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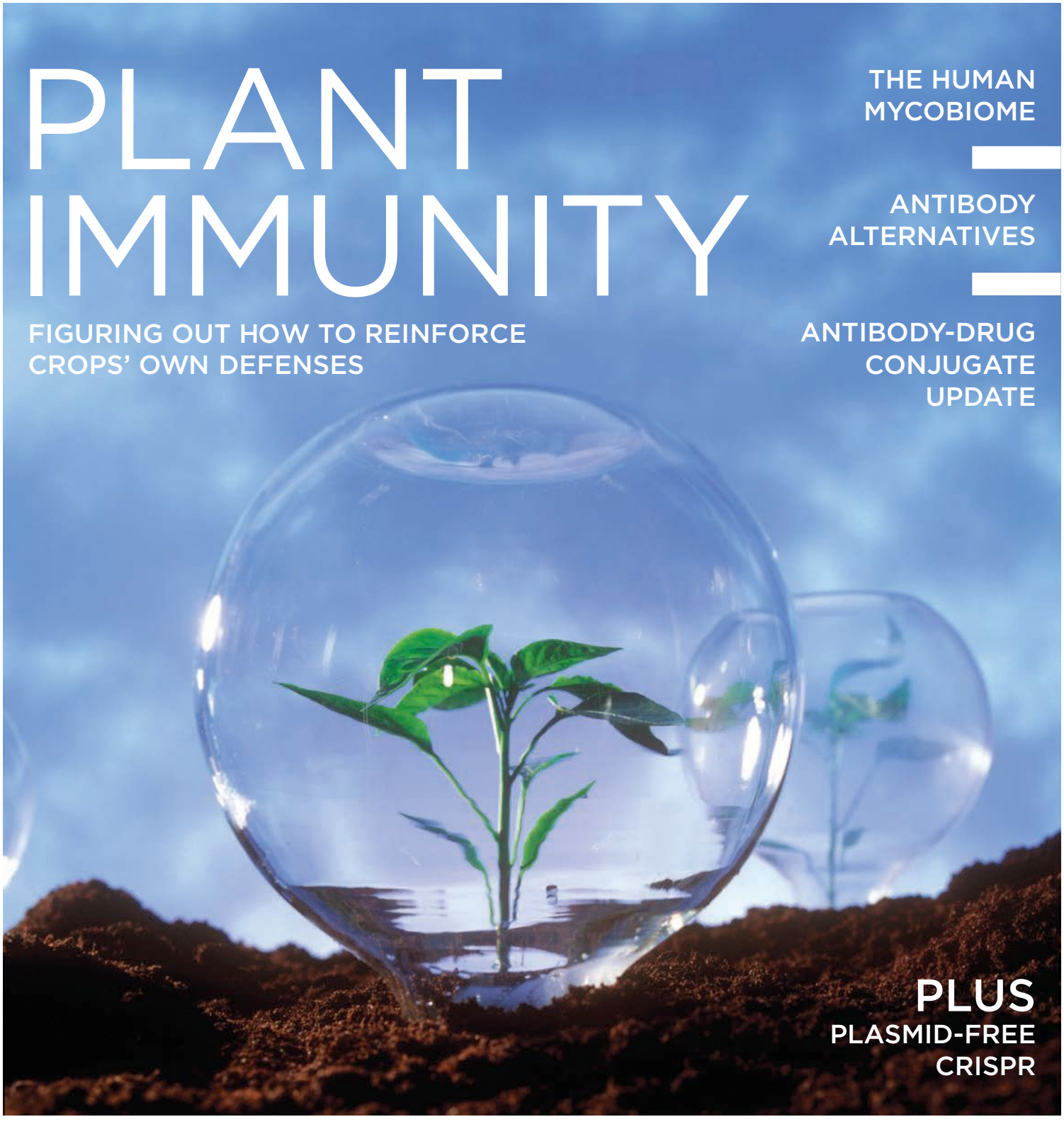
PLANT IMMUNITY

FIGURING OUT HOW TO REINFORCE CROPS' OWN DEFENSES

THE HUMAN MYCOBIOME

ANTIBODY ALTERNATIVES

ANTIBODY-DRUG CONJUGATE UPDATE



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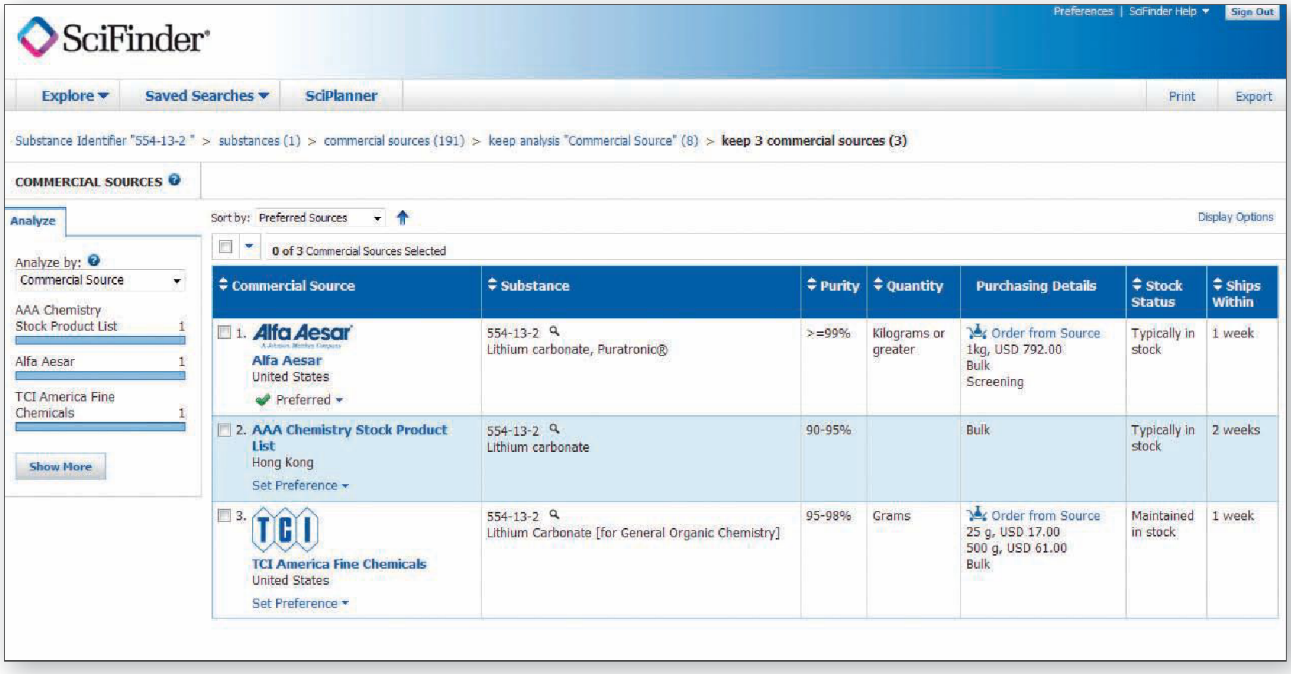

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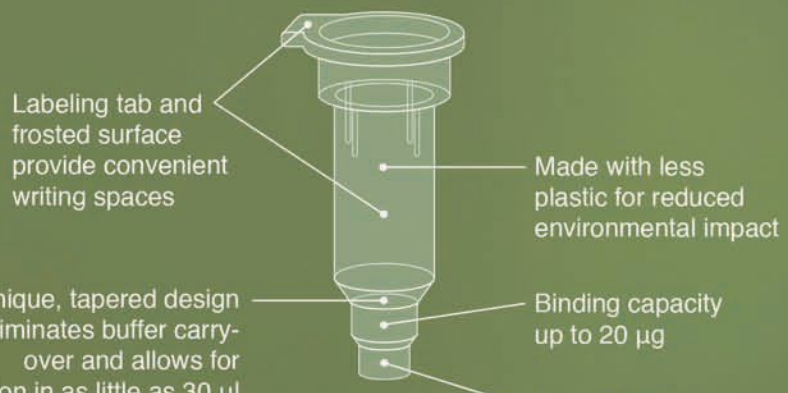

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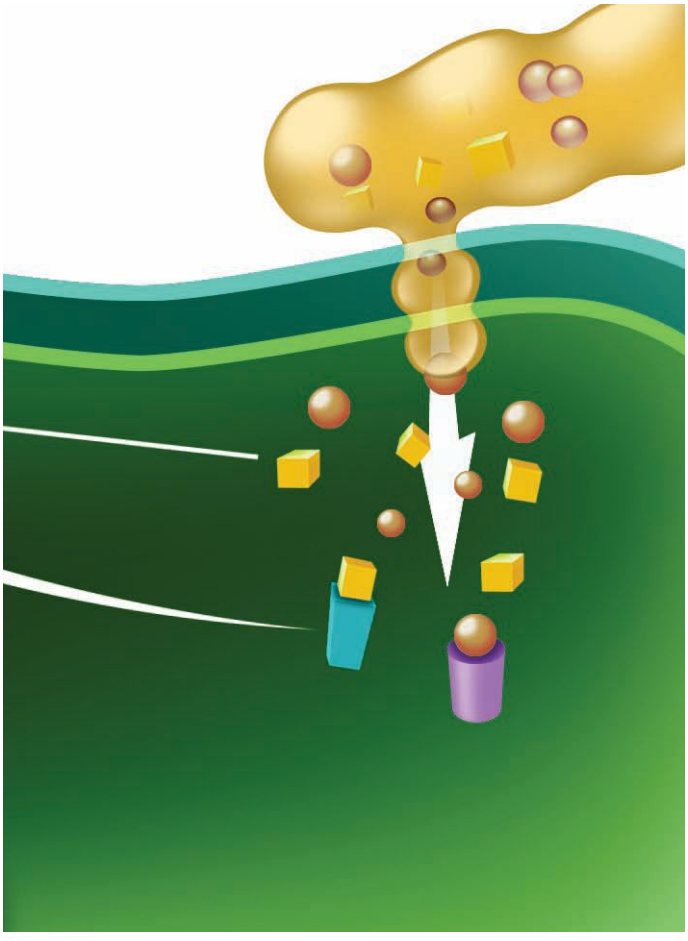

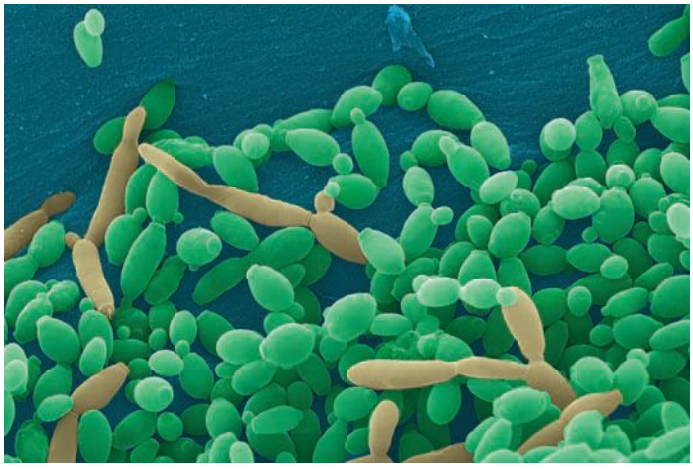

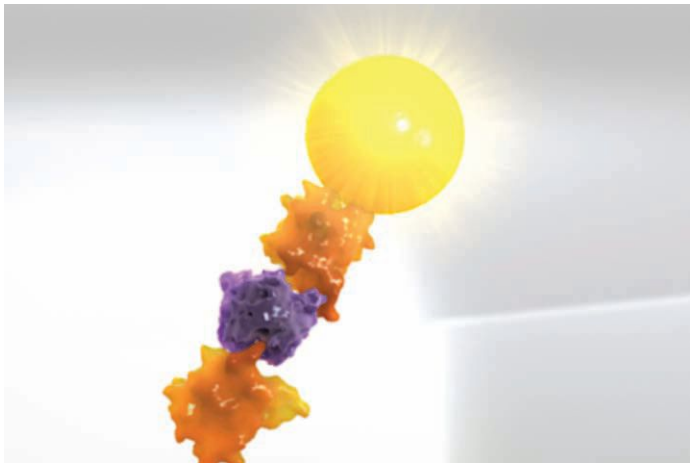

Features

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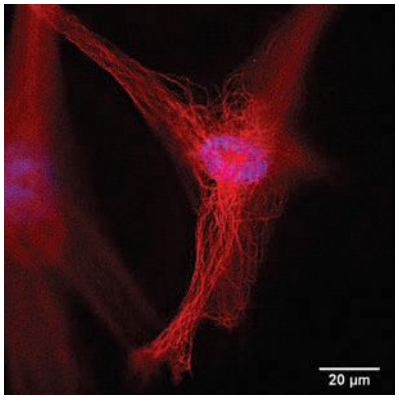

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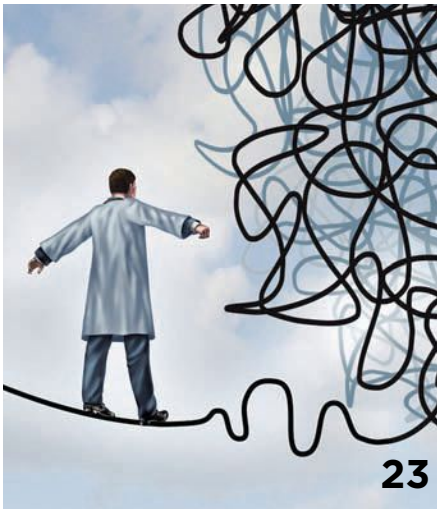

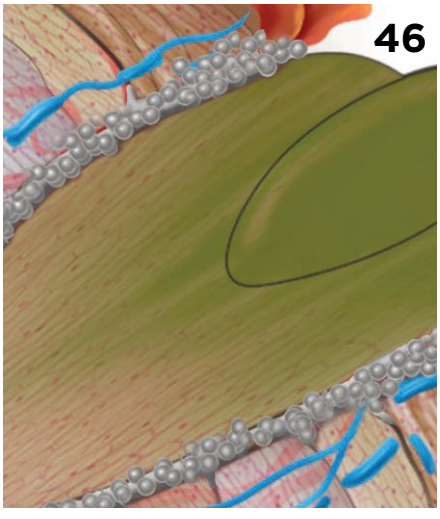

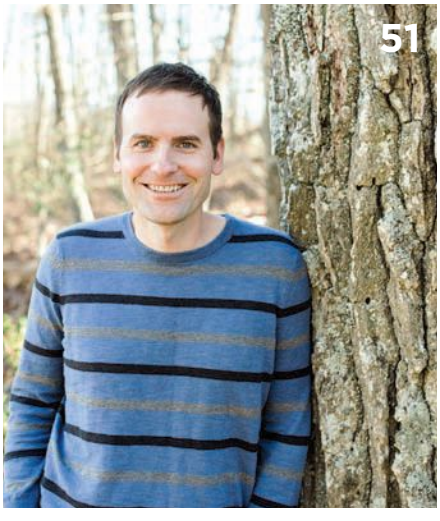

CORRECTIONS:
"Picking Up the Pace" (Bio Business, January 2016) misstated the launch of Ariad's Phase 3 trial; the company plans to launch the trial in 2016, not 2017. Also, Ariad data resubmitted to the FDA in 2015 reported 26 responders to brigatinib out of 51 patients, not 23 out of 57.

The Scientist regrets the errors.

Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
The Importance of Plant Science
Meet February profilee Natasha Raikhel and hear her explain the importance of studying plant genetics.

VIDEO
Giraffe in Half
Watch footage from the public dissection of Marius, the young giraffe at the Copenhagen Zoo who was ultimately fed to predators at the facility.

VIDEO
Chat With Charlie
See a preview of the app that lets you ask questions of a virtual Charles Darwin, played by an actor who answers in the evolutionary master's own words.

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

Coming in March

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

Focus on sleep research

- Sleep variation across the animal kingdom
- The role of neural networks in sleep
- The consequences of sleep deprivation
- Animal models for studying sleep biology
- Insomnia therapies

AND MUCH MORE

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Contributors

The investigative nature of lab work drew **Jane McLeod** into science. She earned her PhD in biochemistry and molecular biology from the University of York in 2007 and went on to a postdoc at the University of Sheffield, but then started questioning her next steps. "It takes a long time to get one result, and then you have to pace yourself for another," McLeod reflects. "While it's really thrilling when you do get that one result, it's so few and far between." She landed a writing job with a public-health policy project at the University of Sheffield in 2012, and now relies on her academic background to inform her work as a science writer for Avacta Life Sciences. "I feel like, handily, I've just fallen on my feet into something that I actually enjoy," she says.

The first draft of the human genome inspired **Paul Ko Ferrigno**, then a postdoc at the Dana-Farber Cancer Institute in Boston, to work on developing a technology that would allow researchers to examine proteins with the same level of detail and speed as they could investigate genes. When a colleague introduced him to peptide aptamers, "I realized they could be the solution to this problem" with some refinement, he says. In 2001, Ko Ferrigno set up his own lab at the Medical Research Council Cancer Cell Unit in the U.K., where he developed the Affimer protein scaffold. After transferring to the University of Leeds in 2007, he was able to commercialize the technology, founding the company Aptuscan in 2008. Avacta Life Sciences, where Ko Ferrigno now serves as chief scientific officer, bought the startup in 2011.

McLeod and Ko Ferrigno discuss the role of nucleic acid and protein-scaffold aptamers as substitutes for antibodies in "Antibody Alternatives" on page 38.

As a master's student studying the fungus *Candida* at Loughborough University of Technology in the U.K., **Mahmoud Ghannoum** didn't know he was entering the field in which he would spend the next 40 years. "I wasn't sure I would be invested in it for my entire career," he says of mycology. "As it happened, I fell in love with it." Shortly after earning his PhD from Loughborough in 1978, Ghannoum joined the faculty of the Department of Botany and Microbiology at Kuwait University, where he researched methods for determining the pathogenicity of fungi. In 1991 he moved to the University of California, Los Angeles, School of Medicine, first as a research scientist and later as a professor, before moving in 1996 to Case Western Reserve University in Cleveland, Ohio, where he is the current director of the Center for Medical Mycology. In recent years, Ghannoum's research interests have broadened to include the biology of fungal communities living in and on healthy humans. Coining the term "mycobiome," he published the first characterization of the oral fungi of healthy individuals in 2010, and now investigates fungal communities living in the human gut. Ghannoum's feature (page 32) discusses this largely overlooked component of the human microbiome.

Jo Marchant originally aspired to a career devoted to research. Her scientific interests were fostered by a patent agent father who, like Marchant's mother, had a background in chemistry. In the late 1980s, her father represented The Wellcome Foundation in a patent dispute/antitrust case involving a genetically engineered heart-attack treatment marketed by Genentech. "He had Jim Watson as an expert witness in the court case," Marchant recounts. "It just all sounded so glamorous."

Enchanted with the promise of genetics, Marchant studied genetics and medical microbiology at Saint Bartholomew's Hospital Medical College in London, earning a PhD in the late 1990s. But she favored the writing process more than the actual conduct of science. "I was one of those few PhD students who actually enjoyed writing my thesis much more than doing the research," she says. "I found it quite a lonely thing, in a way, doing science and working at the bench."

So Marchant got a master's degree in science communication from Imperial College London in 2000. Her subsequent writing career has been marked by a wandering fascination with particle physics, archaeology, the history of science, and, of course, genetics. Her latest book, *Cure*, considers the science of mind over body, and in an essay on page 62 Marchant explores some of the intriguing clinical results that point to a truth that lies somewhere in between conventional dogma and new-age mysticism.

JAMES TOWLER; AVACTA LIFE SCIENCES; MAHMOUD GHANNOUM; GARRY SIMPSON

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TheScientist

Fighting Back

Plants can't run away from attackers, so they've evolved unique immune defenses to protect themselves.

BY MARY BETH ABERLIN

Botany has never been my strong suit, but this month's focus on plant biology reminded me, yet again, what botanists already know: plants are amazing. Darwin, whose birthday is this month, described himself in an 1846 letter to the great botanist Joseph Hooker as "a man who hardly knows a daisy from a Dandelion." But Darwin wrote a number of books about plants in his continuing effort to understand and test his conceptions about some of the more difficult predictions of evolution, including a book titled *The Power of Movement in Plants*. Published in 1880 to scant interest, the book never enjoyed much popularity, and after his death in 1882 was not reprinted for 84 years.

Although they employ various methods of pollination and seed dispersal to propagate their offspring, most terrestrial plants are securely rooted to one spot. They can't run away from bacterial or fungal attackers, and thus have developed a two-pronged immunological system in order to stand up for themselves. Read all about it in "Holding Their Ground" on page 26. Some plants have even recruited a mobile defense in the form of friendly fungi that migrate to the site of pathogen attack (page 46).

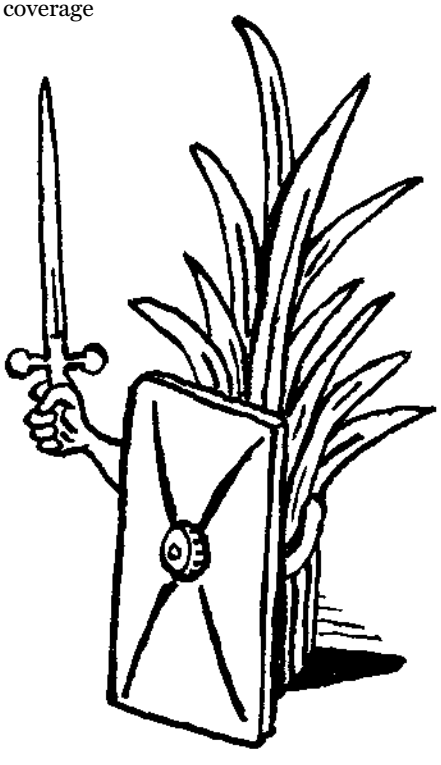
A mnemonic popular with gardeners also touches on content in this issue: "Plant peas on President's Day." Or, as some would have it, St. Patrick's Day. Exactly when you perform this earliest spring gardening task obviously depends on where you live, and it has been suggested that Gregor Mendel may have adhered to a similar dictum in the Brünn monastery garden where he did his famous pea-crossing experiments. This year marks the 150th anniversary of Mendel's "Experiments in Plant Hybridization," another publication that aroused little to no interest for decades before resurfacing to worldwide recognition. In a Foundations article (page 68), *TS* intern Karen Zusi covers the debate that raged in 1902 about Mendel's newly rediscovered data, its veracity, and what it really showed about the inheritance of traits.

