well recognized in academia."

To solve this problem, Levin recommends that academic scientists, in addition to writing more-detailed methods and holding data to higher statistical standards, should begin their collaborations as early as possible in the development of a product. If a lab and an industry partner are already working together on a project then such translational problems should be less severe.

Starting academic-industry cross-talk early in the career of an academic also promotes reproducibility, Levin says, since scientists who know how industrial research works are more likely to carry out studies that can achieve translation. To educate academics about industry research, Levin and Behar-Cohen recommend the establishment of university programs in which academics spend time working in industry labs (see "Making the Most of School," *The Scientist*, 2016).

> **The most successful collaborations with industry are actually growing out of personal relationships.**
>
> —Jon Soderstrom
> Office of Cooperative Research, Yale University

Universities such as Yale offer industry fellowships for grad students, postdocs, and junior faculty. "Academics, particularly younger junior faculty, are totally unaware of what it takes to actually do the translational research that's going to make their observation that they make at their lab bench into a successful product," says Jon Soderstrom, managing director of Yale's Office of Cooperative Research. It's only through experience working with industry that researchers learn to collaborate with industry, he adds. "You can't teach it as a lecture."

Such hands-on early career experience is often linked to another aspect of successful collaborations, Soderstrom says: a gradual development. "The most successful collaborations with industry are actually growing out of personal relationships that the faculty already have with somebody at a company or have developed over time with a company, usually based on some relatively small initiatives to start, and then they build up over time."

Work out the details

Despite the benefits of relations that start early and develop gradually, there are some sources of tension that are likely to be inevitable whatever the nature of the collaboration. Partners may clash over intellectual property or decisions about how much a company will pay to license a patent from a university lab. And then there's liability: who will be legally responsible for what, and under what circumstances either partner could be sued. "Every single collaboration we enter into, [liability] is an issue that has to be discussed and negotiated," Soderstrom says. "Suffice it to say each side is interested in protecting its interests from damages resulting from lawsuits," he adds in an email.

Such tensions can significantly delay a project's progress. For example, several years ago Stephen Waxman, a neurologist at Yale School of Medicine and Veterans Affairs Connecticut Healthcare System, collaborated with Pfizer to test a drug for "man-on-fire syndrome"—in which pain-sensing neurons overreact to mild stimuli, causing searing skin pain—in human patients (see "Channeling the Pain," *The Scientist*, January 2018). Yale and Pfizer investigators had planned to work together to carry out the trial, using Pfizer's drug and patients recruited by Waxman, at a Pfizer clinical research facility in New Haven. Yet for a while, liability concerns prevented Yale researchers from working at the Pfizer lab. "It slowed things down," Waxman says. "That frankly was discouraging, and it took some strong urging on my part to move things ahead."

One common strategy to minimize such logistical difficulties is to draw up master agreements that cover multiple projects. These agreements decrease the strain that per-project negotiations would otherwise place on partnerships by laying out the parameters of the collaboration—for example, whether a company might have first dibs on licensing the resulting intellectual property and to what extent academics are free to publish the results—in writing.

Harvard, for example, engages in long-term collaborations, called strategic alliances, with company partners. The multi-project agreements that govern these collaborations serve to expedite collaborative research, Caroline Perry, director of communications at Harvard's technol-

ogy transfer office, tells *The Scientist* in an email. "Our research alliances encourage ongoing collaboration by establishing a common understanding up front, under which multiple specific projects in various labs can then be initiated more quickly."

Such long-term agreements can also help resolve the conflict between academics' wish to publish results and industry's preference for secrecy. As described in a 2012 article in the *MIT Sloan Management Review* by the University of Bath's Ammon Salter and Imperial College London's Markus Perkmann, who both research academic-industry collaborations, short-term, confidential projects would ordinarily not appeal to academic researchers. But in the context of a longer, more open collaborative project, academics might find short-term secret projects more palatable.