Other plant biology topics covered in articles in the issue include the editing of plant genomes using CRISPR but no plasmids (page 25); a pollen rehydration mechanism (page 47); and profiles of two plant biologists (pages 48 and 51).

From plant immune defenses and defenders/detractors of Mendel, we shift to the second focus in this issue: antibodies. Famous as they are for battling pathogen attacks in vertebrates, our objective was to examine antibodies as research tools and therapeutic vehicles—their validation, their use in drug delivery, and their eventual sharing of the stage in research and therapeutics with molecules that bypass some of their disadvantages.

Over the last few years we've covered the problems arising from the lack of reproducibility of research results, and a lot of the blame seems to lie squarely on the antibodies used to study all sorts of interactions. "Exercises for Your Abs" (page 55) reports on validation procedures and techniques to ensure that your chosen antibody is actually targeting the protein you are studying. "Marriages of Opportunity" (page 52) updates our coverage of advances in drug delivery using antibody-drug conjugates. And the pros and cons of using nucleic acid aptamers and protein scaffolds as antibody alternatives is the subject of a feature (page 38) by Avacta Life Sciences writer Jane McLeod and CSO Paul Ko Ferrigno, who codeveloped and patented engineered protein scaffolds called "affimers" that the company sells.

In March we will be roaring in with a special issue all about sleep research. Rest up; we hope the content will keep you burning the midnight oil. ■



ANDRZEJ KRAUZE

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Speaking of Science

Today, farm production has stopped growing in the United States, and agriculture research is no longer a priority; it constitutes only 2 percent of federal research and development spending. . . . We need another ambitious surge in agricultural science.

—Nobel Prize-winning geneticist **Phillip A. Sharp** and **Alan Leshner**, CEO of the American Association for the Advancement of Science, in a *New York Times* opinion piece on the need to invest in agroscience (January 4)

If we look closely enough, nothing we do in the lab will ever be precisely the same as nature. The question should be whether what we have created in the lab allows us to do something beneficial that nature does not. In other words, can we now do something we want to do in a way that we could not with what nature provided? That is the heart of a life science invention.

—University of California, Hastings College of the Law professor **Robin Feldman** and **Vern Norviel**, a partner at Wilson Sonsini Goodrich & Rosati, writing in the *Yale Journal of Law and Technology* about changing the definition of what constitutes patentable life-science discoveries (January 8)

Each defecation event, which excretes about 1/3 of the colonic bacterial content, may flip the ratio to favor human cells over bacteria. This anecdote serves to highlight that some variation in the ratio of bacterial to human cells occurs not only across individual humans but also over the course of the day.

—Weizmann Institute of Science researchers **Ron Sender**, **Shai Fuchs**, and **Ron Milo**, writing in a *bioaRxiv* paper suggesting a revised estimate for the ratio of the number of human to bacterial cells in healthy people (January 6)

If you take all journals and rank them according to prestige, the most prestigious journals publish the least reliable science (at least when you look at the available evidence from experimental fields).

—**Björn Brembs**, a neurogenetics researcher at the University of Regensburg in Germany, in an email to *Vox* about the link between a scientific journal's clout and its propensity to publish iffy papers (January 11)

THE SEEDS OF SCIENCE: Agricultural science has taken a back seat to biomedical research in the U.S.

Maternal kissing of boo-boos confers no benefit on children with minor traumatic injuries compared to both no intervention and sham kissing. In fact, children in the maternal kissing group were significantly more distressed at 5 minutes than were children in the no intervention group. The practice of maternal kissing of boo-boos is not supported by the evidence and we recommend a moratorium on the practice.

—The Study of Maternal and Child Kissing (SMACK) Working Group, authors of a satirical study published in the *Journal of Evaluation in Clinical Practice* designed to highlight the limitations of evidence-based medicine (December 29)

We call for public debates in which the U.S. presidential and congressional candidates share their views on the issues of science and technology policy, health and medicine, and the environment.

— From an online petition being circulated by the nonprofit **ScienceDebate.org**, which seeks to encourage candidates to address science research and innovation issues on the campaign trail

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Notebook

FEBRUARY 2016

COPENHAGEN ZOO

HOOFING IT: A lion cub at the Copenhagen Zoo munches on a portion of Marius, the young giraffe culled and dissected at the facility in 2014.

Giraffe Diplomacy

In 2014, the Copenhagen Zoo grabbed the world's attention by killing and publicly dissecting Marius, a healthy 18-month-old giraffe. Today, scientists still invoke Marius when talking about public engagement in the social media age, the role of zoos in society, and the complicated choices involved in animal management. Aarhus University's Cathrine Sauer was at the zoo when Marius was put down, dissected, and fed to lions. But she wasn't one of the hundreds of spectators. She was collecting data for her doctorate on the digestive function of giraffes.

Sauer, an animal nutritionist, defended her PhD thesis in November and has published several papers on the anatomy, digestive patterns, and intestinal microbiomes of giraffes in the wild and in zoos. "We found that overall, giraffes are like other browsing ruminants," says Sauer. "They have a smaller omasum [third stomach] than grass-eating grazing ruminants. That correlates with feeding type since giraffes browse—eat foliage—in the wild."

But Sauer says we have much to learn about the physiology of animals such as giraffes. "Cows, goats, and sheep are well studied, but we don't know much about the digestive system of exotic ruminants," she says. "We need basic knowledge to better understand how to feed them."

Tobias Wang also studied Marius for Aarhus University's Danish Cardiovascular Giraffe Research Programme. "Because giraffes are the tallest living animal on earth, they have twice the blood pressure of other mammals," says Wang, zoophysiology professor at Aarhus and scientific adviser for the Copenhagen Zoo. "But they don't get the problems of humans with high blood pressure like heart enlargement, kidney failure, or edema in the legs. We're interested in knowing why."

Sauer's research was unique because of its scope. Opportunities to measure organs from exotic ruminants are rare, so similar studies on their digestive tracts typically include fewer than 10 animals. Sauer used data from 38 wild-caught or zoo-raised

giraffes. This allowed her to do statistical analyses and detect significant variations from expectations. For example, she says, "we found that giraffes have smaller salivary glands than predicted, compared to other browsing ruminants. We're asking ourselves the consequences of that. Maybe they don't tolerate the tannins in browse as well as other species?"

The finding that some parameters deviate from expectations, such as salivary gland size, supports the need for large, comparative studies with comprehensive measurements. "The take-home message," says Sauer, "is if you only examine digestive tracts, you have to evaluate more than a few parameters to predict the feeding ecology of an exotic ruminant." Her work also highlights the fundamental difficulty of conducting basic research on exotic animals. Randomized trials with multiple control groups are impossible, so Sauer collected measurements opportunistically, when wild or zoo giraffes became available.

Cows, goats, and sheep are well studied, but we don't know much about the digestive system of exotic ruminants.

—Cathrine Sauer, Aarhus University

Marius was one such animal. As he matured, his father became increasingly aggressive toward him. Marius's genetic background was already represented in the European zoo giraffe population with close relatives at other zoos, so he was removed from the group and euthanized before a public necropsy. The media attention attracted some protesters, "and lots of people to watch," says Sauer. "We'd done public dissections before, but never with so much fuss." The kids who attended were fascinated, she says, and "asked clever questions."

COPENHAGEN ZOO

GIRAFFE SNACK: One of the Copenhagen Zoo's lions, which were allowed to dine on the body of Marius, the young giraffe dissected at the facility in 2014

Mads Bertelsen, a veterinarian at the zoo's Center for Zoo and Wild Animal Health and an affiliate professor at the University of Copenhagen, explains the justification for the public event: "Most people see zoos as entertainment," he says, "but we see ourselves as also doing education, research, and conservation." Feeding Marius to the carnivores ensured that the entire animal was used, he says.

"That's what it's like in nature," adds Sauer. "Most Danes probably feel the same way." Still, she says, the experience taught her the importance of media training and considering how news and images spread. "I've never met anyone who didn't understand, if you sat and talked with them about what you are doing and why," she says. "But that conversation takes time. On social media, you have only a few lines, so you can't have that conversation."

The Marius controversy wasn't all bad, contends Cheryl Asa, director of the Association of Zoos and Aquariums (AZA) Reproductive Management Center, headquartered at the Saint Louis Zoo. "It's been a chance to raise people's consciousness about the complexity of managing zoos and conserving genetic diversity," she says. The Marius affair also revealed a philosophical divide among zoos: some control breeding using contraception, while others allow natural breeding and cull animals later. The Copenhagen Zoo mainly lets animals mate and raise young, removing offspring when they would normally leave the group and euthanizing them if necessary for space or genetic diversity reasons. In US zoos, however, "the general approach is not to use euthanasia for management," says Asa, stressing the safety of animal contraception. That said, "no one from the zoo community who spoke to me about the incident was critical of the Copenhagen Zoo's euthanasia policies," she adds.

Breeding policy is an evolving discussion as zoos come together to coordinate internationally on conservation, says Bertelsen. "For some species," he says, "all the zoos in Europe, and now sometimes the world, are considered one population with a species coordinator planning breeding to maintain long-term diversity." The coordination of global breeding programs is making zoos more collaborative, says Asa, and talking about policy differences will be good for the field. Even if zoos don't change their practices, "at least they'll have considered the alternatives," she says. In the meantime, Danish zoos continue to use public dissections to educate the public about animal anatomy. In October, the Odense Zoo, 170 kilometers west of Copenhagen, created its own media splash by publicly dissecting a 9-month-old lion.

—Chris Tachibana

iDarwin

"My name is Charles Robert Darwin," the bearded man on my phone's screen says to me. "I think I am most famous for establishing without a doubt that species change over time, and that they do this mostly by a process I discovered that I call natural selection, and that species are related to each other by a long history of common descent."

The man speaking to me is not Charles Darwin, obviously, but he does look like him—right down to the hairdo. He's got the distinctive hairless pate, and the dark gray hair on the sides of his head transitions smoothly into mutton-chop sideburns. He's dressed in typical 19th-century British attire—a black suit with a white shirt, paired with a matching maroon vest and ascot—and he's standing in a digital replica of Darwin's office at Down House. He even *sounds* like the pioneering evolutionary thinker. The lines spoken by actor Randy Kovitz come largely from letters and manuscripts Darwin himself wrote, and Kovitz studied recordings of the historical dialect spoken in Darwin's hometown outside London.

"We need to introduce Darwin as a man, an individual who lived a life. That's as much who he is as anything else," says Duquesne

ANDRZEJ KRAUZE

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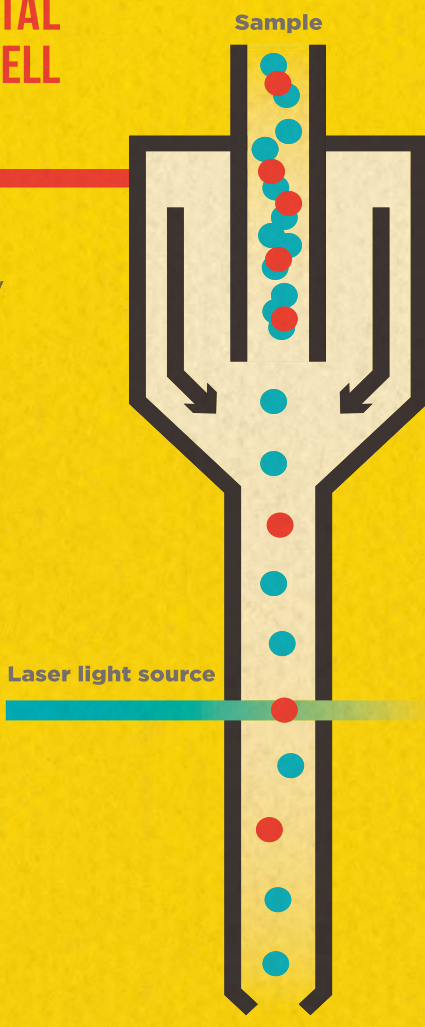

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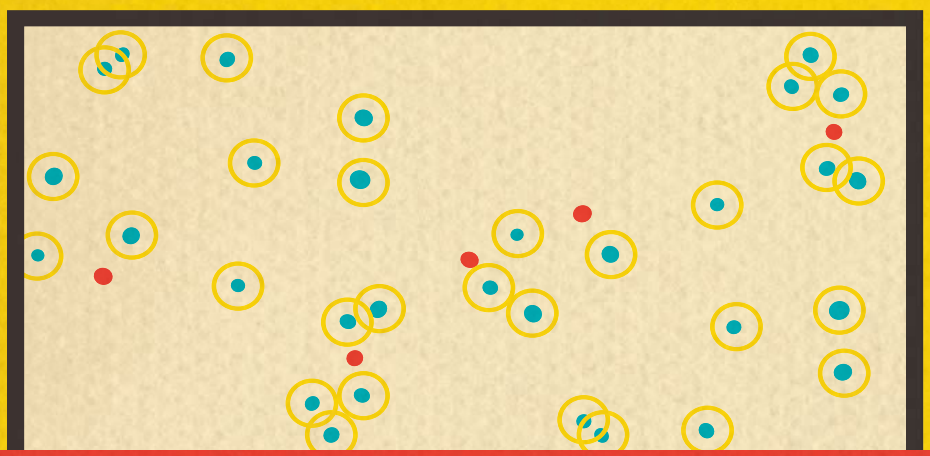

CELL ANALYSIS

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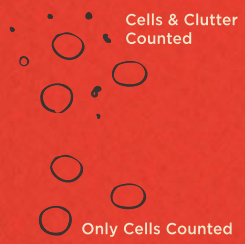

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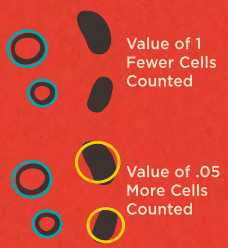

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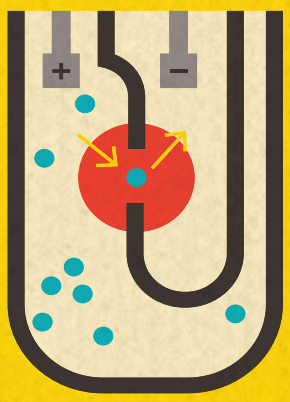

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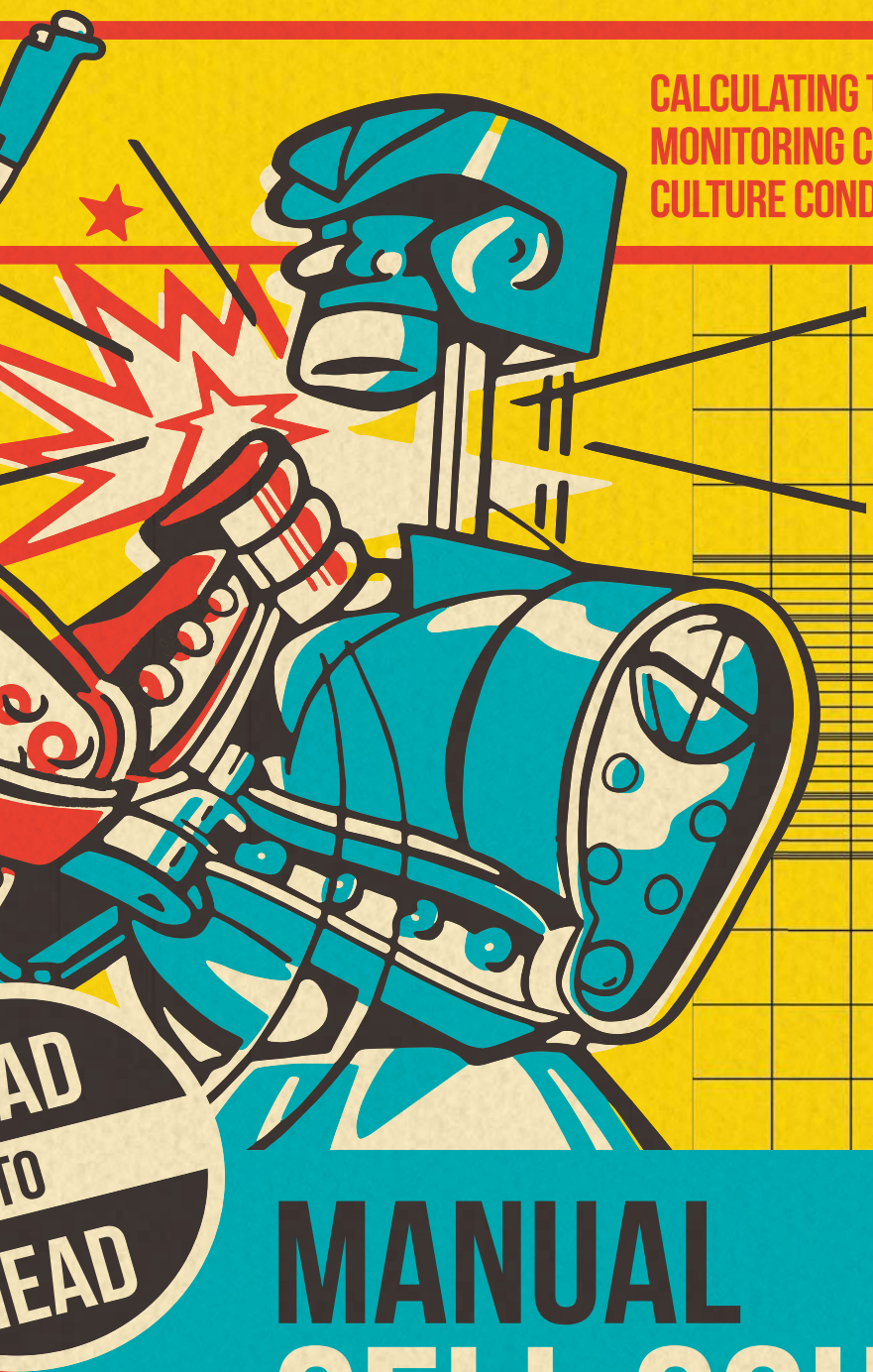

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- **Accurate • Reproducible**
- **Cell type independent, i.e., take into consideration cell property variables such as cell clumping, cell shape, and cell populations of varying size**

HEMOCYTOMETER

DIMENSIONS	AREA	VOLUME AT 0.1 MM DEPTH
1 x 1 mm	1 mm^2	0.0001 mL
0.25 x 0.25 mm	0.0625 mm^2	0.00000625 mL
0.20 x 0.20 mm	0.04 mm^2	0.000004 mL
0.05 x 0.05 mm	0.0025 mm^2	0.00000025 mL

1. Apply the cell suspension so that the sample fills the chamber.
   Tip: Dilute the cell suspension if necessary. Cells should be uniformly distributed without clumping or overlap on the grid.
2. Using a microscope, count the number of cells in 4 squares.
   Tip: The lower the concentration of cells, the more squares should be counted to reduce statistical errors.
   Tip: Only count cells that are within the square or on the bottom or left lines; do not count cells that touch the top or right lines of the square.
3. Calculate the density of cells in the suspension.

$$\text{Cell density (cells/mL)} = \frac{\text{(Average number of cells counted per square) X (Dilution factor)}}{\text{Volume of square (mL)}}$$

Cell viability can be calculated by treating the cell suspension with cellular dyes, such as Trypan Blue. Live cells do not take up Trypan Blue, dead cells will stain blue.

$$\text{Cell viability (\%)} = \frac{\text{Total viable cells (unstained)}}{\text{Total cells (stained + unstained cells)}} \text{ X 100}$$

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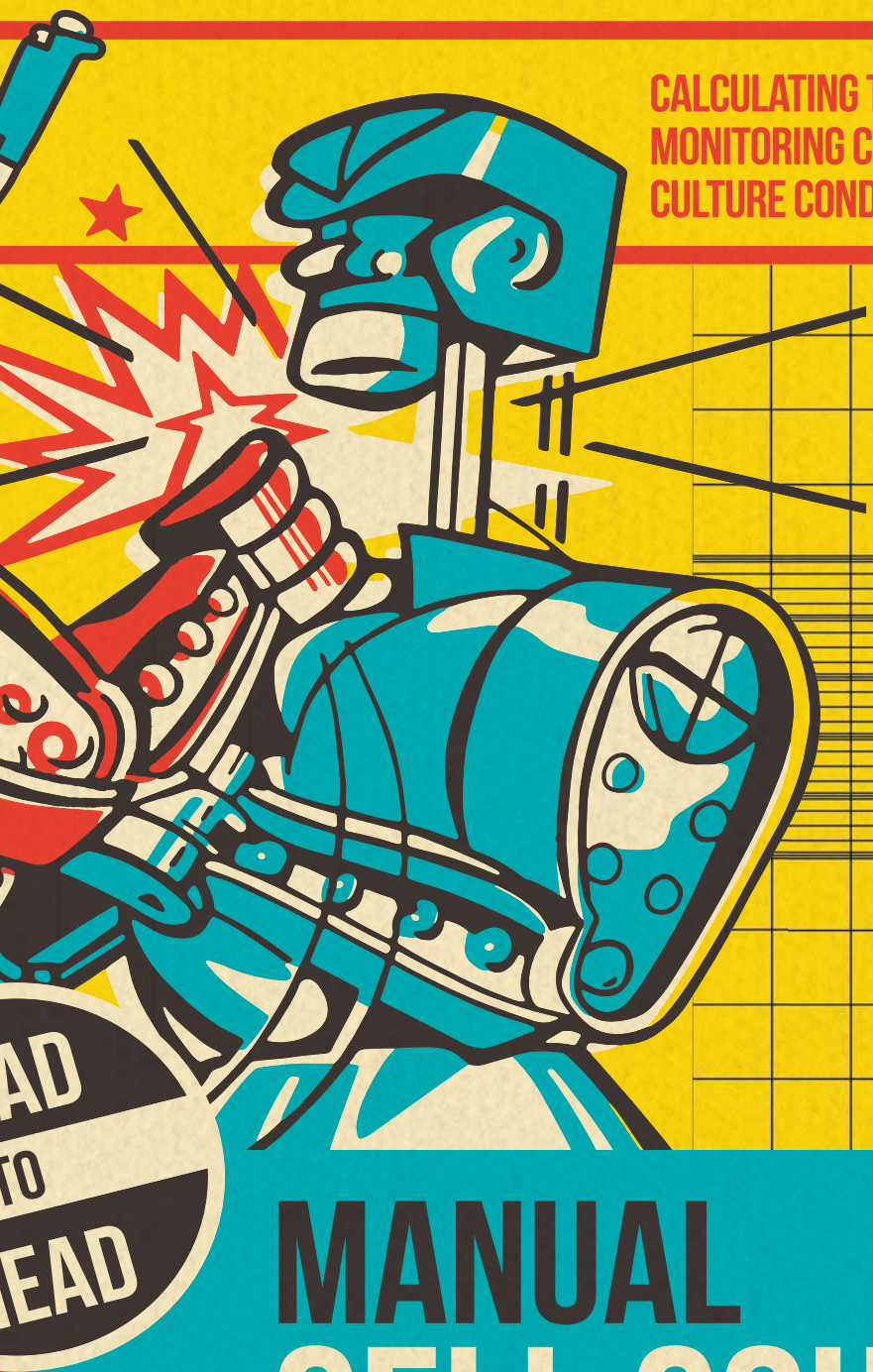