Handled the right way, logistical matters are simply part of the collaborative process. In the case of Waxman's collaboration with Pfizer, "it wasn't that there was an issue," Soderstrom says. Rather, "we had to contractually figure out . . . who was responsible for what," or who was liable for the patients in the trial. In the end, Yale and Pfizer researchers did work together at the Pfizer research unit, and the resulting study appeared in *Science Translational Medicine* in 2016. Waxman looks back on the liability-related delay as a "legal administrative wrinkle."

Boost transparency and trust

Perhaps the most insidious threat to collaborations across the academic-industry boundary, however, is a lack of communication. Barriers to information flow might arise for a number of reasons. For example, a firm might withhold information about an invention it doesn't want the university to take part in, or a university might restrict the flow of information or materials, such as cell lines or plasmids, from its labs out of fear that the companies might use them without giving the institution credit. That "only leads to frustration," says Soderstrom.

To avoid such outcomes, he recommends keeping in frequent contact with industrial partners. Key to success, he says, is "free flow of information" in both directions. "Regular exchanges of telephone calls, emails, visits, et cetera. . . . If you don't have full and open transparent communication it tends to make people suspicious and distrustful. And if you have that, it's going to fail."

The value of trust in academic-industry collaborations is supported by research. For a pair of studies published in 2010 and 2012, Salter and colleagues surveyed both academic and industry researchers in STEM fields about two types of barriers to collaboration: "orientation-related barriers," such as academia and industry's different perspectives on publishing results, and "transaction-related barriers," or those related to intellectual property and other logistical details. Survey responses revealed that the greater trust industrial and academic partners reported feeling toward their collaborators, the lower both sides perceived both types of barriers to collaboration to be.

But whatever your collaborative strategies may be, patience and perseverance will also be required. "A message for young people thinking about academic-industry interactions is to be prepared for a long ride, sometimes a ride that has bumps in the road, but to be persistent," says Waxman. Of his collaboration with Pfizer, he says, "It took a long time. It required the coming together of two cultures, the academic culture and the biopharma culture, but neither of us . . . could have done this alone." ■

Ashley P. Taylor is a freelance writer and science journalist in Brooklyn, New York. She has written for Yale Medicine, the Yale School of Medicine alumni magazine, since 2014 and Medicine@Yale, *the medical school's newsletter, since 2017.*

TIPS FOR SUCCESSFUL COLLABORATION

Focus on reproducibility: To increase the likelihood that preclinical findings can be translated, determine study outcomes and statistical analyses in advance of projects, and publish detailed methods. "It's very possible that an industry laboratory may not be able to reproduce [a paper's results] completely because of the lack of enough specific detail," says McGill University ophthalmologist Leonard Levin. "Don't just say, 'This is what we did.'" Instead, "say 'This is how we do it.'"

Don't wait: Instead of approaching industry in the late stages of preclinical research, form collaborations early on. Partnerships that start small and personal, then build gradually, tend to be more successful than those arranged late in product development, or by people higher up the administrative ladder, says Jon Soderstrom, managing director of Yale's Office of Cooperative Research. "It's hard to do 'top down,' [where] a company decides that their people ought to work with somebody from a university, or a university decides that they ought to go plug somebody in to work with a company."

Find the middle ground: To overcome differences in publishing culture, seek compromises between publishing results and protecting intellectual property by filing for patents prior to publication and combining short-term, confidential projects with long-term, more-open research partnerships. "Relationships are successful when the two parties understand each other's needs and concerns . . . and reach a structure and a conclusion that is acceptable to all," says Isaac Kohlberg, chief technology development officer of Harvard's Office of Technology Development.

Talk it out: Avoid conflict by keeping industry partners informed of developments in research projects. Transparent and frequent communications are key to developing and maintaining trust, a crucial ingredient in any collaboration, says Soderstrom. "At the base of everything is trust and mutual respect."