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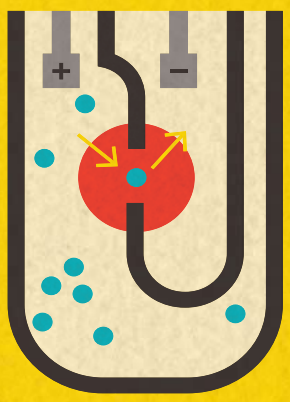

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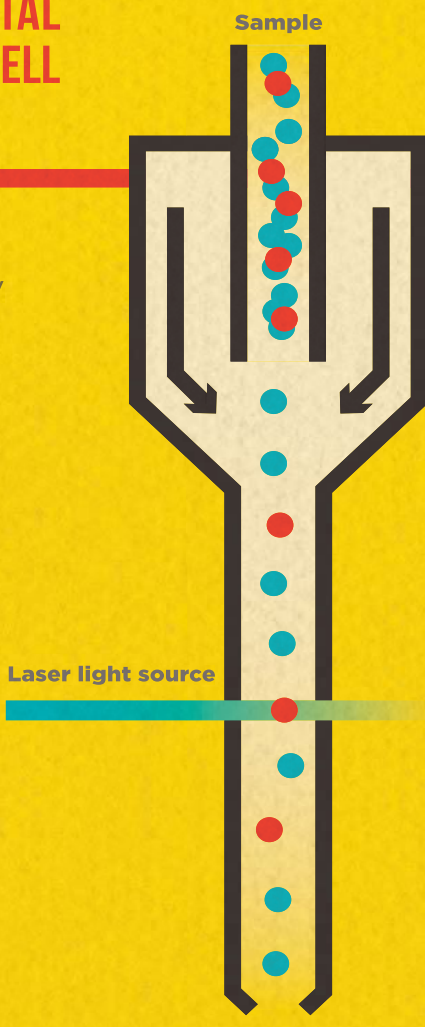

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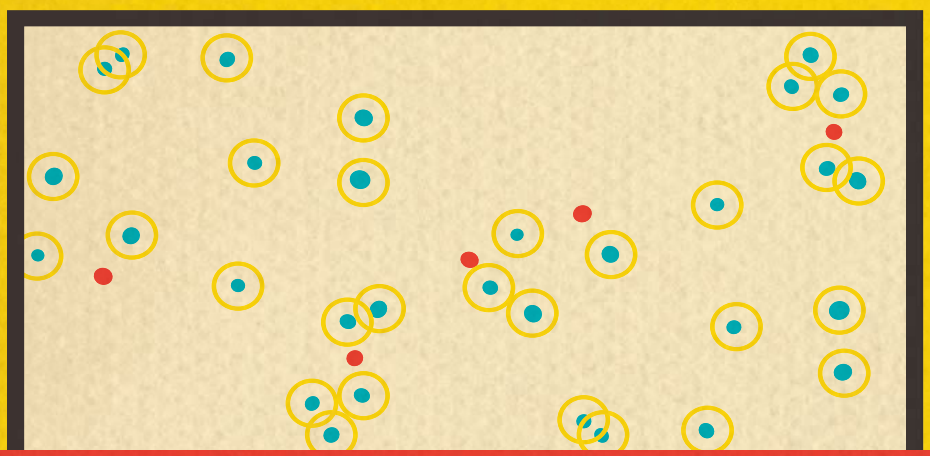

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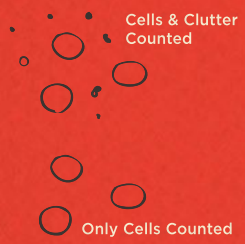

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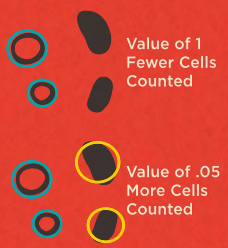

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University neuroscientist John Pollock, who has been planning this "synthetic interview" with Darwin for more than a decade.

In 2004, the Entertainment Technology Center at Carnegie Mellon University created a similar synthetic interview with Ben Franklin. "The very first day I saw Ben I was thinking we really should do this with Darwin," says Pollock.

We need to introduce Darwin as a man, an individual who lived a life. That's as much who he is as anything else.

—John Pollock, Duquesne University

Visitors could ask Franklin any question they wanted, and if they asked nonsensical questions they'd get nonsensical answers. Pollock wanted to limit users' experience to a reasonable conversation with Darwin—so he moderated their interactions. Pollock and his colleagues took to the streets of Pittsburgh to ask people what they would ask Charles Darwin. They also spoke with local teachers and kids at the Carnegie Science Center.

The exercise confirmed to Pollock that the project was worthwhile. "What saddened me and shocked me—about half the people said, 'Who are you talking about?' By not showing the picture of old man Darwin, by not saying Darwin in the context of evolution, people had no idea who we were talking about."

The researchers added a few additional questions themselves—"questions we knew people should be asking," Pollock says—and whittled the list down to 199 queries grouped into 10 categories.

Pollock's Duquesne colleague David Lampe then scripted the answers, relying heavily on Darwin's own writings. As luck would have it, the Darwin Correspondence Project had recently made more than 1,000 of Darwin's letters available online. "Because he was a prolific writer of letters and kept everything, and they had just gone online, we could answer every single question in Darwin's own words," Pollock says. Of course, he added, "Darwin was long-winded, so we had to soundbite him a little."

After the script was vetted by three Darwin historians, Pollock's team put out a call for auditions. Pittsburgh local Kovitz landed the role. The Darwin Synthetic Interview debuted on February 12, 2009—Darwin's 200th birthday and the 150th anniversary of the publication of *On the Origin of Species*—at the Carnegie Science Center and is now used in all Pittsburgh public middle schools. "[It can get] kids, or anyone really, to identify with Darwin as a person . . . which can make it more interesting and easy to learn," says science educator Kirsten Sanford, producer and host of This Week in Science podcast and radio show.

The "interview" was so popular that Pollock wanted to make it more broadly available, but at 4.5 gigabytes, the videos were not amendable to streaming. Pollock decided to enlist the help of Carnegie Mellon professor Jessica Trybus, who had worked on the Ben Franklin synthetic interview and had since spun out the company Simcoach Games, which specializes in making interactive educational programs. Trybus and her team of engineers worked to pare the software down and make it mobile-friendly, and last August the Charles Darwin Synthetic Interview was released as an app for iPhones and Androids.

"[This] will make it more available to more people on their own time," says Sanford. "Anyone can access this information and learn about Darwin." She does wish that users could craft their own questions to Darwin, though. "By forcing students to think a particular way and ask very particular questions, you're really limiting their actual interest in the process."

"There's a lot you can do with the nascent idea of synthetic interview," says Trybus. "It's a way to immerse people in living history." —Jef Akst

Keep Off the Grass

Among people who realize the threat posed by global climate change, you might be hard-pressed to find anyone arguing against planting trees. Unless you happened to be sitting in a conference room last fall listening to grassland ecologists lament a lack of social and political support for the conser-

TREELESS TREASURE: Brazilian ecologists question whether their country's historical focus on conserving the Amazon may have contributed to past neglect of the Cerrado, 1.2 million square miles of savanna grassland located in the country's central high plains and mostly fringed by forest.

BÉRIA L. RODRÍGUEZ/WIKIMEDIA COMMONS

vation of grassy biomes. Political scientist Forrest Fleischman, a Texas A&M University researcher who studies how government officials devise land-management plans, was among a handful of academics invited to speak at a Stanford University symposium on nonforest ecosystems held in November 2015. He talked about the drivers of tree-planting efforts and, by his own account, was one of the least impassioned scholars in the room.

But the grassland folks were fired up. So were the nongovernmental organization (NGO) representatives, whose interactive map of global opportunities for forest restoration—posted online in 2011 and updated in 2014—had already been lambasted in the literature. Both groups want the same thing: ecosystem restoration and preservation. So why was one accusing the other of promulgating unscientific, potentially eco-unfriendly policies?

"The people who have decision-making authority over ecosystems—government officials and international donors—have been very focused on forests," says Fleischman. "Other ecosystems are [often viewed as] 'Oh, that's something we can convert to forest,' or 'That's degraded, and something we can use for agriculture.'"

The organizations that contributed to the creation of the interactive forest-restoration map—the Washington, DC–based World Resources Institute (WRI); the International Union for Conservation of Nature (IUCN), headquartered in Gland, Switzerland; and the University of Maryland—"mean well, 100 percent," Fleischman says. "But there's a bit of a disconnect between what [NGOs are promoting and what] ecologists are telling us we should be doing, which is restoring ecosystems as opposed to planting trees."

Such "forest bias," as some grassland ecologists have come to call it, has pervaded nearly every level of environmental policymaking. Because anthropogenic deforestation is known to contribute to global climate change, funders such as the United Nations and the World Bank are encouraging tree planting, to the tune of billions of dollars annually. International programs such as the Bonn Challenge and Initiative 20 × 20 have incentivized both reforestation—planting trees where woodlands were destroyed—and afforestation—planting trees where there weren't any.

While trees help sequester anthropogenic carbon from the atmosphere, afforestation of grasslands is known to affect soil stores of carbon, phosphorus, and nitrogen over the long term, impacting root systems and underground microbial communities, among other things. It's not fully known how changes to these critical nutrients beneath the Earth's surface could impact plant life—and, therefore, carbon sequestration—aboveground.

"Tree planting in grassy systems would result in a number of changes, including ecosystem functioning, change in biodiversity, and a change in ecosystem services,

[such as] water availability," ecologist Kate Parr of the University of Liverpool, U.K., wrote in an email.

For its Atlas of Forest and Landscape Restoration Opportunities, the WRI-led team used satellite imaging data to identify approximately 23 million square kilometers of land as potential forestation targets. The problem is, ecologists have shown, more than 9 million square kilometers of that area overlap with grassy biomes.

"The tricky bit is mapping whether the open ecosystems are ancient, old-growth systems or secondary products of deforestation," says William Bond, professor emeritus at the University of Cape Town. That, he says, will require more field-level data in addition to the satellite images the WRI-led team analyzed to produce its map.

Bond, Joseph Veldman of Iowa State University, and eight other colleagues wrote in a letter published in *Science* last year that projects like the WRI-led Atlas have undervalued grassy biomes. "That such a scientifically flawed analysis is poised to promote misinformed tree planting is emblematic of deep misunderstandings about the grassy biomes, as well as their devaluation relative to forests," the authors wrote. "Deforestation and forest degradation are critical problems that must be addressed, but with due consideration of the value of the many naturally nonforest biomes that also face tremendous pressure from human-caused environmental change."

The letter, says Veldman, was meant to highlight "issues that are not unique to the WRI, IUCN, or their partnership." The map, he says, just happened to be a compelling visual representation of forest bias.

In their response, published two months later in the same journal, WRI's Lars Laestadius and his colleagues pointed out that their map was not meant to guide policy. "The map shows areas with a higher probability of finding [forestation opportunities] if you were to drill down, which we say, very specifically, you have to do," Laestadius told *The Scientist*. "[Veldman and his colleagues] say that our work has basically said that grasslands ought to be forested for climate-change reasons, or something like that, and we have never said that . . . and we certainly cannot say it with a global map."

"I think grasslands *are* being undervalued," Laestadius continues, citing a lack of global data on these biomes. But, he says, his team's efforts are not to blame. In its published reply, the WRI-led group called for ecologists "to collaborate on a more in-depth mapping of ecosystem restoration opportunities, which can incorporate new information on the world's old-growth grasslands and other important biomes as it becomes available."

The disagreement—which spilled over into a September 2015 *BioScience* paper, the symposium at Stanford, and a handful of blog posts—highlights a key issue in environmental policymaking, says Veldman: that, quite often, it outpaces the relevant science.

"Some of these political and conservation efforts, in some regards, may be trying to take actions faster than scientists can provide some better information," he says. "In some cases, I think science can make very clear recommendations. I think this is a situation in which science is suggesting we should be cautious in how we proceed, to avoid unintended consequences."

To catch up with their colleagues focused on forests, grassland ecologists have their work cut out for them. "We are beginning to identify the attributes of old-growth grasslands, particularly in the tropics and subtropics, but the studies are still few and geographically restricted and useful only at a local scale," says Bond. With additional field work, "it should be possible to use ground-based vegetation maps from the 20th century to separate deforested [areas] from ancient grasslands," he says. "The science has not been done yet linking local to remote-sensing scales."

Meanwhile, countries continue to commit to planting trees. While most forestation efforts have yet to cause substantial harm, grassland ecologists are concerned. "In Africa, the savannas are . . . our equivalent of the castles and cathedrals of Europe," says Bond. "Turning them into forests is like flattening Notre Dame for a parking lot."

—Tracy Vence

Life After Sequencing

The students in John Burton's grade 7 to 11 science classes have never lived in a world without knowledge of how the human genome is strung together. That their teacher was one of the people involved in unravelling this mystery means little to them. "I have told them, to try to generate enthusiasm, but have not gone into detail," says Burton, who mainly teaches physics.

Fifteen years ago, as a member of the International Human Genome Sequencing Consortium, Burton managed one of the high-throughput production teams at the Sanger Centre. "The Human Genome Project (HGP) was a fantastic piece of work to be involved in, and we always felt that we were helping to leave a worthwhile legacy," he says. Altogether, researchers from more than 40 institutions took part in this international effort, led by MIT's Eric Lander.

Human genomics has come a long way in 15 years, and so have the people who began the task of elucidating that sequence.

The publicly funded consortium had commercial competition from Craig Venter and his company Celera, however, and in February 2001, both groups published first drafts of the human genome sequence. The consortium's work appeared in *Nature*, and Celera's article was published in *Science* the next day. Fifteen years and thousands of citations later, how have these landmark papers affected some of the more than 500 people on the author lists?

For many, the HGP marked the beginning of a career in genomics. Aoife McLysaght was a PhD student at Trinity College Dublin at the time, looking for whole-genome duplications in animals at the base of the vertebrate evolutionary tree. Now a professor of genetics at Trin-

ity, she studies evolutionary constraints on gene dosage.

The project also motivated Andy Mungall and Bill Majoros, who were both inspired to start PhD programs after working on the sequencing efforts. Mungall enrolled in a PhD program to study genomic imprinting soon after co-leading the sequencing of chromosome 6 at the Sanger Centre. "I was keen to apply my genomics expertise to health care," says Mungall, who now works at the British Columbia Cancer Agency in Vancouver. Majoros wrote a book on gene-prediction methods after developing gene-discovery software for Celera. Now he is a PhD student at Duke University Medical Center, where he studies how genomic sequence variants affect gene splicing. "The reference genome was only the beginning," he says. "Extrapolating what we've learned about the reference genome to personal genomes is an exciting new frontier with enormous implications for health care."

Several companies based on personal genomics have popped up in recent years, such as Invitae, which carries out genetic testing for hereditary disorders. Years back, Invitae cofounder Michele Cargill contributed to the population genetics section of Celera's *Science* paper, which taught her more than just genomics. "I learned to work with people from different disciplines and learned to not avoid efforts that seem impossible."

The project also helped Kimmen Sjölander, who developed algorithms to assign genes to functional subfamilies while at Celera. "The paper in *Science* increased my visibility, allowing me to return to academia." Sjölander is now at the University of California, Berkeley, where she studies the evolutionary development of new functions and structures across protein superfamilies. She points out that we still don't really understand what all the genes in the human genome do. "We have a serious problem with the accuracy and information content of gene functional annotations. Since functional annotations are foundational to biomedical research, this problem has to be addressed."

It's a problem that extends to other organisms' genomes. A few years ago, Kevin McKernan's company Medicinal Genomics sequenced the cannabis genome, to better understand the plant's pharmaceutical potential. McKernan has been involved in genomics for years. He was team leader for R&D at the Whitehead Institute during the HGP, and afterwards started various companies focused on sequencing technologies. One of these systems was acquired by Life Technologies, where McKernan worked for several years. When he left, he was asked to sign a noncompete agreement, which led him to start working on the cannabis genome instead. McKernan is now CSO at Courtagen Life Sciences—a medical genetics testing company founded by his brothers—which acquired Medical Genomics in 2011.

External circumstances also steered the career path of Trevor Woodage. At Celera, he was involved in genome annotation and single nucleotide polymorphism (SNP) analysis, but when a move to Minnesota left him with little choice in scientific jobs, he retrained in patent law. Now, Woodage is an associate attorney at Fish & Richardson, in the Twin Cities. "I've had the opportunity to work on a number of patent litigation cases, primarily in the life sciences," he said. "I've worked on some cases that have overlapped with some of the work that I did at Celera, but more often, the cases have involved drug development."

Neha Desai (née Garg) made a big career change as well. "I was especially interested in ensuring children have no fear of math." She worked on template preparation and marketing at Celera, but now runs a Mathnasium franchise in Arlington, Virginia, offering math tutoring to kids. "One of my biggest goals is to see more children go into STEM fields, especially girls."

Human genomics has come a long way in 15 years, and so have the people who began the task of elucidating that sequence. Some are now working on annotating the genome or developing new techniques, others are educating the next generation or taking their scientific experience elsewhere, but all of them have been shaped by their contribution to science history. "Being part of such an obviously important project at such an early career stage was very exciting," says Trinity's McLysaght, "and that type of excitement motivates me still." —Eva Amsen

SEQUENCING PAST AND PRESENT: Sequencers that were used for the human genome project at the Sanger Centre (left) and the sequencers the facility uses today (right).

GENOME RESEARCH LIMITED

Scientific Literacy Redefined

Researchers could become better at engaging in public discourse by more fully considering the social and cultural contexts of their work.

BY CYNTHIA BRANDENBURG

During a Republican primary debate this past fall, some of the presidential candidates publicly perpetuated the false notion that autism is linked to vaccinations. Understanding science includes appreciating the nature of its process. Over time, science corrects itself as theories change to account for new data. But when science is communicated in the contemporary world, factual errors, misrepresentations, and misappropriations can get perpetuated through a series of shares, copies, likes, and reposts. The viral nature of today's information sharing makes a new kind of scientific literacy all the more imperative.

The same week as the debate, a colleague sent me a link to a "Science Knowledge Quiz" available through the Pew Research Center's website. Looking at the report, I was not surprised to learn that only 6 percent of respondents obtained a perfect score. But reading the results of the Pew survey more closely, along with the accompanying thoughtful analysis, I couldn't help but think about the overly simplistic way scientific literacy tends to be framed. Literacy can be broadly understood as the ability to interpret representations of information in complex and nuanced ways, accounting for the multiple meanings that result. By focusing on whether or not enough people know the "right" answers, we are missing something far more important.

Choosing the correct answer to a question from a list of multiple choices hardly indicates the kind of literacy skills necessary to critically evaluate scientific information in the context of society. It is here—at the intersection of scientists and the public—that scientific literacy (or a lack thereof) has its greatest impact, from individual choices related to personal health behaviors to wide-reaching decisions on public policy.

I am a scientist by training, but I no longer do science in a traditional sense. Instead, I teach a range of required interdisciplinary general education courses at a professionally focused college where no one majors in a basic science. As such, I put a heavy emphasis on fostering scientific literacy among my students.

It's a complex process. For example, in a course called "Scientific Revolutions," we discuss what science is, why it matters, and how it changes over time. We situate science within historical and cultural contexts and learn to appreciate science through the lenses of race, class, and gender. The rhetorical nature of scientific communication is another topic of interest. Whether we are considering the immunization misinformation, the recent approval of the first drug for female sexual dysfunction, or the political discourse around climate change, I have discovered that getting students to understand the science is relatively easy. Connecting the dots between a scientific discovery and its implications for society is where the real challenge begins.

This lesson is not just for students. Understanding science in and of itself cannot account for the complex ways in which scientific knowledge impacts the lived human experience. This is where scientists suffer their own form of scientific illiteracy. I scored perfectly on that Pew quiz, but that doesn't make me an expert when it comes to understanding science in the context of society. Intense immersion in research can be blinding when it comes to the broader social and ethical implications of scientific questions and the actual human costs involved.

Furthermore, our cultural norms may bias our science. In addition to shap-

ing how we understand the world, which drives the questions we ask and the experiments we conduct, our experiences influence our ability to see alternatives. Think of Charles Darwin and how hard he tried to account for his Victorian-era beliefs about the lesser intellectual capabilities of women in *The Descent of Man*. His culture blinded him to the problematic premise upon which this research question was based. As neuroscience suggests, it is very difficult to see things we are not looking for.