Holding Down the Lab

During World War I, women scientists stepped in to keep research labs running. But a century later, the struggle for equality in science still rages.

BY PATRICIA FARA

When Marie Skłodowska Curie embarked on a fund-raising tour of the United States in 1921, she was amazed to see female students being taught alongside the men. In Curie's adopted home country of France, married women belonged to their husbands and were not allowed to earn money in their own right. In the U.S., many thousands of independent American women greeted her with rapturous applause, lavishly donating the equivalent of more than $1 million to buy a whole gram of radium for her research institute in Paris. Yet despite their relative liberation, American women by and large regarded Curie as exceptional, too extraordinary to provide a realistic role model.

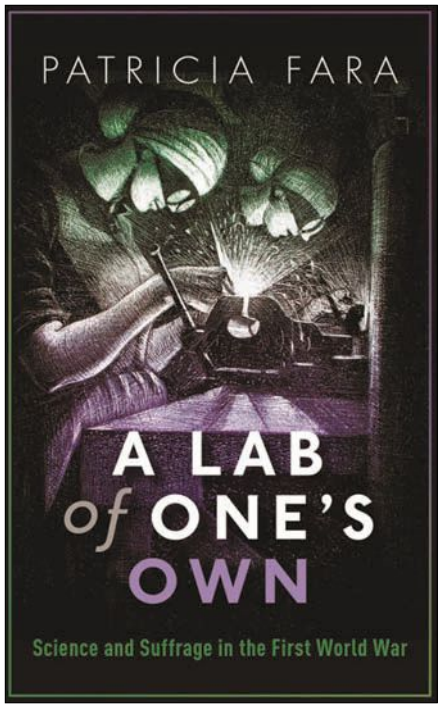
One of Curie's closest friends was Hertha Ayrton, an English physicist and militant suffragette. She had won a Royal Society medal for her groundbreaking research into electric lighting, but was turned down for membership of the Society on the grounds that she was married. Defiantly, Ayrton told a journalist that she did "not agree with sex being brought into science at all. The idea of 'woman and science' is completely irrelevant. Either a woman is a good scientist, or she is not." Brave words, but are they any truer now than then?

These days, the principle of gender equality is widely enshrined in law, but the problems of unequal numbers and unequal salaries remain unresolved. When I started writing *A Lab of One's Own: Science and Suffrage in the First World War*, I wanted it to be more than just a book about long-gone events. My fundamental aim as a science historian is to analyze how the past led to the present—and the whole point of doing that is to improve the future. Through exploring the setbacks and successes of my grandmothers' generation, I came to understand more about my own experiences in science. Armed with those insights, I hoped to ensure better opportunities for up-and-coming female scientists.

Researching those wonderful suffrage pioneers made me appreciate their effects on my life. Deep inside an experimental trench dug in the grounds of London University, the chemist Martha Whiteley headed a seven-woman team researching tear gas and explosives—and after the War was over, she continued to fight against reactionary scientists, insisting on equal terms regardless of gender. Suffrage leader Ray Strachey's passion was mathematics, but she gave it up to campaign for the vote and train women for scientific work. The first female science lecturer at the University of Manchester was Marie Stopes; she later transformed millions of lives with her practical advice about birth control. And Mabel Elliott opened up opportunities for women in the Civil Service when she foiled a German spy ring by heating a letter to reveal secret messages written in lemon juice.

Blatant discrimination may have disappeared in the intervening decades, but there are other ways of making women feel like outsiders. My teachers encouraged me to aim as high as Curie, but modern shops and toys are color-coded pink and blue, while the only two-time female Nobel laureate is caricaturized as a resolutely unsentimental woman working long, lonely hours without concern for food or comfort. As an Oxford physics student, I was not surprised to discover that I was outnumbered by men 25 to 1, but the concealed prejudice was harder to bear. Even now, university reading lists include few articles by women, and female portraits are absent from corridor walls. No wonder schoolgirls feel ambivalent about a scientific career.

During the War, women proved that they could carry out—or even do better—the scientific jobs normally reserved for men. Strachey declared in 1927: "The establishment of equality of pay and opportunity for women may lie far ahead in the future; but that it does lie there is beyond question." To reward her confidence, the scientists of today must continue fighting to make her prophecy come true. ■



Oxford University Press, March 2018

Patricia Fara is President of the British Society for the History of Science and a Fellow of Clare College, Cambridge. Read an excerpt of A Lab of One's Own *at the-scientist.com.*

Barrier Function of Endothelial Cells

When endothelial cell monolayers are grown in tissue culture, ECIS (Electric Cell-substrate Impedance Sensing) can be used to electrically probe changes in the paracellular pathways between the cells (red arrows in the diagram). ECIS has been used to monitor the effects of molecules effecting barrier function including VEGF, thrombin, TNF alpha, histamine and sphingosine-1-phosphate.

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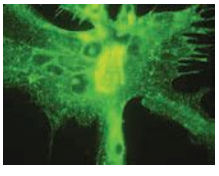
Herpes Virus Monoclonal Antibodies



- Herpes viruses produce a number of diagnostically significant antigens including glycoproteins D and G
- Recent ViroStat antibody release includes new monoclonal antibodies to HSV glycoproteins D and G
- Provide useful tools for developing rapid assays to detect these viral antigens
- Data sheets of these new antibodies are available on the ViroStat website

ViroStat
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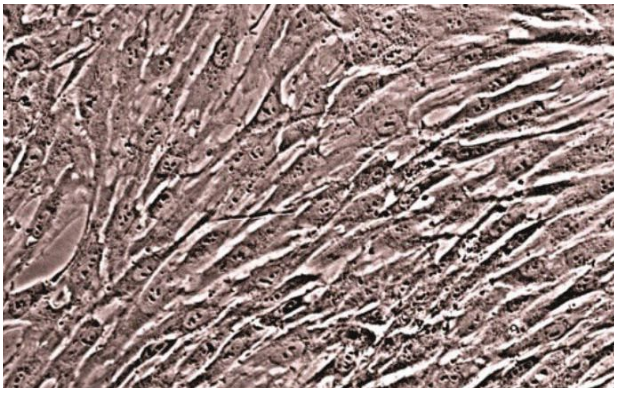
Lonza's Mesenchymal Stem Cells for Gene Therapy & Drug Discovery



Bone marrow contains a population of rare progenitor cells known as mesenchymal stem cells (MSC), which are capable of differentiating into multiple cell types, including bone, cartilage, and fat. MSCs play a critical role in many research applications, such as gene therapy, transplantation, cell differentiation and gene regulation, as well as in cell-based screening assays for drug discovery.

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Cold Spring Harbor Laboratory 2018 Meetings & Courses Program