But awareness of cultural contexts becomes arguably most important when communicating with the public about science. Resistance to science and ignorance of science, while frustrating, can often be understood within a broader framework. In the past, many traditionally nondominant cultural groups—including women, minorities, and those living in poverty—found themselves on the losing end when it came to scientific "progress" and understanding. They and others have good reason to be suspicious of new discoveries. Grappling with contexts such as these is critical if we hope to understand why objective science is understood as anything but in a subjective world. And while today's vaccine skeptics might include a presidential candidate, a recent immigrant to the country, or a concerned mother from the Pacific Northwest, any truly productive conversation about why their fears are scientifically unfounded must be predicated upon an understanding of what caused those fears in the first place.

Let us continue to work toward promoting meaningful and comprehensive scientific literacy among students to help them become informed citizens who can effectively engage in public discourse. But let us also begin to advocate for a parallel track, in which we embrace a deeper and more complex discussion about science in the real world—and recognize that this type of scientific literacy is important for those of us who work in the sciences, too. ■

Cynthia Brandenburg is an associate professor at Champlain College in Burlington, Vermont.

The Postdoc Crisis

A lack of jobs leaves postdocs without a future in academia in the United States. Meanwhile, other challenges threaten the postdoc community abroad.

BY MUHAMMAD Z. AHMED

Postdoctoral fellows play a critical role in the research productivity of any country. Currently, the United States has a relatively strong postdoc infrastructure, offering higher salaries and more benefits than most other countries. Postdocs also have support from the National Postdoctoral Association (NPA) and postdoc offices in most American universities. However, limited growth in federal research funding during the last decade has made it increasingly hard for postdocs to find permanent jobs. The limited funding has also created a highly competitive environment for those who do find positions as principal investigators (PIs). Under constant pressure to produce high-impact papers and secure large grants, many PIs no longer invest adequate time and attention in the development of their postdocs, treating them instead as a skilled labor force.

Conversely, research and development (R&D) funds have increased nearly tenfold in China over that same period, and the number of postdocs has risen with it. But postdoc salaries and benefits remain low. According to a recent survey, most Chinese scientists felt that they received insufficient mentoring during their progression from PhD to postdoc to independent researcher in China, and there is no organized infrastructure to deal with challenges postdocs face there. If the lack of investment in postdocs continues, both the U.S. and China could see their reputations as research powerhouses diminished, which would only serve to exacerbate the postdoc problem.

My colleagues and I recently surveyed the postdoc community, the amount of federal R&D funds, and the annual number of publications in both the U.S. and China (*BioScience,* 65:1088-95, 2015).

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Between 1993 and 2012, China exhibited exceptional growth in all three of these areas: the total number of domestic and international postdocs increased substantially each year; government funding rose at a rate of approximately 18.7 percent per year, resulting in an increase of approximately 2,273 percent (from $7.08 billion to $168.2 billion) over the 20-year period examined; and the number of publications has increased fourfold. In the U.S., on the other hand, the number of postdocs has not increased; federal funding has decreased approximately 0.2 percent per year, resulting in a total decline of 4.7 percent between 1993 and 2012; and the annual publication output has remained relatively constant. The relative difference in publication trends held true even when examining only those papers published in the high-impact journals *Science* and *Nature*.

There is no doubt that these three factors—postdocs, federal funding, and publications—are correlated. There is a stark difference between China's upward trends and the U.S.'s neutral and slightly downward trends, which raises an interesting question: Could increased funding for postdocs spur greater scientific productivity in the U.S.?

In a survey conducted at Harvard Medical School, 70–97 percent of papers published between 1990 and 1999 from select high-profile labs, including 43 percent of papers published in *Science,* had a postdoc as the first author (*Science,* 285:1531-32, 1999). This trend, which continues in the U.S., indicates that postdocs remain one of the most productive groups in research. In addition to publications, postdocs bring new research ideas, mentor graduate and undergraduate students, and help PIs write grants.

ONLINE FIRST

One trend we noted in our recent study is the significant movement of young researchers from China to the U.S. for their postdoctoral training. One half of current postdocs in the United States are non-US citizens with temporary visas. Only 11 percent of China's PhD recipients continue their research as postdocs at Chinese institutions (*Nature,* 452:1028-29, 2008); many head to the United States. Some Chinese researchers choose to expatriate even earlier in their training; the number of Chinese graduate students in the U.S. more than tripled from 1987 to 2010. China is now the largest source of foreign science doctoral graduates in the U.S. China's notoriously low stipends are pushing talented young researchers toward postdoctoral opportunities abroad, and the widespread use of Mandarin has created a language barrier that often deters international postdocs from accepting positions in China. On the other hand, international postdocs are often attracted to the U.S. because they believe it provides a better opportunity than their home countries for advancing their research career. The U.S. is able to offer higher postdoc salaries and greater benefits, and the country's cultural heterogeneity makes integrating into the new environment much easier for international postdocs.

However, postdocs in the U.S. are facing increasing uncertainty of landing a permanent job. Instead of creating job openings for permanent staff scientists, PIs and some funding agencies tend to favor hiring postdocs, partly due to postdocs' relatively low salaries and high research productivity. A recent estimate showed that only around 15 percent of U.S. postdocs secure tenure-track jobs, while another survey found that the unemployment rate after completing a postdoctoral fellowship has more than doubled from four percent in 2008 to 10 percent in 2012.

As a result, postdocs are staying in these low-paying positions much longer. While a typical postdoc position used to average just one to two years, many young researchers today remain postdocs for upwards of three to five years (*Nature,* 452:1028-29, 2008; *Nature,* 471:578, 2011). Others are becoming discouraged by the prospect of a life in academia and choosing to take positions in industry, or to abandon primary research altogether.

In order to maintain their high output of top-tier research, both the U.S. and China must enact significant changes. China must direct more funds toward increasing postdoc stipends and improving the quality of research training, and the U.S. needs an influx of financial support, along with maintenance of current postdoc standards. In addition, both governments should offer specialized counseling for postdocs and open all postdoc fellowship programs to national and international researchers.

Changes must also occur at the university level. Academic institutions should teach students about the academic career path, collaborative research, and time and laboratory management; revise the selection criteria for incoming graduate students to ensure that only the most talented candidates enroll; and conduct surveys to determine which types of students tend to perform better in industry or academic settings, then redesign graduate programs accordingly. Additionally, if institutions are unwilling or unable to create new tenure-track faculty positions, they should create additional permanent staff research positions.

Recent trends in the U.S. and China are keeping postdocs undervalued and unemployed. This ultimately will drive bright minds away from research, and limit or cease altogether our advancements in science. My coauthors and I believe that acting on these recommendations will help to raise standards for the global postdoc community and the scientific community as a whole. ■

Muhammad Z. Ahmed is a postdoctoral associate in the Tropical Research and Educational Center at the University of Florida's Institute of Food and Agricultural Sciences. He formerly held postdoc positions at the University of Pretoria in South Africa and at South China Agricultural University in Guangzhou, China.

Gene Editing Without Foreign DNA

Scientists perform plant-genome modifications on crops without using plasmids.

BY RUTH WILLIAMS

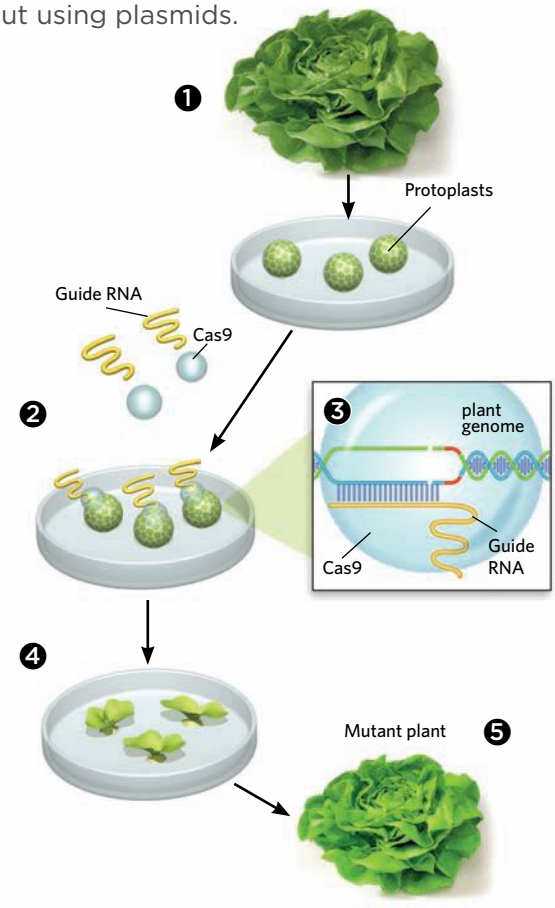
The ongoing quest to increase the yield of crops and produce varieties resistant to disease, drought, and pests has been aided by the development of gene-editing technologies. These days, probably the most commonly used gene-editing approach in labs is the CRISPR/Cas9 system, in which a guide RNA—specially designed to match part of the sequence of a target gene—positions the Cas9 nuclease at that gene, enabling it to chop the DNA.

To date, researchers have been using DNA plasmids, both naked and inside infecting bacteria, to transfer Cas9 and guide RNAs into plant tissues and cells. However, says geneticist Jen Sheen of Harvard Medical School, this approach runs the risk of creating additional mutations—either from the integration of the plasmid itself into the plant genome, or from the persistence of the encoded gene-editing factors, which can "continue to make mutations."

Sunghwa Choe of Seoul National University and colleagues have therefore devised a technique that avoids the use of plasmids altogether. They preassemble the Cas9 protein and guide RNA complex in vitro and then mix the complex with polyethylene glycol, which allows direct transfer by endocytosis into protoplasts—plant cells that have had their cell walls removed.

The edited protoplasts can then be cultured into small clumps of plant tissue called calli, from which a mature plant can be regenerated. Choe and colleagues have created genetically modified lettuce plants using this approach and have also edited genes in the protoplasts of three other species.

Avoiding the use of plasmids should not only prevent any additional unwanted DNA damage, but might also allow the genetically modified plants to skip regulatory oversight, explains Choe. (*Nature Biotechnol*, 33:1162-64, 2015)



PLASMID-FREE EDITING: To mutate a specific gene of interest in a plant (lettuce shown here), scientists first grow protoplasts—plant cells lacking a cell wall ❶. Preassembled CRISPR complexes, including a tailor-made stretch of guide RNA and the nuclease Cas9, are introduced into the protoplasts ❷. The complex homes in on the target gene and cuts the DNA at a locus specified by the guide RNA ❸. Protoplasts are then grown in clumps called calli ❹, which are regenerated into a mature, genetically modified specimen ❺.

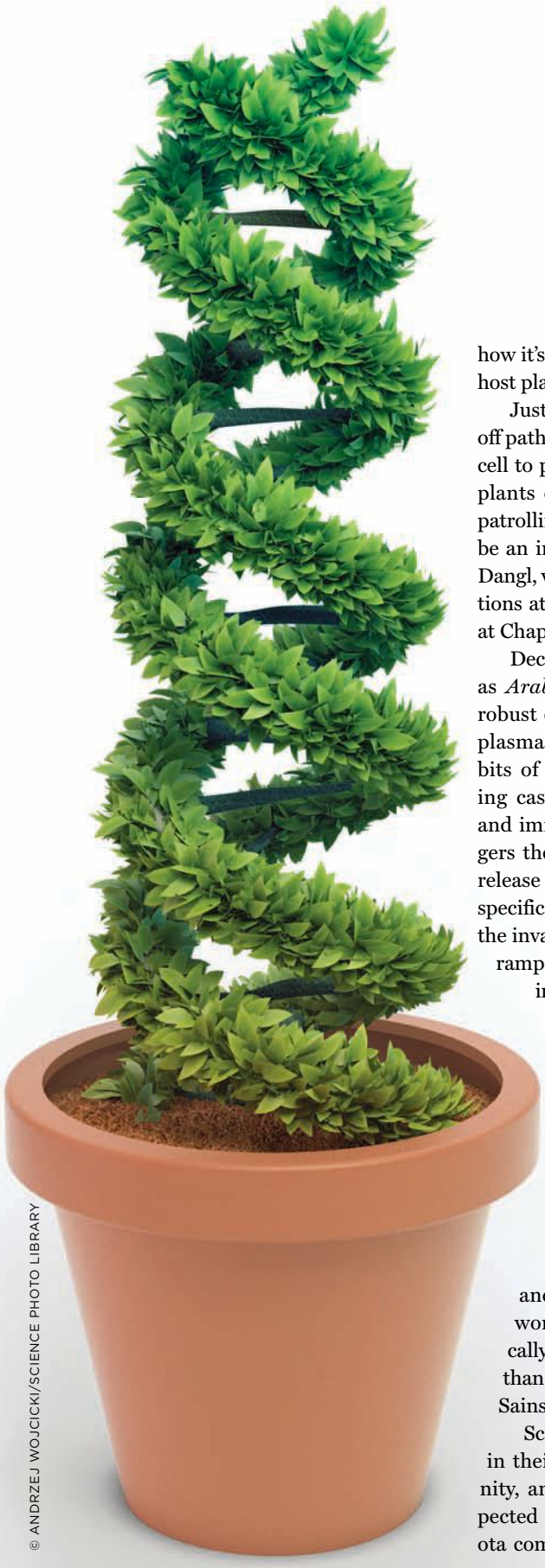
AT A GLANCE

PLANT GENOME EDITING APPROACH	METHOD OF TRANSFER	OFF-TARGET MUTATIONS	EDITING EFFICIENCY (MUTATION FREQUENCY)	SUITABILITY FOR SEXUAL AND ASEXUAL CROPS
Agrobacterium tumefaciens–mediated transfer of plasmid (T-DNA) encoding Cas9 and guide RNA	*A. tumefaciens* bacteria carrying the plasmid infect plant tissue.	Possible: plasmid DNA integrates randomly into plant genome. Also, persistence of Cas9 and guide RNA genes may lead to additional off-target edits.	Approximately 2 percent (*Nature Biotechol,* 31:691-93, 2013)	Sexually reproducing crops, such as wheat, rice, and maize, can be crossbred to remove the T-DNA. Asexual crops, such as grapes, potatoes, and bananas, will retain the T-DNA.
Transfection of preassembled Cas9/guide RNA complex	Assembled particles are transferred into protoplasts using solvents.	None detected. Once complex degrades, there's no chance of unwanted edits.	Up to 46 percent	Suitable for sexual and asexual crops alike

To protect the global food supply, scientists want to understand—and enhance—plants' natural resistance to pathogens.

BY AMANDA B. KEENER

Plant pathologist Jean Ristaino hunts down crop-threatening diseases all over the world. Last year, in the span of two months, she visited India, Uganda, and Taiwan to help colleagues track the fungus *Phytophthora infestans*, which infects tomatoes and potatoes and caused numerous famines in 19th-century Europe. Ristaino tracks the pathogen's modern march using farmers' online reports of outbreaks of the disease, called late blight; then she travels to those locations to collect fungal samples. In her lab at North Carolina State University in Raleigh, Ristaino's team genotypes fungi from these farms to trace their origins and monitor how *P. infestans*'s genome is changing in response to fungicide use and



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how it's subverting immune strategies the host plants use to defend themselves.

Just like animals, plants have to fight off pathogens looking for an unsuspecting cell to prey on. Unlike animals, however, plants don't have mobile immune cells patrolling for invaders. "Every cell has to be an immune-competent cell," says Jeff Dangl, who studies plant-microbe interactions at the University of North Carolina at Chapel Hill.

Decades of work on model plants such as *Arabidopsis thaliana* have revealed robust cellular immune pathways. First, plasma membrane receptors recognize bits of pathogen and kick-start signaling cascades that alter hormone levels and immune-gene expression. This triggers the cell to reinforce its wall and to release reactive oxygen species and nonspecific antimicrobial compounds to fight the invaders. These responses can also be ramped up and prolonged by a second immune pathway, which can lead to localized plant cell death. Some plant defense compounds even manipulate bacterial communication. The polyphenol rosmarinic acid, for example, was recently found to disrupt a quorum-sensing pathway that *Psuedomonas aeriginosa* uses to form biofilms.[1] The molecular details of these and other pathways have yet to be worked out, however. "Mechanistically, it's still rather opaque," says Jonathan Jones, a plant immunologist at the Sainsbury Laboratory in Norwich, U.K.

Scientists are now filling in the gaps in their understanding of plant immunity, and discovering previously unsuspected roles for factors such as microbiota composition and circadian rhythms. If they can understand a plant's defenses, maybe they can engineer more-robust crops, introducing immune genes that may have been inadvertently bred out of modern varieties. Some are also looking to alter known immune receptors so that plants can recognize pathogens despite adaptations that help the invaders fly under the immune radar. Collectively, these strategies could help plant breeders keep up with economically devastating pathogens like *P. infestans*.

One-two punch

A plant's first line of defense is recognizing pathogen-associated molecular patterns (PAMPs), which may be found within proteins such as flagellin, the lipopolysaccharides of the gram-negative bacterial outer cell membranes, or the complex carbohydrates of fungal cell walls. Cell-surface pattern recognition receptors (PRRs) bind to PAMPs and activate the production of nonspecific antimicrobial compounds, such as flavonoids and alkaloids, as well as enzymes including proteases and lipases. But the PAMP response does not always go as planned, Dangl says. "Pathogens have learned ways to subvert that . . . system."

By inserting so-called effector proteins directly into a plant cell's cytoplasm, bacterial and fungal pathogens can interfere with signaling cascades downstream of PRRs, or directly target hormone pathways and transcription factors to prevent PAMP-triggered immunity. That's when the plant's second line of defense kicks into gear. The cells sense the bacterial effectors by means of other receptors, called intracellular nucleotide-binding domain, leucine-rich repeat receptors (NLRs), that trigger secondary immune cascades.

NLRs provide flexibility in the plant immune system. *Arabidopsis* only has

about 150 NLR proteins—not nearly enough to cover the wide range of potential pathogen effectors the plant may encounter. But NLRs don't just recognize pathogen effectors; many recognize plant proteins targeted by those effectors.[2] For example, the bacterium *Pseudomonas syringae* produces a protease that degrades a plant protein called RIN4, which is involved in PAMP-triggered immunity. RIN4 binds to an NLR called RPS2, so when the bacterial protease results in lowered levels of RIN4, RPS2 notices the protein's absence and initiates an alarm signal.[3,4] "If the host figures out how to recognize your action as a protease activity, then you're useless," says Dangl. By recognizing damaged proteins as "modified self," one NLR can detect the presence of many effectors, which often go after the same host targets.

In the last decade, researchers have found several examples of NLRs that operate in pairs: one binds a pathogen effector and the other mediates downstream signaling. In *Arabidopsis*, for example, the NLRs RRS1 and RPS4 work together to sense effectors from several pathogens: RRS1 binds to them, while RPS4 activates the defense response. RRS1 contains a domain that looks like a member of the WRKY transcription factor protein family—a group of major immune gene regulators in plants and the targets of several bacterial effectors.[5] Subsequent research revealed that it's common for one member of an NLR pair to contain a domain borrowed from an effector target. This led some researchers to hypothesize that these extra domains can act as decoys: the effectors bind the NLR, alerting the plant's immune system to the bacterium's presence before it can wreak too much damage. Sure enough, a bacterial effector called PopP2, which acetylates WRKYs, also acetylates the WRKY domain of RRS1 to activate RPS4-mediated immunity.[6,7]

Jones says decoy NLRs can offer a helpful shortcut for identifying the signaling proteins that link immune receptors and defense-gene activation. Any decoy domain fused with an NLR is likely to be a target of a pathogen effector, and therefore likely to be involved in plant immunity.

Cataloging plant immune genes and understanding how they work are also vital to breeding and engineering crops that can stand up to rapidly mutating pathogens. Although diverse genetically modified (GM) crops are now widely sold and consumed, the vast majority of today's growers still rely on chemical pesticides. In the U.S., farmers spend an estimated $77.1 million per year on fungicide to combat late blight alone.[8] Such treatments are often too expensive for growers in the developing world, says Ristaino. So researchers are turning to genetic methods to shore up the plants' defenses. "Host resistance [is] probably the best way to reduce losses," she says.

FIGHTING BLIGHT: Strategies to fight *Phytophthora infestans*, an oomycete that causes late blight in tomatoes and potatoes, cost US farmers tens of millions of dollars each year.

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Putting plant defense to use

The most direct way to implement knowledge of plant immune pathways in agriculture is to introduce the immune genes themselves into plants. Many wild relatives of domesticated crops still harbor so-called resistance (R) genes that defend plants against specific pathogens. Once these genes are identified, researchers can breed or engineer them into the genomes of modern fruits, vegetables, and grains.

By recognizing damaged proteins as "modified self," one NLR can detect the presence of many effectors, which often go after the same host targets.

One of the first R genes bred into crops, which codes for an NLR called R3a, came from a wild relative of the potato called *Solanum demissum*. In the early 20th century, researchers discovered that the wild potato plant was resistant to *P. infestans* and began crossing it with cultivated potato varieties to transfer that resistance into the crop.