photo: course picnic at CSHL beach

Meetings

Systems Biology: Global Regulation of Gene Expression
March 20 - 23

The PARP Family & ADP-ribosylation
April 3 - 6

Neuronal Circuits
April 11 - 14

Protein Homeostasis in Health & Disease
April 17 - 21

Gene Expression and Signaling in the Immune System
April 24 - 28

Nuclear Organization & Function
May 1 - 5

Biology & Genomics of Social Insects
May 5 - 8

The Biology of Genomes
May 8 - 12

Regulatory & Non-Coding RNAs
May 15 - 19

Retroviruses
May 21 - 26

Brains & Behavior: Order & Disorder in the Nervous System
May 30 - June 4

Glia in Health & Disease
July 19 - 23

Mechanisms & Models of Cancer
August 14 - 18

Genome Engineering: The CRISPR/Cas Revolution
August 22 - 25

Single Biomolecules
August 28 - September 1

Translational Control
September 4 - 8

Epigenetics & Chromatin
September 11 - 15

Molecular Mechanisms of Neuronal Connectivity
September 25 - 29

Mechanisms of Aging
October 1 - 5

Germ Cells
October 9 - 13

Nutrient Signaling
October 25 - 28

Transposable Elements
November 1 - 4

Probabilistic Modeling in Genomics
November 4 - 7

Biological Data Science
November 7 - 10

Neurodegenerative Diseases: Biology & Therapeutics
November 28 - December 1

web: meetings.cshl.edu

Courses

Advanced Bacterial Genetics
June 5 - 25

Ion Channels in Synaptic and Neural Circuit Physiology
June 5 - 25

Workshop on Schizophrenia & Related Disorders
June 6 - 13

Mouse Development, Stem Cells & Cancer
June 6 - 25

Metabolomics
June 9 - 25

Statistical Methods for Functional Genomics
June 29 - July 12

Advanced Techniques in Molecular Neuroscience
June 29 - July 14

Single Cell Analysis
June 29 - July 14

Drosophila Neurobiology: Genes, Circuits & Behavior
June 29 - July 19

Frontiers & Techniques in Plant Science
June 29 - July 19

Computational Neuroscience: Vision
July 9 - 22

Synthetic Biology
July 24 - August 6

Chromatin, Epigenetics and Gene Expression
July 24 - August 12

Imaging Structure & Function in the Nervous System
July 24 - August 13

Yeast Genetics & Genomics
July 24 - August 13

Cellular Biology of Addiction (in UK)
July 29 - August 5

Genetics & Neurobiology of Language
July 30 - August 5

Brain Tumors
August 7 - 13

Proteomics
August 7 - 21

Programming for Biology
October 15 - 30

X-Ray Methods in Structural Biology
October 15 - 30

Advanced Sequencing Technologies & Applications
November 6 - 18

Computational Genomics
November 28 - December 5

The Genome Access Course
March 26 – 28 & September 23 - 25

Professional Development: Scientific Writing Retreat November 14 - 18

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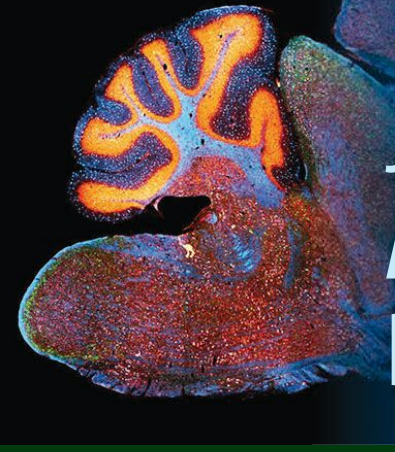

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Li Gan, Gladstone Institutes and University of California, San Francisco, USA
Leonard Petrucelli, Mayo Clinic Jacksonville, USA
Morgan H. Sheng, Genentech, Inc., USA

Joint with the conference on ***New Frontiers in Neuroinflammation: What Happens When CNS and Periphery Meet?***

As the world population ages, neurodegenerative diseases are becoming the new epidemic in both developed and developing countries. Progress made in human genetics has revealed increasingly more disease-associated genes for Alzheimer's disease (AD), Parkinson's disease (PD), Frontotemoral dementia (FTD), amyotrophic lateral sclerosis (ALS) and other neurodegenerative disorders. These discoveries have linked neurodegenerative diseases with several common key biological processes that have so far been under-studied, including RNA metabolism, protein trafficking and innate immune responses. By focusing on these emerging areas in neurodegenerative disease research, this conference seeks to challenge the current research paradigms and to inspire in-depth discussion and exploration. The program assembles leaders from various fields to facilitate interactions between groups using diverse approaches, by providing a platform to cross-fertilize ideas and to encourage new collaborations that could lead to novel mechanisms and therapeutic targets against neurodegenerative diseases.