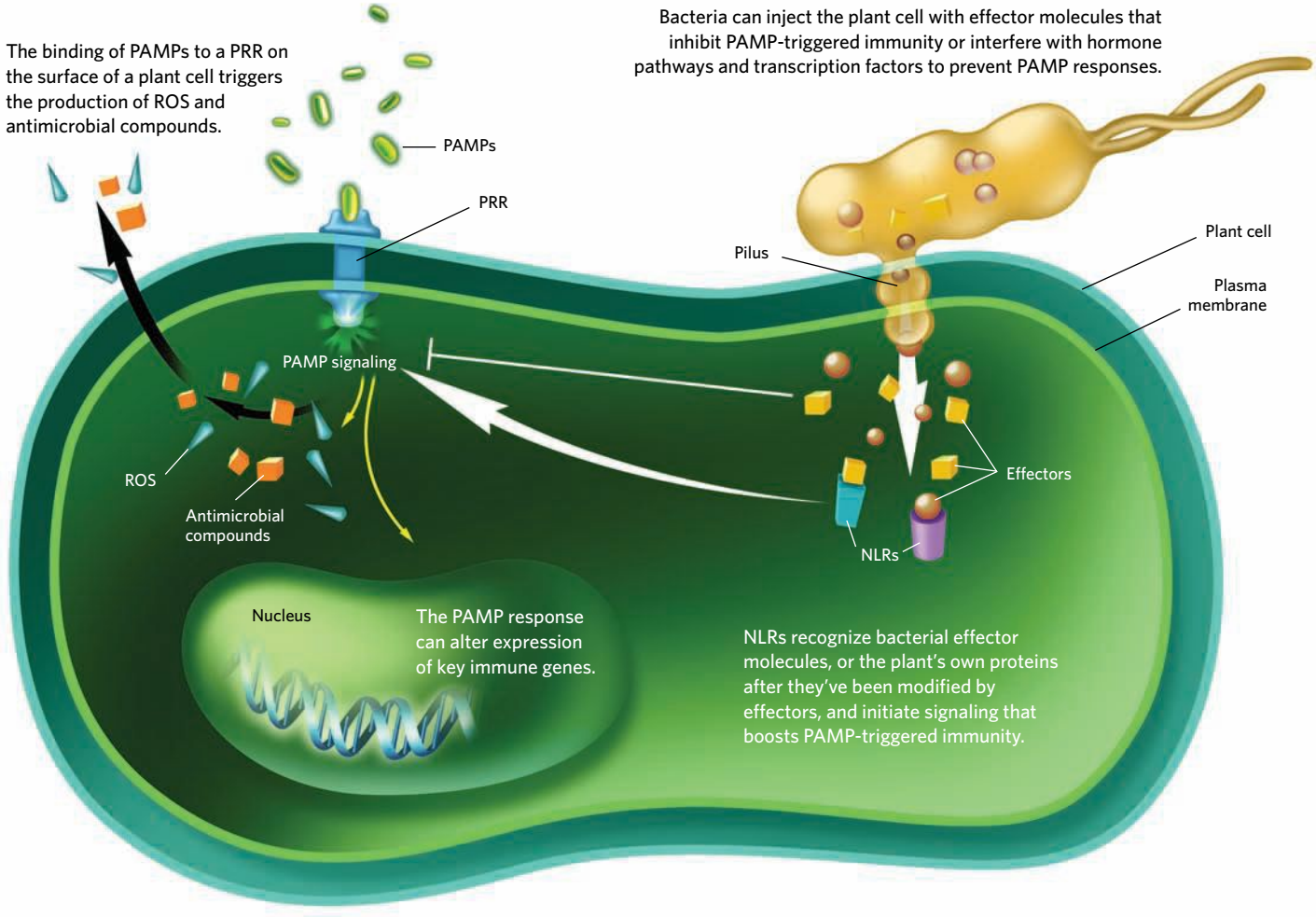
R3a recognizes a *P. infestans* effector called AVR3a, but since R3a was introduced into domestic potatoes, a fungal variant that evades R3a detection has become more prevalent. To address this issue, Sophien Kamoun of the Sainsbury Laboratory is looking to alter R3a so it can bind this stealthy effector, called AVR3a^{EM}. In 2014, his group used random mutagenesis to make a series of single amino acid changes to R3a and identified several that enabled the NLR to recognize AVR3a^{EM}.[9] The researchers also noticed that one of the mutant receptors bound an effector from a different fungal pathogen. "The really cool thing about this concept is it does open the door to engineering totally new synthetic receptors," Kamoun says.

Engineering NLRs to expand the list of effectors they can sense could be an efficient way to improve resistance to many pathogens at once. Recently, Kamoun and his colleagues applied what they learned

HOW PLANTS FIGHT OFF PATHOGENS

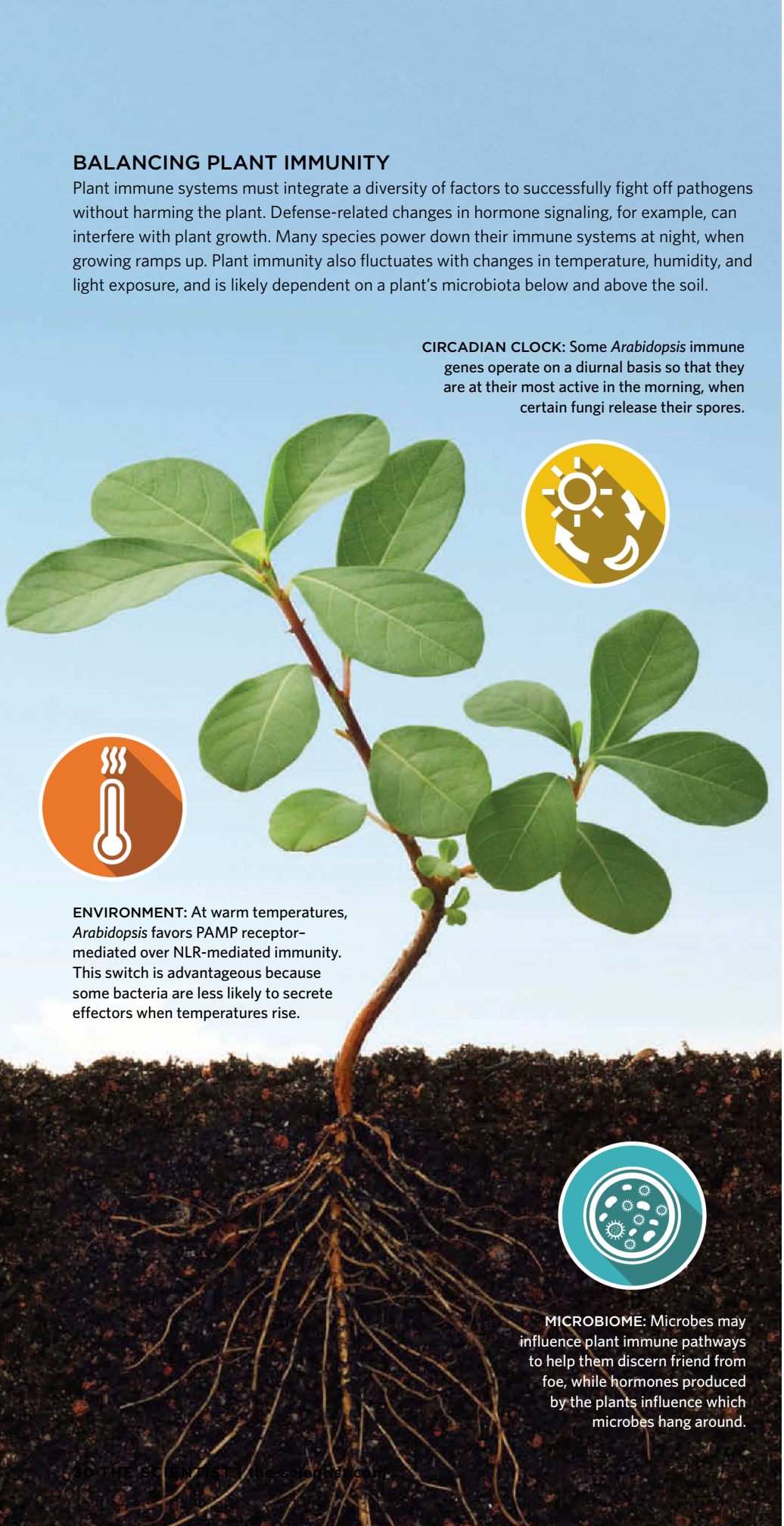
Plants have two basic immune pathways. First, a pattern recognition receptor (PRR) on the plant cell's surface recognizes pathogen-associated molecular patterns (PAMPs) released by invaders—say, the flagellar proteins from pathogenic bacteria. This jump-starts signaling pathways inside the cell that spur the production of reactive oxygen species (ROS) and antimicrobial compounds, which are secreted to combat the pathogen. PAMP-triggered pathways can also lead to changes in gene expression and hormone levels.

But bacteria can interfere with PAMP-triggered immunity by injecting effector molecules into the plant cell. Intracellular plant protein complexes called nucleotide-binding domain, leucine-rich repeat receptors (NLRs) bind bacterial effectors and set off secondary immune cascades that boost the PAMP-triggered responses. NLR-binding can also lead to plant cell death, limiting the infection.



from randomly mutating the gene that codes for R3a to selectively mutate a homologous tomato NLR gene called *I2*. They enhanced *I2*'s sensitivity to late blight and to an effector produced by a fungus that causes tomato wilt. Expressing the mutated *I2* in the leaves of the model plant *Nicotiana benthamiana* protected the leaves from late blight infection.[10]

NLRs are not the only group of receptors that researchers are mutating to enhance pathogen resistance; they also engineer effector targets. Last fall, Michigan State University plant scientist Sheng Yang He and his colleagues described a single amino acid change in a plant hormone receptor called coronatine-insensitive 1 (COI1) that protected *Arabidopsis* plants from *P. syringae* infection.[11] When the plant hormone jasmonate binds the COI1 receptor, it activates defense pathways against chewing insects at the expense of the plant's immune response to bacteria. *P. syringae*, which causes a disease called leaf speck on tomatoes, produces a mimic of jasmonate, called coronatine, that binds COI1 to keep antibacterial immu-



nity repressed.[12] But a mutation in *COI1* introduced by the researchers prevented binding with the bacterial mimic while maintaining normal jasmonate binding, making the plants resistant to *P. syringae* without compromising jasmonate-dependent defense against predatory insects.

He's approach of mutating host targets to make plants less susceptible to pathogen attacks bypasses a major hurdle to breeding in new resistance genes. "There's always this trade-off," says Imre Somssich, a plant immunologist at the Max Planck Institute for Plant Breeding Research in Cologne, Germany. "If the R gene's activated constantly, you get small plants." Making plants impervious to bacterial subversion avoids the need for such heightened immune surveillance, conferring protection without compromising growth.

But to really strike a balance between plant growth and immunity, scientists need to know how the cellular pathways regulating these processes converge. Patrick Schäfer, who studies plant immunity at the University of Warwick in the U.K., is examining how immune activation by bacterial flagellin affects cell-cycle pathways in *Arabidopsis* root cells. At the moment, he says, the endocrine system appears to be the strongest link between a plant's growth and its resistance to pathogens. "It looks like the hormone pathways that are used by immunity are in part also used by growth signaling pathways," says Schäfer.

Recent work by plant researcher Xinnian Dong and her team at Duke University suggests that another way plants juggle defense and growth is through the use of internal clocks. In 2011, they unexpectedly found a correlation between the expression of immune genes and the internal circadian clock of *Arabidopsis* plants. "We were puzzled at the time," she says. "We thought these genes were just pathogen-induced, but then we found this connection to the clock."

Dong's group found that a central clock transcription factor called circadian clock-associated 1 (CCA1) activates resistance genes involved in defense against the fungus *Hyaloperonospora arabidopsidis* first thing in the morning, when the pathogen

typically releases its spores.[13] Last year, they reported that the plant's redox clock, which is driven by changes in plant cell metabolism and hormone levels, works with the circadian clock to boost plant immunity in the morning and repress it in the evening, when plants do most of their growing.[14] When the researchers perturbed the cycle by artificially inducing immunity of plants grown in the dark for a few nights in a row, the plants shriveled up and died.

The findings could help farmers who treat crops with the hormone salicylic acid to boost immunity, says Dong. "If you induce immunity at the wrong time of the day, that can cause much more damage."

Another important factor in a plant's resistance to pathogens is its microbiome. He's team has found that germ-free *Arabidopsis* plants express lower levels of many immune genes and exhibit impaired immune responses such as reactive oxygen species production compared to their microbe-colonized counterparts—findings that he hopes to publish this year. And Dangl's group recently reported that the *Arabidopsis* microbiome is shaped by the plant's hormones, especially salicylic acid.[15]

But how these microbial communities interact with the plant immune system is still a mystery. Just as many microbiologists would like to know how the human body tells the good microbes from the bad, those studying plant immunity are trying to understand how plants make peace with beneficial inhabitants. "All of these microbes are going to have PAMPs," says Dangl. "You have to know who your friends are."

Assisting evolution

Historically, resistance genes have been bred into crops one gene at a time. But with just one mutation that lets it bypass a new resistance gene, a pathogen can decimate a field of genetically identical crops. "Late blight has been particularly notorious for doing that," Ristaino says.

So instead of arming plants with individual genes, researchers are now looking to give plants whole suites, or "stacks," of resistance genes. Although this can be done with conventional breeding, researchers and agriscience companies are increasingly drawn to new precision gene-editing techniques such as the CRISPR/Cas9 system. Last October, scientists in South Korea demonstrated that they could make precise genetic changes to several plant species using CRISPR guide RNA and Cas9 enzymes, without leaving behind any bacterial DNA.[16] That same month, DuPont announced it would collaborate and share patents with Berkeley, California–based Caribou Biosciences to apply CRISPR technology to agricultural products in the next 5 to 10 years.

Host resistance is probably the best way to reduce losses.

—Jean Ristaino, North Carolina State University

In addition to being dramatically more efficient than conventional breeding, gene editing allows researchers to introduce genes from wild varieties that won't breed with their domesticated relatives because the strains have diverged too much and their offspring are not viable. And when resistance genes are successfully bred into plants using conventional methods, they bring in a lot of unwanted extras, which then have to be painstakingly bred out. "When you're crossing you have no idea what other genes you're bringing in," says Somssich; gene editing is "much cleaner."

Like conventional breeding, however, genetic engineering methods still face the challenge of keeping up with a pathogen's rapid adaptation. "You really can't deploy stable resistance in the host unless you understand how the pathogen's evolving in response to the genes being thrown at it," Ristaino says.

But if plant scientists can predict how pathogens might evolve, as virologists do to generate a flu vaccine each year, gene-editing techniques could allow them to generate new crop varieties as quickly as the pathogens mutate, says Kamoun. "My personal vision is that we turn this into an arms race between us and the pathogen—not the plant and the pathogen." ■

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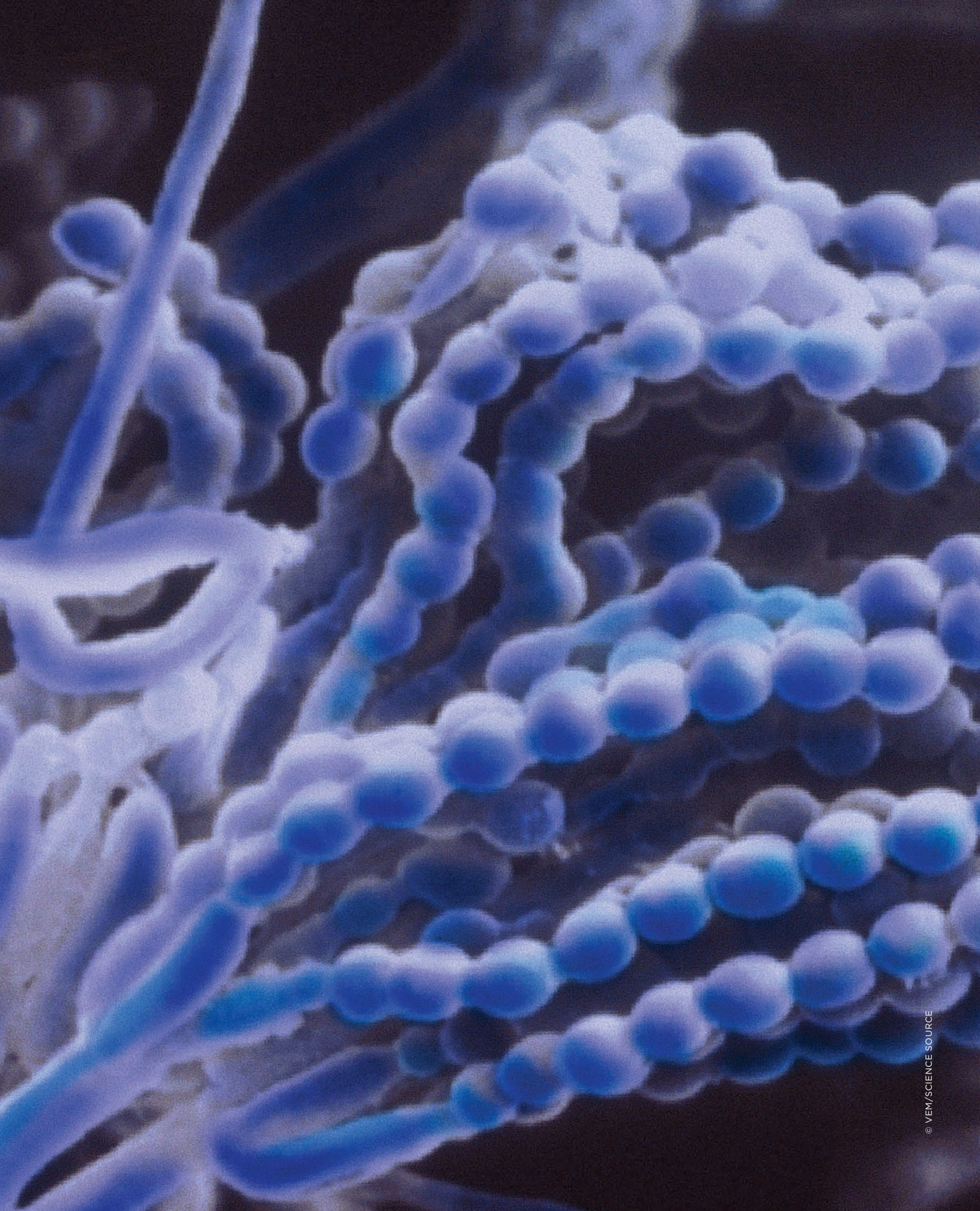


The Mycobiome

The largely overlooked resident fungal community plays a critical role in human health and disease.

BY MAHMOUD GHANNOUM

At a workshop held at the National Institutes of Health (NIH) last September on the role of human microbiota in infectious disease, I was disheartened not to hear a single talk on the fungal community—the mycobiome. Disheartened, but not surprised. Ignoring the fungal kingdom is nothing new. More than five years ago, my colleague and I tried to draw attention to this issue in a letter published in *Microbe*, recommending that the Human Microbiome Project should investigate not just people's bacterial inhabitants, but the fungal and viral commensal communities as well. While research on the human virome has increased in recent years, the scientific community has not heeded our advice with regard to the fungal components of the microbiome. As of November 2015, only 269 of more than 6,000 Web of Science search results for the term "microbiome" even mention "fungus," and the scientific search engine returns only 55 papers pertaining to the "mycobiome."

Despite this lack of attention, a handful of recent studies point to the importance of our commensal fungal inhabitants as critical players in human health and disease. The oral mycobiome of HIV-infected patients differs from that of uninfected controls, for example. Abundance of *Candida* and *Saccharomyces* species correlates with increased severity of hepatitis B infections. And an overabundance of the gut fungal pathogen *Candida tropicalis* aggravates inflammatory bowel disease. As widespread surveys of the mycobiome become available, such correlational data can be further explored by experimental manipulations in the lab. One day, perhaps, researchers will include fungal species in fecal transplants to treat infections by *Clostridium difficile* and other bacteria, and companies will begin to market probiotic products that contain live cultures of friendly fungi.

Characterization of the human mycobiome

In 1974, my master's advisor handed me a paper showing that rabbits treated with antibiotics to kill bacteria or with anti-inflammatory steroids to suppress the immune system developed the fungal infection candidiasis, commonly known as thrush.[1] It made me realize that not only could fungi in the environment negatively impact our health, but fungal species also inhabit the mammalian body, alongside diverse commensal bacteria. And when one microbial community is knocked out, another can cause illness. If the communities are undisturbed, however, the fungal inhabitants appear to be harmless or perhaps even beneficial.

The medical mycology community didn't attempt to define the fungal component of the human microbiome until 2010. My colleagues and I found a complex fungal community in the human oral cavity that went beyond *Candida* and *Saccharomyces*, fast growers that can be easily cultured. Across 20 volunteers, we identified 101 fungal species, each person harboring between 9 and 23 fungal species in their mouth. To eliminate transient fungi, we considered the core mycobiome to be those organisms that were present in at least four participants (20 percent of the population studied). This core included 15 genera, of which *Candida* species were the most frequent, followed by *Cladosporium* (a known allergen and trigger for asthmatic attacks), *Aureobasi-*

dium (which can cause fungal infections in solid-organ transplant recipients), and genera of the order Saccharomycetales (which includes beneficial species, such as *Saccharomyces boulardii*, which has been suggested as a potential probiotic).[2]

We also found several pathogenic fungi, including *Aspergillus* (which can cause invasive and devastating fungal infections), *Fusarium* (which can cause superficial and systemic infections that are difficult to treat), and *Cryptococcus* (a major cause of meningitis in AIDS patients). These pathogens may render individuals at increased risk of infection, especially if the host immune status is compromised, though such a link has yet to be demonstrated experimentally. Of the species we identified, more than a third could not be cultured, making it difficult to study their roles in health and disease.

Investigators have started to look at the fungal communities residing in body sites other than the oral cavity. In 2012, a team in Japan profiled the distribution of *Malassezia* species, which can lead to a superficial skin disease, in the external auditory canal and on the sole of the foot. *M. slooffiae*, known to cause skin discoloration, was the most common species in both body sites, followed by *M. restricta,* which predominated in scalp dandruff. Despite some similarities to the mycobiomes of other body sites, however, the researchers found distinctive *Malassezia* communities present in the outer ear canal and on the sole.[3]

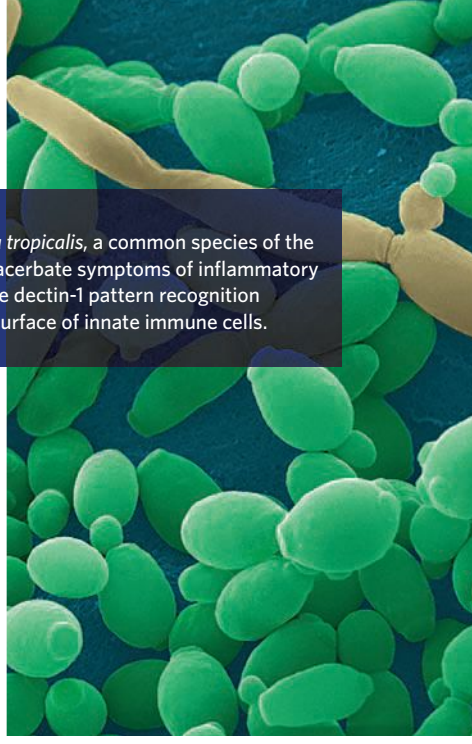
In 2013, Keisha Findley of the National Human Genome Research Institute and other NIH researchers characterized the mycobiome of the skin, which varied depending on the body part sampled.[4] On 10 healthy adults, the research team found that fungi of the genus *Malassezia* dominated 11 torso and arm sites, while greater diversities of fungal genera were observed on the plantar heel, toenail, and toe web. Importantly, the researchers showed that while bacteria were grouped based on whether the skin was sebaceous, moist, or dry, the fungal community appeared to be determined by location—foot, arm, head, or torso.

Most recently, a group of French researchers characterized the mycobiome in the lungs and reported that environmental fungi such as *Aspergillus* dominate in healthy people.[5] The lungs of patients with cystic fibrosis, pulmonary fibrosis, and other lung diseases, as well as of those suffering from cardiovascular disease or who had received a lung transplant, were dominated by *Candida* species such as *C. albicans*.

Collectively, these and other surveys have demonstrated the presence of diverse fungal communities throughout the human body, and suggest their importance in health and disease.

Fungi and disease

In 2014, to go beyond listing the fungal species found at various sites in the body and move towards mechanistic studies, my lab conducted an analysis of fungal-fungal and fungal-bacterial interactions in the oral cavity of HIV-infected patients.[6] Among diverse microbial interactions, we discovered an antagonistic relationship between *Pichia*, a nonpathogenic yeast used as a biocontrol agent to protect crops from fungal growth, and pathogenic *Candida*: a decrease in *Pichia* abundance coincided with an increase in *Candida* colonization.



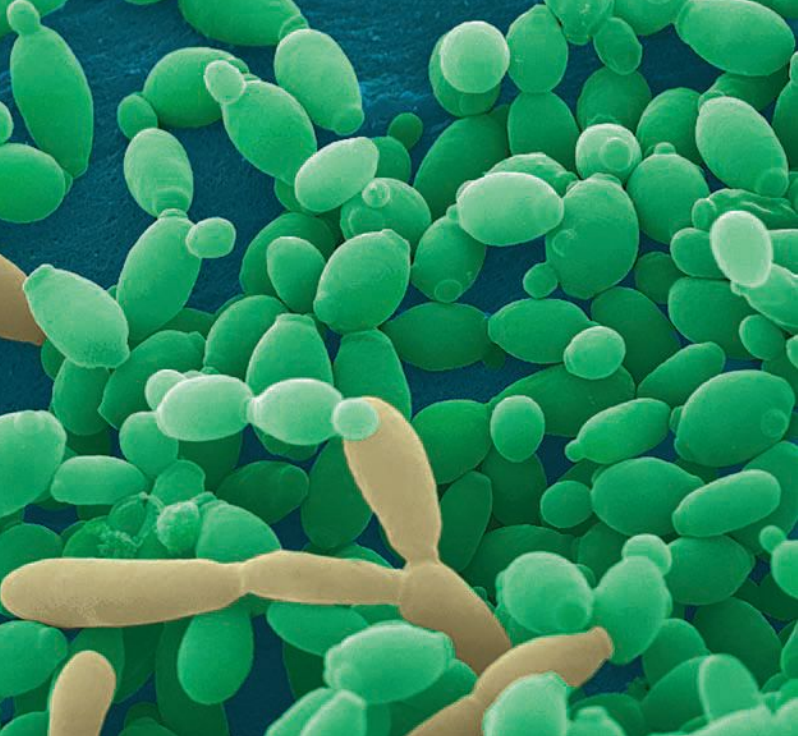
TWO-FACED FUNGUS: *Candida tropicalis*, a common species of the mammalian mycobiome, can exacerbate symptoms of inflammatory bowel disease in mice lacking the dectin-1 pattern recognition receptor typically found on the surface of innate immune cells.

Growing these species in the lab, we found that *Pichia* released a growth inhibitory agent into culture media that was capable of limiting the growth of *Candida*, *Aspergillus*, and *Fusarium*. Moreover, adding *Pichia* cells or the medium they had been grown in (*Pichia* spent media, PSM) to *Candida* cultures significantly inhibited biofilm formation. And when immunosuppressed mice were challenged with *C. albicans*, those treated orally with PSM developed more-benign oral infections and had a decrease in the number of fungi invading the tongue mucosal tissues compared with untreated mice.[6]

Looking more closely at the fungal growth in PSM-treated mice, we found fewer hyphae growing on their tongues, and their oral mucosa appeared intact. Control mice, on the other hand, showed evidence of extensive fungal invasion of the tissue. Identification of the active *Pichia* component responsible for the inhibition of these pathogenic fungi is currently under investigation; preliminary studies suggest that it is a protein.

Fungal species inhabit the mammalian body, alongside diverse commensal bacteria. And when one microbial community is knocked out, another can cause illness.

Recent work has also pointed to the importance of the mycobiome in gastrointestinal health. In 2008, Stephan Ott of Christian-Albrechts-University and the University Hospital Schleswig-Holstein in Kiel, Germany, and colleagues found that the fecal fungal community in patients with inflammatory bowel disease (IBD) was substantially different from that of healthy controls.[7] And in a mouse model of colitis, David Underhill's group at the Cedars-Sinai Medical Center in Los Angeles found that dectin-1, a pattern recognition receptor present on the cell surface of innate immune cells (e.g. macrophages, neutrophils, and dendritic cells) that mediates the biological effects of fungal wall polysaccharides called glucans, significantly influences the animals' IBD symptoms.[8] Specifically, Underhill and his colleagues found that mice



deficient in dectin-1 suffered increased weight loss, histological alterations, and production of proinflammatory cytokines compared with wild-type mice. Additional testing demonstrated that *Candida tropicalis*, a dominant species in the gut of wild-type mice, exploited the lack of dectin-1, and that treatment of dectin-1–deficient mice with the antifungal fluconazole attenuated disease severity.

Disruptions to the mycobiome can also exacerbate graft versus host disease (GVHD), a complex and multidirectional process that involves interactions between the host innate immune system, the gut microbiota, and donor T cells. In 2000, Kieren Marr, then at the University of Washington and the Fred Hutchinson Cancer Research Center, and colleagues were among the first to show an association between treating candidiasis and improvement of GVHD. Specifically, they showed that bone marrow transplant patients undergoing long-term fluconazole treatment had decreased gastrointestinal GVHD, persistent protection against *Candida* infections and candidiasis-related death, and improved survival.[9]

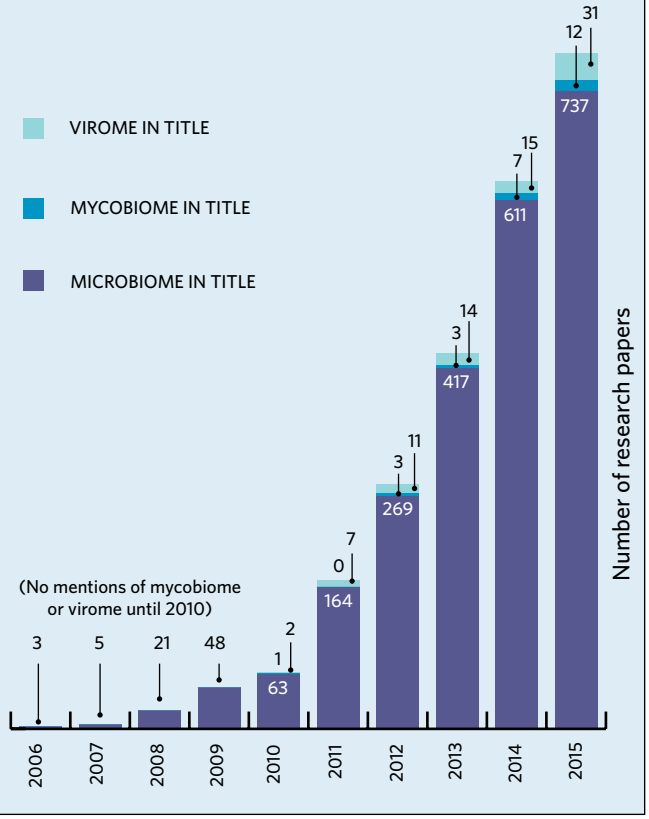
Ten years later, Walter van der Velden of the Radboud University Nijmegen Medical Center in the Netherlands and colleagues examined whether *Candida* colonization and dectin-1 function had an effect on the development of GVHD through a retrospective analysis of patients who had undergone allogeneic stem cell transplantation.[10] The patients had been evaluated for *Candida* colonization in fecal and mouthwash samples. Van der Velden's group found that *Candida*-colonized patients had a significantly higher rate of severe acute GVHD than those that were *Candida* free, and that colonization was the only factor they looked at that was associated with GVHD; stem cell source and the method by which the researchers depleted T cells had no significant impact. When treated with fluconazole, patients colonized with *Candida* had a lower likelihood of developing GVHD.

The composition of the human mycobiome may also influence much more common diseases, such as obesity. Last year, a group in Spain reported that the fungal community of obese people has an increased abundance of fungi belonging to the phylum Ascomycota, classes Saccharomycetes and Tremellomycetes, and families Dipodascaceae and Saccharomycetaceae compared with non-obese subjects.[11] There was also a tendency towards decreased fungal diversity in obese individuals, which is consistent with published data on bacteria. Alterations in the mycobiome of obese subjects seemed to be associated with higher amounts of body fat and related metabolic disorders such as insulin resistance, high blood pressure, and inflammation.

The researchers found that *Mucor* was the most prevalent fungal genus in non-obese participants, and that the relative abundance of *Mucor* increased after weight loss in obese patients. Researchers have reported similar results with regard to Bacteroidetes bacteria, which tend to be associated with weight loss. The new finding may point to the potential use of *Mucor* with or without Bacteroidetes to combat obesity. Notably, similar to our findings of an antagonistic relationship between

RESEARCHING THE HUMAN MICROBIOME

Prior to 2010, there were no mentions of the mycobiome or the virome in the literature. Even in the last five years, research on the human microbiome has been dominated by surveys and studies of bacterial species.

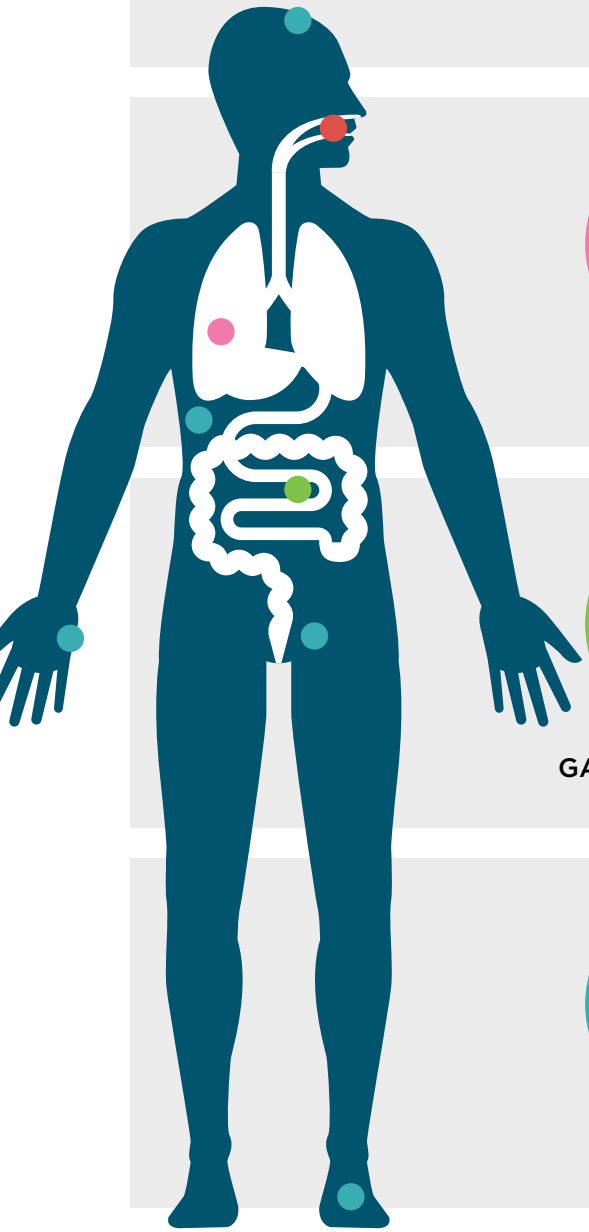


Data collected from ISI Web of Science on December 21, 2015

THE HUMAN MYCOBIOME

Diverse fungal species live in and on the human body. Preliminary surveys have revealed several pathogenic species that may increase one's risk of disease when the healthy microbiome is disrupted.

Candida species are among the most common members of the human mycobiome. When the balance of a microbial community is disrupted, *Candida* species can flourish and cause disease (candidiasis, or "thrush," when it develops in the mouth or throat).



ORAL CAVITY

Genera Identified
Potentially pathogenic lineages

- *Alternaria* • *Aspergillus*
- *Aureobasidium* • *Candida*
- *Cladosporium* • *Cryptococcus*
- *Fusarium* • *Gibberella*
- *Glomus* • *Pichia*
- *Saccharomyces*
- *Teratosphaeria*

Pathogenic fungi such as *Aspergillus*, *Fusarium*, and *Cryptococcus* species are common residents, and may increase the risk of invasive fungal infections, especially in immuno-compromised patients.

LUNGS

- *Aspergillus*
- *Candida*
- *Cladosporium*
- *Penicillium*
- *Cryptococcus*

Pathogenic species such as *Candida albicans* are found in patients with cystic fibrosis, pulmonary fibrosis, and other lung diseases, as well as in lung transplant patients and those suffering from cardiovascular disease.

GASTROINTESTINAL TRACT

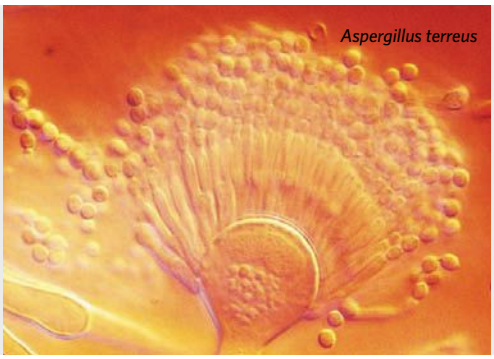
- *Aspergillus*
- *Candida*
- *Cladosporium*
- *Cryptococcus*
- *Fusarium*
- *Penicillium*
- *Pneumocystis*
- *Mucor*
- *Saccharomyces*

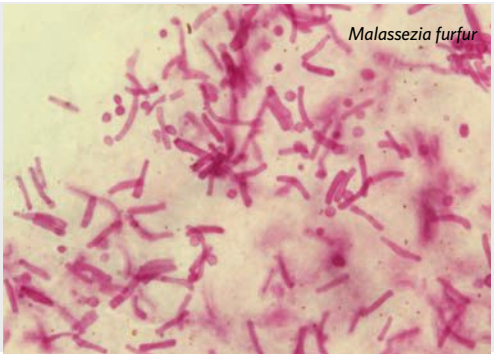
Alterations in the composition of the commensal mycobiome in the gut have been implicated in the exacerbation of inflammatory bowel disorders such as Crohn's disease.

SKIN

- *Candida* • *Cryptococcus*
- *Debaryomyces*
- *Epidermophyton* • *Malassezia*
- *Microsporum* • *Rhodotorula*
- *Trichophyton* • *Aspergillus*
- *Chrysosporium* • *Epicoccum*
- *Leptosphaerulina* • *Penicillium*
- *Phoma* • *Saccharomyces*
- *Ustilago*

Malassezia species, which can lead to superficial skin disease, have been found in the external auditory canal and on the skin, particularly on the torso and arms.





ASPERGILLUS: MEDMYCO/WIKIMEDIA COMMONS; *CANDIDA*: MICHAELFRANCISCO/FLICKR; *FUSARIUM*: CDC/DR. LIBERO AJELLO; *MALASSEZIA* :CDC/DR. LUCILLE K. GEORG

Candida and *Pichia* in the oral mycobiota of HIV-infected patients, the French group that surveyed the lung mycobiome found a significant negative association between the families Pichiaceae (which includes *Pichia*), and Dipodascaceae (which includes *Candida*) among nonobese subjects.[5]

Given that the field of mycobiome research is so young, these examples are undoubtedly but a few of many diseases in which our commensal fungi play significant roles. Already, additional links have been found between the fungal community and at least two such conditions, atopic dermatitis[12] and allergic responses in the lung.[13]

Clearly, the human mycobiome has an influence on both health and disease. The scientific community must adopt an all-inclusive characterization of the human microbiome going forward; studies that focus solely on bacteria are myopic and doomed to failure, and they squander precious research funds. ■

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Antibody Alternatives

Nucleic acid aptamers and protein scaffolds could change the way researchers study biological processes and treat disease.

BY JANE McLEOD AND PAUL KO FERRIGNO

There is a growing reproducibility problem across the life sciences. The retraction rate of published papers has increased tenfold over the past decade, and researchers have reported only being able to replicate published results in 11 percent[1] or 25 percent[2] of attempts. It's become known as the "reproducibility crisis," and science is in a race to fix it.

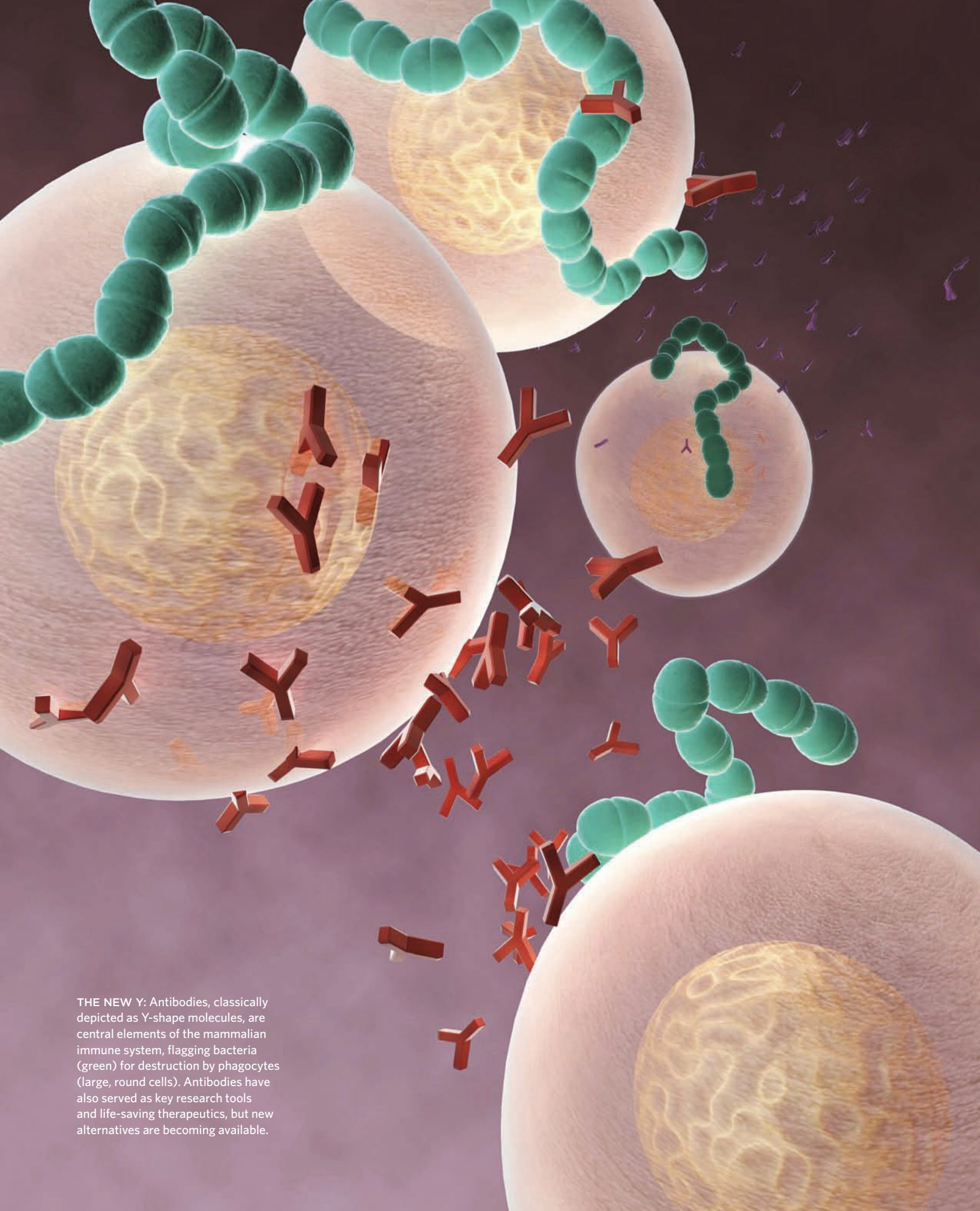
One major factor contributing to this problem is the use of poorly validated research antibodies. Lot to lot, antibodies can vary wildly. Some may not bind specifically to their target, or they may bind a different cellular protein altogether. According to one estimate, researchers around the world spend $800 million each year on poorly performing antibodies.[3] (See "Exercises for Your Abs" on page 55.)