Session Topics:
- Genetics to Epigenetics in Neurodegenerative Diseases
- Aging in Health and Disease
- Innate Immunity
- Neuronal and Network Dysfunction in Neurodegeneration
- Protein Trafficking and Degradation
- Disease Modeling
- RNA Metabolisms and New Mechanisms of Toxicity
- Biomarkers and Therapeutics

Abstract Deadline (for Short Talk consideration): March 15, 2018
Discounted Registration Deadline: April 18, 2018

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CONFIRMED SPEAKERS
(as of February 6, 2018):
Adriano M. Aguzzi
Rita Balice-Gordon
C. Frank Bennett
Baris Bingol
Guojun Bu
Ana Maria Cuervo
Bart De Strooper
Andrew G. Dillin
Li Gan
Aaron D. Gitler
Christopher K. Glass
Alison Goate
Christian Haass
John Hardy
David M. Holtzman
Yadong Huang
Bradley T. Hyman
William J. Jagust
Zayd M. Khaliq
Albert R. La Spada
Leonard Petrucelli
Morgan H. Sheng
J. Paul Taylor
Leslie M. Thompson
Li-Huei Tsai
Tony Wyss-Coray
Huaxi Xu

Submitting an abstract is an excellent opportunity to gain exposure for your work. Abstracts submitted by the abstract deadline will also be considered for short talks on the program.

Upper image of mouse brain with the neurodegenerative disease Niemann-Pick disease courtesy of I. Williams, National Institute of Child Health and Human Development, National Institutes of Health

Meeting Hashtag: #KSneurodegen

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The Child Hatchery, 1896

BY CATHERINE OFFORD

In the 1880s, premature birth was mostly a mother's issue. Some hospitals used simple technology, inspired by chicken incubators and heated by hot water bottles, to keep premature babies warm. And studies conducted by the makeshift incubators' creator, Stéphane Tarnier, showed that such care reduced infant mortality. Still, most hospitals focused "just as much on breastfeeding and maternal care to save the baby," says Jeffrey Baker, a medical historian at Duke University .

French physician Alexandre Lion took a different approach. In 1889, he patented an incubator in which temperature was regulated by a thermostat—"a tricky technology" back then, says Baker—and ventilation was provided by an electric fan. Rather than extra support, Lion's incubator was "almost an artificial mother."

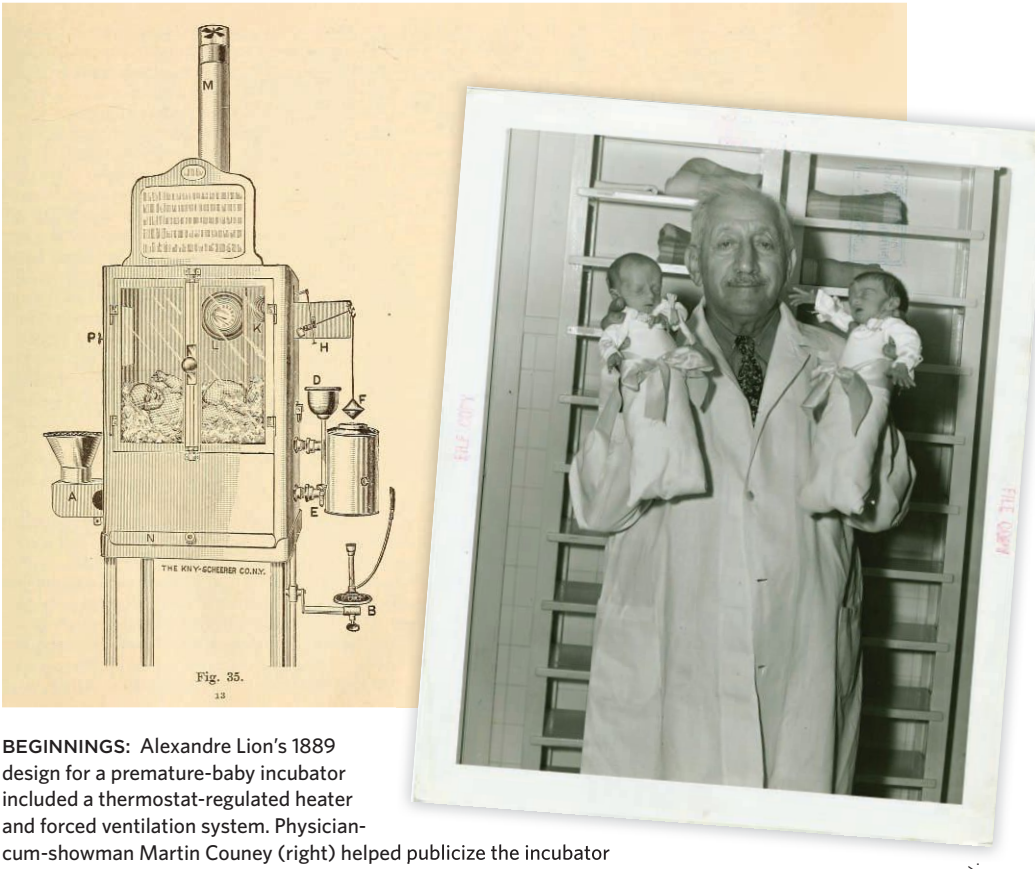
But such newfangled technology wasn't cheap. And this was in a time when hospitals by and large functioned like charities. Lion, however, had an unusual solution. To popularize the invention, he organized a wildly successful *Kinderbrutenstalt*, or "child hatchery," at the Berlin Exposition of 1896. An article in London's *The Strand Magazine* reported that in two months, more than 100,000 people viewed Lion's show, which consisted of nurses caring for six incubated premature infants, and called it the exposition's "most attractive exhibit." The article included photos of babies "saved" by the incubator intervention, though unlike Tarnier, Lion didn't conduct research comparing infant survival rates between babies who used his device and those subjected to lower-tech treatments.

Incubator shows quickly became a popular storefront attraction in Paris, with ticket sales funding incubator installation and maintenance. Interest also rose across the Atlantic; in 1901, one of Lion's associates, physician Martin Couney, held a show at the Pan-American Exposition in Buffalo, New York—part of what Couney would later refer to as "propaganda for the proper care of preemies." A handful of the devices were installed in maternity wards from Chicago to New York.

Yet excitement didn't last. Incubators remained inaccessibly expensive, and the growing popularity of eugenics in the early 20th century ran counter to the idea of taking measures to save premature infants, then still known as "weaklings." Several exhibition mishaps in the early 1900s—including a gastroenteritis epidemic in Louisiana and a fire at Coney Island—didn't help.

It would be decades before incubators would make a comeback in US hospitals following World War II—only to fall out of favor again in the 1970s, and then return with oxygen provision and other features by the end of the century, Baker says. "It's a reminder that technology does not evolve on a line of progress."

Now, with access to modern neonatal intensive care units, babies born as early as 23 weeks of gestation have a 50 percent chance of survival. In Lion's time, many if not most babies born under 36 weeks died. While he published no research about infant care, Lion retains a legacy as a "popularizer of the idea that premature babies were medically treatable," says Baker. "That was not a given before." ■



BEGINNINGS: Alexandre Lion's 1889 design for a premature-baby incubator included a thermostat-regulated heater and forced ventilation system. Physician-cum-showman Martin Couney (right) helped publicize the incubator technologies designed by Lion and others for premature infant care. However, some of his shows in amusement parks at Coney Island and elsewhere caused him to be associated by many with exploitative "freak shows"—an image he spent most of his life trying to shake.

L. FISCHER, *INFANT-FEEDING IN ITS RELATION TO HEALTH AND DISEASE*, F. A. DAVIS COMPANY, 1901; NEW YORK WORLD'S FAIR, 1939-1940, NEW YORK, N.Y.

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