While many researchers debate the best way to weed out the good antibodies from the bad, others are developing alternatives. Nucleic acid aptamers and protein scaffolds are increasingly being used to detect proteins of interest. Although they currently constitute only a fraction of affinity reagents, with the lion's share of the market still going to traditional antibodies, these newer options offer an opportunity to rectify the problems stemming from using poorly validated antibodies in research. Researchers can engineer RNA or DNA aptamers and protein scaffolds to a specific target and function, the molecules are consistent from batch to batch, and they can be produced at a fraction of the cost of antibodies. These new reagents can target proteins that remain inaccessible to antibodies. And researchers have designed them to be functional in a wider range of conditions, including intracellular environments that degrade the antibody structure, opening up applications such as super-resolution microscopy and intracellular live-cell imaging to investigate the molecular dynamics of diverse cellular processes.

So, rather than complain about the poor performance of antibodies, perhaps the scientific community should embrace the new antibody alternatives designed to overcome this problem—and, by doing so, begin to resolve the ongoing reproducibility crisis.

The rise—and pitfalls—of antibodies

Antibodies are large protein molecules composed of two heavy and two light chains linked by disulfide bonds. They play a crucial part in the immune system's ongoing battle to keep our bodies from falling prey to deadly diseases. Through the diversification of gene segments in the antibody sequence, the mammalian immune system produces different combinations of heavy and light chains to bind a wide variety of foreign proteins. When an invader is detected, those B cells that produce the most specific antibodies undergo hypersomatic mutation to fine-tune the antibody's affinity to a particular antigen, then differentiate into plasma cells that generate the targeted antibody molecules by the million to mark the disease-causing target for destruction. It has been estimated that the human body can



THE NEW Y: Antibodies, classically depicted as Y-shape molecules, are central elements of the mammalian immune system, flagging bacteria (green) for destruction by phagocytes (large, round cells). Antibodies have also served as key research tools and life-saving therapeutics, but new alternatives are becoming available.

create enough different antibodies to recognize 10^{12} distinct pathogens.[4]

For decades, life-science researchers have taken advantage of this natural process to develop tags and assays for a wide array of proteins. In the early 1900s, researchers began to cultivate protein-specific antibodies by immunizing rabbits, chickens, goats, donkeys, and other animals with a desired target protein. B cells within the animal host generate antibodies to different antigenic areas (epitopes) on the protein of interest. The antibodies targeting the desired protein can then be isolated and purified for use in biochemical and cell-based assays to document protein expression under different conditions or to identify potential disease biomarkers. But the reliance on an animal host system for production meant lot-to-lot heterogeneity for such polyclonal antibodies. (See illustration on page 42.)

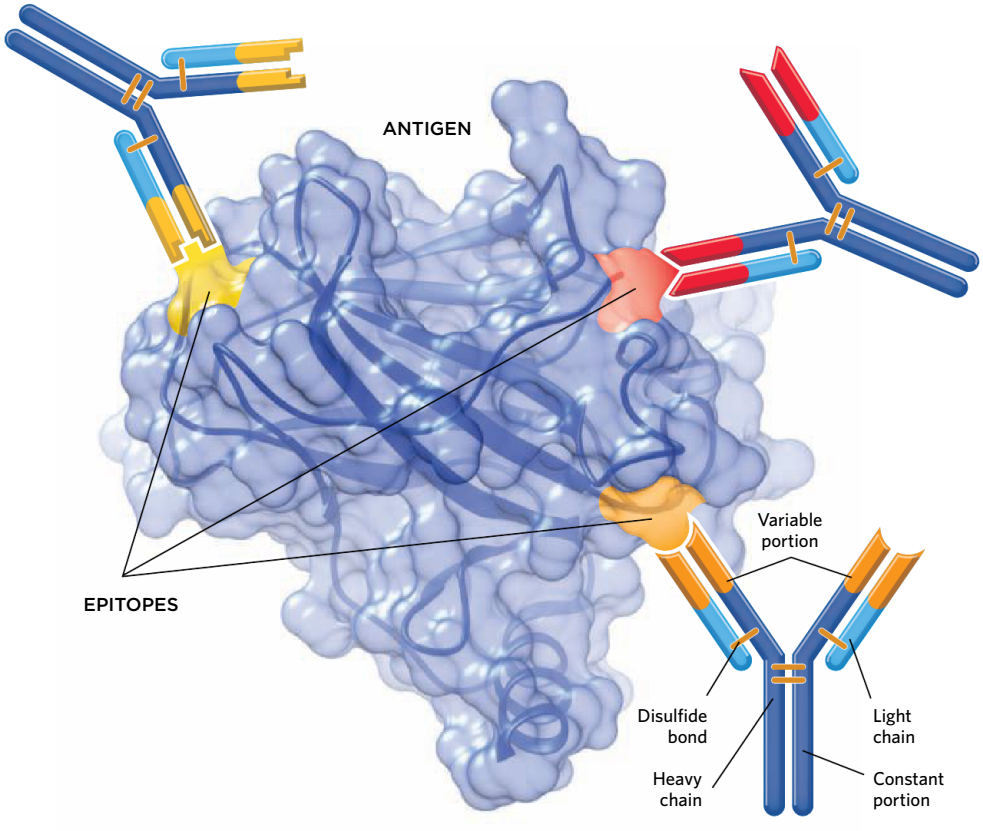
In 1975, Argentine biochemist César Milstein and German biologist Georges Köhler discovered how to generate batches of individual antibodies, produced by a single B cell to target a specific antigen. Once an animal host produces antibodies to a target, the antibody-producing B cells are isolated from the spleen or lymph nodes and fused with tumor cells to generate immortal hybridoma lines. These lines are then screened to identify clones producing antibodies that bind with a high affinity to a specific epitope on the target protein. These cells are then cultured in large-scale bioreactors.

While heterogeneity can arise from drift in the cell line's antibody expression and downstream production processes, monoclonal antibodies exhibit far less lot-to-lot variation than polyclonal antibodies, and have become the affinity tool of choice in modern research laboratories. Monoclonal antibodies are now routinely employed to localize proteins within tissues, determine protein network interactions, and analyze protein function. They are now being pushed to the limits of their performance in applications such as nanoimmunoassays and in vivo cell imaging. In medicine, antibody therapeutics represents the fastest growing sector of pharmaceutical sales, with 47 monoclonal antibodies currently on the market and a further 300 in clinical trials.[5]

But there are many examples where the use of antibodies has actually hindered scientific progress, by providing misleading or inaccurate results. Antibodies have evolved to execute their biological function perfectly, but this does not make them foolproof as investigative tools or therapeutic agents. In fact, many of the very characteristics that aid in antibodies' function as part of the immune system limit their use in research and medicine.

In the context of an immune reaction, for example, not all B cells produce antibodies that are exquisitely specific. So long as the antibodies exceed a certain threshold of binding affinity for the target, they remain part of the immune system's defense. In the body, this is a good thing: these less-specific antibodies cross-react with a variety of related antigens, making the antibody defense force more versatile.[6] If an invading pathogen mutates or a similar pathogen invades, potentially effective antibodies may already be in circulation. As part of an assay to specifically identify a particular protein, however, such cross-reactivity can be the downfall of the experiment or therapy.

Examined in this light, it is easy to see why taking a molecule that is derived for one purpose and applying it to another may not yield the best results. A clear example of the shortcomings of antibody use in life-science research comes from the Human



A CLASSIC FIT

Antibodies are large proteins, weighing in at about 150 kDa. Four polypeptides—two heavy chains and two light chains—are linked by disulfide bonds to form a Y-shape molecule. The amino acid sequences at tips of the short ends of the Y vary greatly between antibodies produced by different B cells, while the rest of the molecule is relatively consistent. The variable portion of the antibody binds in a specific region (epitope) on a foreign protein (antigen) and signals the immune system to the presence of an invader.

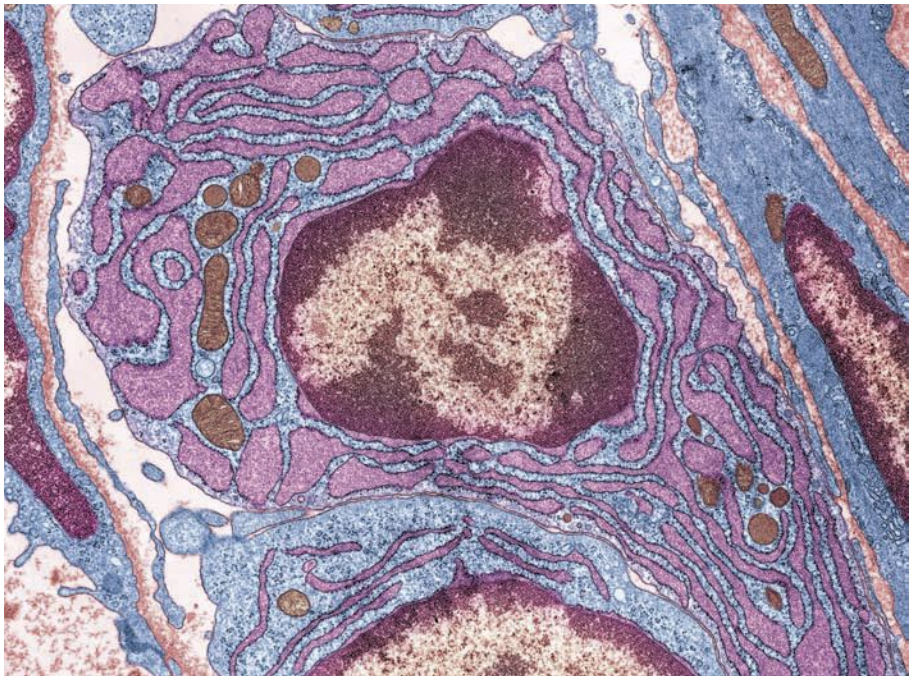
Protein Atlas project. Mathias Uhlén of the Royal Institute of Technology in Stockholm, Sweden, and colleagues set out to catalog protein expression and localization data across 44 normal human tissue types, 20 different cancers, and 46 cell lines. The team sourced antibodies from 51 different commercial vendors for validation. Of the 5,436 antibodies received, about half failed to detect their target in a Western blot or standard immunohistochemistry assay.[7]

The development of novel antibodies that bind new protein targets continues to face several challenges. For example, using the conventional route of immunizing lab animals to produce an antibody against a toxic target molecule will often kill the host animal prior to the generation of sufficiently specific antibodies. Conversely, if a protein target is highly homologous to a host protein, the immune system may not recognize the target as foreign in order to generate antibodies against it.

Given the rapid pace at which molecular biology proceeds in the modern era, new protein-binding reagents are desperately needed. A review of 20 million published research articles from 1950 to 2009 showed that three-quarters of the research focused on just 10 percent of the proteins that were known prior to the mapping of the human genome.[8] Rational design of antibody alternatives will allow us to target a broader swath of proteins and function across more platforms to better investigate the scientific questions at hand.

Engineering a solution

Alternative affinity reagents developed over the past few decades include both nucleic acid– and protein-based molecules. Aptamers are short molecules of single-stranded DNA or RNA, typically less than 100 nucleotides in length, that form 3-D structures capable of binding specific target proteins. Protein scaffolds, formed from polypeptide fragments or whole proteins, have similarly precise interactions with target molecules. Both types of affinity reagents are produced entirely in vitro, so in principle they are not subject to the limitations of antibody production by animal immune systems, allowing researchers to study proteins for which it is impossible to generate antibodies. And even when antibodies do exist, aptamers and protein scaffolds offer more-precise targeting, because they have been engineered for a specific purpose.



ANTIBODY FACTORY: A close-up view of a mature B lymphocyte, which produces and secretes antibodies during an immune response. In the cytoplasm (blue), an extensive network of rough endoplasmic reticulum (light purple) manufactures, modifies, and transports the antibodies.

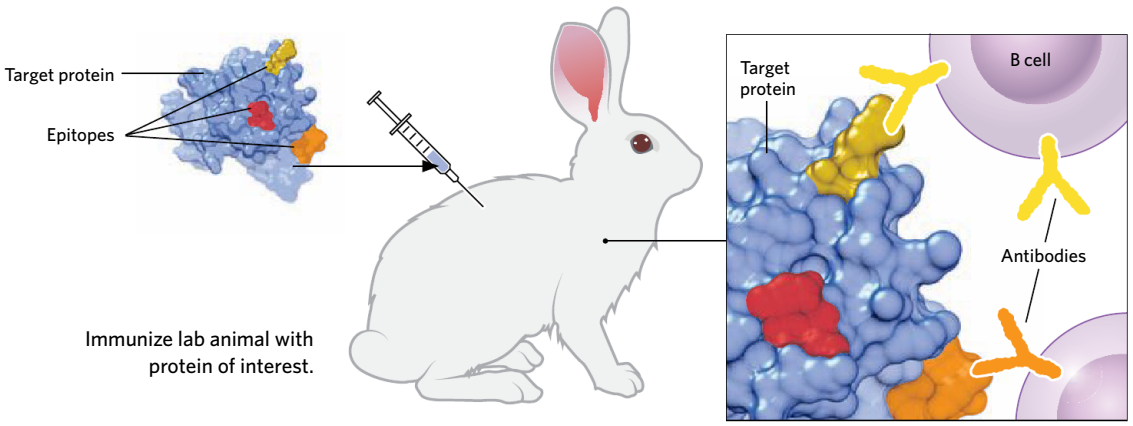
These novel affinity reagents also offer other benefits over antibodies. Both nucleic acid aptamers and protein scaffolds are much smaller than natural antibodies, which typically weigh about 150 kDa. Aptamers and scaffolds are as little as one-tenth that size. This means that their distribution is not restricted in the same manner as that of antibodies, opening up new targets that were previously inaccessible, such as epitopes hidden inside molecular grooves and pockets where antibodies simply can't fit. Labeling target proteins with these smaller tags in cytochemistry experiments reduces the chance of the target protein being dragged around the cell according to the tag's biochemistry, and increases the chances of identifying the correct protein localization. Additionally, their smaller size increases these affinity reagents' tissue penetration, enhancing access to epitopes within tissue sections and decreasing false negative immunohistochemistry results. Smaller molecules are also cleared more rapidly from the body, especially when their size is below the renal cut-off of 45 kDa, making these molecules ideal as imaging agents in the clinic.

Researchers first developed nucleic acid aptamers in 1990 as RNA-based molecules, though DNA variants quickly followed to deal with the low stability of the RNA backbone. Aptamers offer simple chemistry that can be easily functionalized, but they lack stability across a range of temperatures and pH, and in the presence of common buffer components or DNases and RNases found in many media and cell environments. Researchers have used various chemical modifications to increase aptamers' resistance to nuclease activity, to improve aptamer binding, and to increase their structural diversity, but others have turned to yet another option: protein scaffolds.

BUILDING BETTER REAGENTS

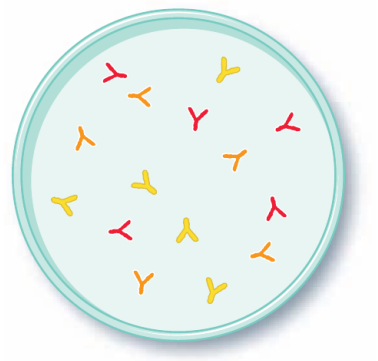
Antibody therapeutics represents the fastest growing sector of pharmaceutical sales, with 47 monoclonal antibodies currently on the market and 300 more in clinical trials. But facing problems of inconsistent, time-consuming, and costly antibody production, some researchers are turning to alternatives—nucleic acid aptamers and protein scaffolds—to target specific proteins of interest, in the lab and in the clinic.

ANTIBODIES



POLYCLONAL ANTIBODIES

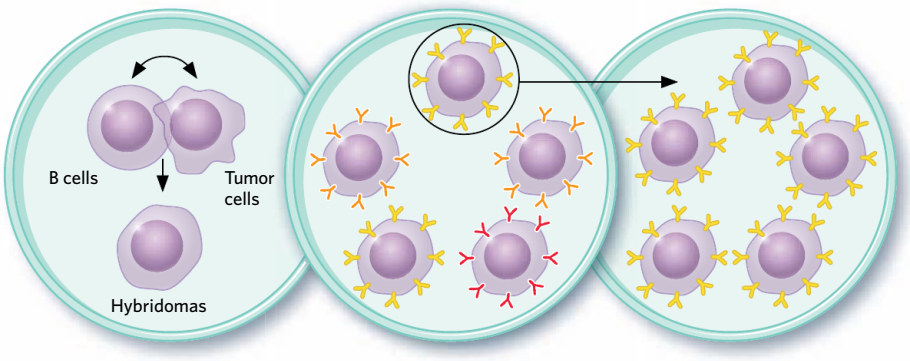
The diverse antibodies that bind to the target protein's numerous epitopes can then be isolated and purified for use.



- **Size:** Large (about 150 kDa)
- **Binds specific epitope?:** Typically no. Diverse antibodies against different epitopes, making them less sensitive to antigen changes than monoclonal antibodies. Antibodies will also vary in affinity and specificity for a given target.
- **Production:** 2–4 months; entirely in animal models
- **Lot-to-lot heterogeneity:** High
- **Shelf life:** Limited

MONOCLONAL ANTIBODIES

Alternatively, the immunized animals' B cells can be isolated from the spleen or lymph nodes and fused with a tumor cell to generate immortal hybridoma lines. Those cell lines that produce the desired antibody against a specific epitope of the target protein can then be grown in large bioreactors to scale up production of the antibody.

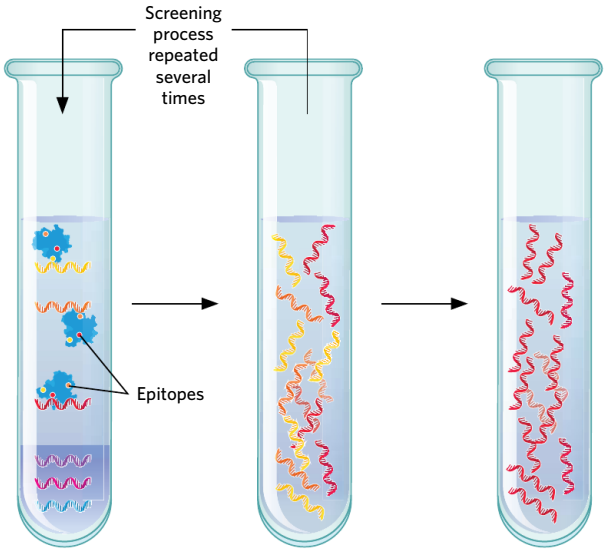


- **Size:** Large (about 150 kDa)
- **Binds specific epitope?:** Yes. As they offer specific recognition of a single epitope on the target protein, monoclonal antibodies are sensitive to molecular changes of that epitope and offer precise molecular recognition of a group of structurally similar molecules.
- **Production:** Six months; requires animal models and the use of expensive cell cultures of higher eukaryotes for growth in bioreactors of up to 2,000 L
- **Lot-to-lot heterogeneity:** Low, though downstream production processes and drift in the cell line's antibody expression can introduce variation
- **Shelf life:** Limited

ANTIBODY ALTERNATIVES

NUCLEIC ACID APTAMERS

Aptamers are short molecules of single-stranded DNA or RNA, typically less than 100 nucleotides in length, that form specific 3-D structures capable of binding target proteins.

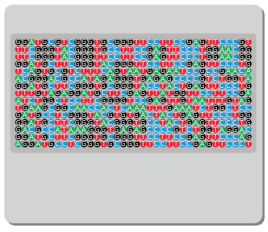


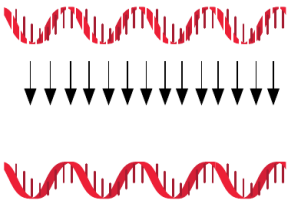
Screen a target protein against a library of nucleic acids. Filter for bound reagents.

Amplify remaining aptamers with PCR, and screen again.

After many rounds of screening, an aptamer that efficiently binds a single epitope is chosen.

Sequence and chemically synthesize the chosen aptamer.

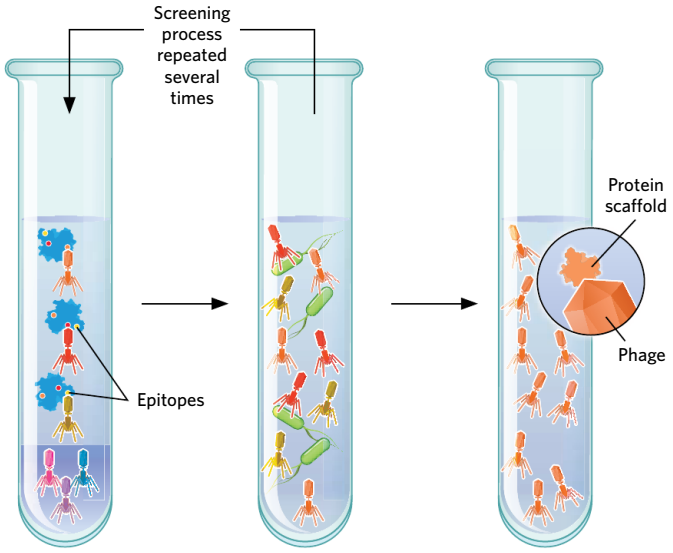




- **Size:** Small (<25 kDa), opening up new targets that were previously inaccessible to antibodies
- **Binds specific epitope?:** Yes
- **Production:** Weeks; chemically synthesized
- **Lot-to-lot heterogeneity:** Very low
- **Shelf life:** Stable at room temperature for months

PROTEIN SCAFFOLDS

Protein scaffolds, formed from polypeptide fragments or whole proteins, have similarly specific interactions with desired target molecules.

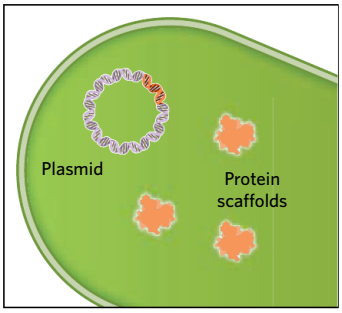


Screen a target against a library of protein scaffolds presented on the surface of bacteriophages. Filter for bound reagents and rinse away unbound ones.

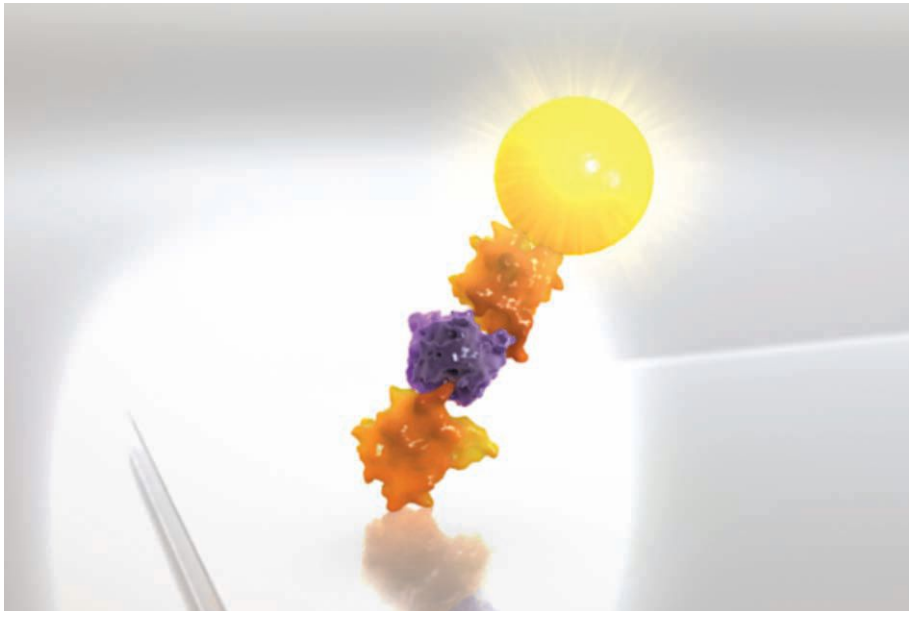
Infect *E. coli* with bacteriophage carrying positive binders to amplify a new, enriched library and screen again.

After many rounds of screening, a scaffold that efficiently binds a single epitope is chosen.

Introduce a plasmid encoding the desired scaffold into *E. coli* to scale up production.



- **Size:** Small (~15 kDa), opening up new targets that were previously inaccessible to antibodies
- **Binds specific epitope?:** Yes
- **Production:** Weeks; entirely in vitro with lower organisms such as bacteria
- **Lot-to-lot heterogeneity:** Very low
- **Shelf life:** Stable at room temperature for months



A PROTEIN BUILT FOR YOU: Protein scaffolds (orange) can be developed to bind specific protein targets (purple) and can be produced in a matter of weeks. Once the scaffolds bind the protein of interest, fluorescent or color reporter tags (yellow ball) can be used to label and isolate them.

Developed around the same time as aptamers, protein scaffold affinity reagents were originally designed to identify potential therapeutic targets. Researchers soon began to apply this technology to screening for binders to completely novel proteins, by presenting a random sequence as the binding surface. Because protein scaffolds lack the disulfide bonds of antibodies, they retain their structure in a greater variety of cell culture and assay environments, without being attacked by other proteins that break these bonds and cause antibodies to fall apart. Scaffolds maintain function and target affinity at temperatures up to 80 °C and in solutions with a pH as low as 2 and as high as 13.

Because protein scaffolds can be delivered to the inside of the cell, researchers can use them in live-cell imaging, ultimately allowing use of the same reagent in both biochemical and cell biology assays. Additionally, protein scaffolds could help to deliver drugs directly into cells, improving targeting of pharmaceutical payloads and reducing side effects. And as new protein scaffolds are often engineered to lack cysteine residues, aberrant folding during their production within the cell factory is unlikely, increasing reproducibility within the reagents.

Importantly, both nucleic acid aptamers and protein scaffolds are far easier to consistently produce than antibodies. In addition to requiring animal hosts to provide an antibody-producing B cell, functional antibodies can only be expressed in higher eukaryotic cell systems. Antibodies are extensively glycosylated with a complex range of sugars that are critical to their function. Lower eukaryotic organisms, such as insects and yeast, and prokaryotic cells are not capable of the full range of complex glycosylation. As a result of these complexities, production times for monoclonal antibodies are six months on average, often making the generation of new antibodies the rate-limiting step in the advance of new research. This production process is also extremely expensive, and the use of such intractable biological systems breeds batch-to-batch inconsistencies.

Aptamers and protein scaffolds can be made without a host immune system. Now that robust and scalable methodologies for creating custom DNA and RNA molecules exist, effective aptamer binders can be chemically synthesized at a fraction of the cost of producing protein-based affinity molecules. And because protein scaffolds do not contain any posttranslational modifications, they can be expressed in bacterial cells, which are cheaper and easier to control than the eukaryotic systems used for antibody production. Both aptamers and scaffolds can often be available to researchers in a matter of weeks. (See illustration on previous page.)

Alternatives at work

So far, the majority of the industry attention for antibody alternatives has largely focused on the therapeutic development of antibody alternatives. Many companies now have initiated Phase 2 and 3 clinical trials of candidate molecules to treat conditions from vision problems to cancer, and two such molecules have already been approved for therapeutic use. In 2004, the RNA aptamer–based therapeutic pegaptanib (Macugen), originally developed by NeXstar Pharmaceuticals, became the first antibody alternative to gain US Food and Drug Administration (FDA) approval for the treatment of neovascular age-related macular degeneration. Pegaptanib is a 28-base-long RNA oligonucleotide with modifications to protect the aptamer from endogenous nucleases and extend its half-life in vivo to 10 days.[9] Administered directly into the eye, this aptamer selectively binds the most common isoform of vascular endothelial growth factor (VEGF), preventing angiogenesis and the increased permeability of the blood vessels within the eye associated with neovascular age-related macular degeneration.[10] Five years later, in 2009, the FDA approved a protein scaffold called ecallantide (Kalbitor) for the treatment of sudden hereditary angioedema attacks.

In a therapeutic context, the most important characteristic of aptamers and scaffolds is that they lack immunogenicity, thus avoiding harmful immune responses in patients. One way to ensure that protein scaffolds do not trigger host immunity is to model one's scaffolds after proteins found in the human body. For example, the FDA-approved Kalbitor is based on the common Kunitz domain of protease inhibitors, and clinical trial participants have not suffered immunogenic responses. When brought to market, Kalbitor was one of only two approved therapies to treat cardiovascular attacks of this sort,

which can cause rapid and serious swelling of the face or other parts of the body that may result in permanent disfigurement, disability, or death; the other is a protein therapeutic derived from human blood.

A major factor holding back the field of antibody alternatives as therapeutics is their small size. While this improves their intracellular function and use in research applications, their low molecular weight means that they are rapidly cleared from the body via the kidneys, reducing their potential therapeutic impact. Various strategies have been employed by the industry to overcome this rapid renal clearance, such as adding an antibody domain or an albumin-binding domain to the scaffold, or increasing the molecular weight of the protein scaffolds (though they still remain significantly smaller than a corresponding antibody). The fusion of an antibody domain to a protein scaffold can also help engage the immune system for improved therapeutic benefit.

While their small size can be a hurdle in developing antibody alternatives in a clinical setting, it is a big advantage in their use as laboratory tools, allowing them to penetrate bodily tissues that are inaccessible to antibodies and offering more-precise molecular labeling. In 2012, for example, Silvio Rizzoli of the European Neuroscience Institute and Center for Molecular Physiology of the Brain in Göttingen, Germany, and colleagues used 15 kDa aptamers to capture the dynamics of endosomal trafficking in live cells using super-resolution imaging.[11] Doubling the molecular weight of the aptamer resulted in a substantial reduction in image quality, showing the importance of the small size of intracellular labels in accurate imaging of the intracellular space.

The increased intracellular stability of protein scaffolds as compared with antibodies is also critical to their function as research tools and offers potential therapeutic benefit for intracellular targets. For instance, protein scaffolds have been used to investigate the function of the small intracellular domain of a matrix metalloproteinase, which was shown to determine protein turnover to help regulate protein function in cell movement.[12] Using antibodies in the reducing environment of the cell interior in such a study would be impossible.

When developing antibody alternatives for research, scientists are purposefully mimicking natural proteins to avoid interference by the immune system. For example, Janssen produces protein scaffolds called Centyrins that are based on the fibronectin glycoprotein of the extracellular matrix. Our own company, Avacta Life Sciences, recently introduced Affimer scaffolds, which are based on the cystatin protein family of common protease inhibitors. The use of consensus sequences from a number of species may allow these reagents to be used across a variety of different model systems.

Nucleic acid aptamers and protein scaffolds may also help fight emerging outbreaks of acute infectious disease. Examples of recent outbreaks that have caused considerable social, economic, and political stress are not hard to come by—SARS in 2003, the H1N1 flu pandemic in 2009, the Ebola crisis of recent years, and the continued emerging threat of MERS. It is impossible to predict such episodes, and alternative affinity reagents could be crucial tools in quickly stemming the spread of pandemic diseases. Screening libraries of 10 billion sequences can take as little as 7 weeks. And while the processes required for optimization, scale-up, and subsequent culture and validation of substantial quantities of the required affinity reagent remain to be explored, this all may take only a matter of months.

Over the past few decades, the rate of advancement of genomic technologies has outpaced proteomics. Yet it is the expressed protein within a cell, not the underlying genetic blueprint, that executes correct or aberrant function. In order to unify and make sense of the numerous data sets being produced, scientists need tools that enable the unraveling of proteomics. This requires affinity reagents that can specifically target individual protein isoforms and glycoforms, and that can tag all the proteins within a cell or organism. While the concerns over antibody irreproducibility are increasing, the solution may already be available. ■

Nucleic acid aptamers and protein scaffolds can target proteins that remain inaccessible to antibodies, and researchers have designed them to be functional in a wider range of conditions.

Jane McLeod is a science writer at Avacta Life Sciences, where Paul Ko Ferrigno is the chief scientific officer. Avacta Life Sciences sells peptide aptamers, one of the main forms of antibody alternative.

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The Literature

EDITOR'S CHOICE IN PLANT BIOLOGY

Fungal Security Force

THE PAPER

S.S.M. Soliman et al., "An endophyte constructs fungicide-containing extracellular barriers for its host plant," *Curr Biol*, 25:2570-76, 2015.

Taxol (paclitaxel) is a potent cell-division inhibitor and anticancer drug produced naturally by yew trees (*Taxus*) and their resident nonpathogenic fungi, called endophytes. In 2008, Sameh Soliman, a PhD student in Manish Raizada's lab at the University of Guelph in Ontario, was trying to coax a *Taxus* endophyte (*Paraconiothyrium*) to ramp up its Taxol production. To Soliman's surprise, he not only detected Taxol in the growth media, but also a resinous substance that turned out to be hydrophobic spheres containing the compound. "It was a really unique structure," says Soliman.

To find out more, Soliman and Raizada stepped out of the lab and into the woods, sampling yews from the university's arboretum. They knew the endophytic fungus grew in the plant's vascular system, but DNA and microscopy analyses revealed that it also accumulated where branches emerged from the tree trunk.

When new yew branches start growing, they cause a deep crack in the wood, breaching vascular tissue. "It's a dumb mechanism," leaving the plant vulnerable to pathogens, says Raizada. But the endophyte's presence suggested it might not be so dumb after all.

Raizada's team most recently found that Taxol-filled spheres aggregated outside the endophytic fungi at these wood cracks, forming a barrier that sealed off the vascular system. To see whether *Paraconiothyrium* could detect and affect pathogens, the researchers grew the endophyte and a wood-decaying fungus together. The endophyte released its Taxol-containing hydrophobic bodies near points of contact, inhibiting the pathogen's growth. The researchers also injected one-year-old yew seedlings with a fungicide to kill the endophyte; these seedlings later succumbed when inoculated with a pathogen, which control seedlings resisted. "That experiment showed, sure enough, that the endophyte protects against the pathogen in the plant," says Raizada.

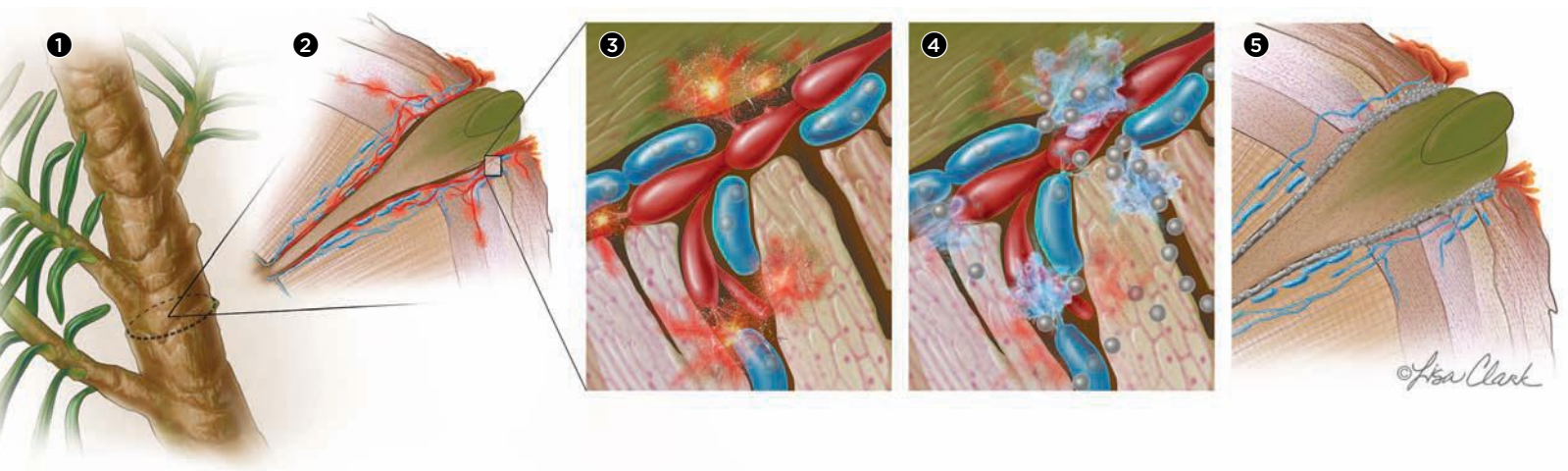
James White, a plant pathologist at Rutgers University, says the result "fits one of the hypotheses regarding endophytic microbes—that in some cases they are defenses, bodyguards for the host." Raizada points out that plant cells are immobile. With an endophytic fungus, he speculates, the plant gets a mobile immune system in exchange for nutrients.

To identify a trigger for Taxol release, the researchers exposed the endophytic fungus to a gaseous compound called chloromethane that pathogenic fungi produce during wood decay. The compound upregulated Taxol production and the expression of genes responsible for exocytosis. Chloromethane also acted as a solvent to pull the Taxol out of its sequestration.

"The localization of the Taxol in these bodies, and the fact that they proliferate and are released in a very targeted way against these pathogenic fungi, was pretty amazing," says Keith Clay, an ecologist at Indiana University. "It seems like one of the most dramatic cases that I'm familiar with where an endophyte associated with a plant is producing these bioactive compounds in such a precise way."

—Karen Zusi

INTRUDER ALERT: Buds growing from under the bark in a yew tree ❶ split the wood open down to the vascular tissue, allowing pathogenic fungi (red) to enter. Endophytic fungi (blue) grow towards the crack to combat the invasion ❷. The pathogen gives off chloromethane (red cloud) as it starts to decay the surrounding wood ❸. This induces the endophytes to release hydrophobic spheres (gray balls) containing the antifungal chemical Taxol. The chloromethane encounters the spheres and causes the Taxol to be released (blue spray), killing the pathogenic fungi ❹. The hydrophobic spheres also accumulate and seal up the crack to prevent future infection ❺.



ADJUST OR BUST: Pollen grains (green) and their tubes (white) that lack the MSL8 ion channel leak their cellular contents (blue) during germination.

PLANT BIOLOGY

Hydropowered Pollen

THE PAPER

E.S. Hamilton et al., "Mechanosensitive channel MSL8 regulates osmotic forces during pollen hydration and germination," *Science*, 350:438-41, 2015.

GETTING HYDRATED

When a dry pollen grain first lands on a flower's stigma, secretions from the flower rehydrate the grain. Eventually, enough inner pressure builds up for the pollen to grow a tube towards the flower's ovaries. Elizabeth Haswell, a plant molecular biologist at Washington University in Saint Louis, wanted to know how the grain's cells sense and manage these mechanical changes.

ELECTROPHYSIOLOGY

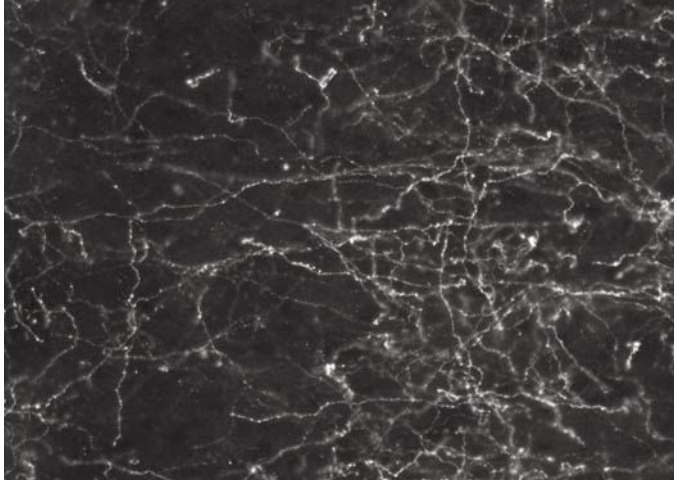
Haswell suspected an ion channel was involved, given that membrane tension-sensing channels protect *Escherichia coli* from taking in too much water. Fluorescent tagging revealed that a related gene, *MSL8*, was active in *Arabidopsis thaliana* pollen grains and tubes. When Haswell's team expressed the gene in *Xenopus laevis* eggs, the ion channel's conductance changed in response to membrane tension.

UNDER PRESSURE

When Haswell's group grew *A. thaliana* strains with reduced levels of MSL8 and immersed the pollen in a germination solution, the grains burst, along with the pollen tubes if they had begun growing. When pollen grains overexpressed *MSL8* instead, they were unable to form tubes. The team concluded that MSL8 controls the osmotic potential for the cell. "There's a balance, between having too little or too much, that the pollen grain needs so it can survive hydration but build up enough turgor to germinate," says Haswell.

BALANCING OUT

"The main component of pollen rehydration is how to control the water going into the pollen grain," says José Feijó, a plant cell biologist at the University of Maryland, College Park. "This is a mechanism to accommodate the changes in volume and allow them not to be too abrupt or too drastic." —Karen Zusi



NETWORKING: Newborn rats treated with a gestation-lengthening hormone had more dopamine-reactive neurons.

DEVELOPMENTAL BIOLOGY

Hormone Hangover

THE PAPER

J. Willing, C.K. Wagner, "Exposure to the synthetic progestin, 17α-hydroxyprogesterone caproate, during development impairs cognitive flexibility in adulthood," *Endocrinology*, doi:10.1210/en.2015-1775, 2015.

PREVENTING PREMATURITY

Expectant moms at risk of premature delivery may receive a steroid hormone boost in the form of a synthetic progesterone, 17α-hydroxyprogesterone caproate (17-OHPC), which lengthens gestation. The developing brain is sensitive to steroid hormones, but few studies have looked at whether these drugs affect cognition. So Jari Willing and Christine Wagner of the University at Albany-SUNY exposed newborn rats to 17-OHPC to model a human fetal phase when cognitive flexibility develops.

DOPING RATS

The team injected rats with 17-OHPC or a control solution for 14 days after birth. Some animals were raised to young adulthood and given a behavioral task to assess their ability to locate food in a new situation. Other animals were killed on day 25 so the researchers could examine dopamine-reactive neurons in the prefrontal cortex, which require progesterone in early life for normal behavioral development.

FIBER FAILINGS

The team found that hormone-treated rats had an increased number of dopamine-sensitive nerve fibers in the medial prefrontal cortex and were slower to find the food, implying impaired cognitive flexibility. "They show that exposure to these synthetic hormones during certain critical periods can have long-term consequences on cognitive development," says Donald Stein of Emory University. The results suggest that although the brain requires progesterone for normal development, too much or too little can cause dopamine-sensitive neural pathways to develop abnormally.

IN THE REAL WORLD

In humans, 17-OHPC does pass from the mother to the placenta. But whether the results are relevant clinically is unknown, says Wagner. "Nobody's studied this in humans yet, but our work suggests the need for that research." —Jyoti Madhusoodanan

GREG JENSEN AND ERIC HAMILTON, CHRISTINE WAGNER AND JARI WILLING

PROFILE

Putting Down Roots

A survivor and a pioneer, Natasha Raikhel emigrated to the U.S. from Soviet Russia and made a career of studying protein trafficking in plants.

BY ANNA AZVOLINSKY

In 1977, Natasha Raikhel, then an assistant research scientist working at the Institute of Cytology in Leningrad (now Saint Petersburg), made a trip to Baku in Azerbaijan to collect ciliate samples from the Caspian Sea. On the return flight, the plane crashed in a potato field between Moscow and Leningrad, killing some on board. Raikhel, her husband, and their three-year-old son survived, but her perspective on life changed drastically. "It was a large plane, and it was horrific. I needed to get a statement for my institute of how the equipment, microscopes, and everything I had collected there went missing, and the airline first told me to go back to Baku, because as far as they were concerned, there was no airplane crash," says Raikhel.

After much prodding by Raikhel, the airline provided her with a statement that the airplane had had to make an "unexpected landing." "After that, I decided that I could not live in the Soviet Union anymore. This was the last drop," says Raikhel, now director of the Institute for Integrative Genome Biology and a distinguished professor in the Center for Plant Cell Biology at the University of California, Riverside. She and her then husband, Alexander Raikhel, at the time a scientist at the Zoological Institute of the Academy of Sciences in Leningrad, asked to be fired from their positions—a way to protect their coworkers who could be punished by the regime for being associated with émigrés, as emigration was frowned upon in the Soviet state—and began to pack their bags. After only four months of waiting, they received permission to emigrate to Israel, says Raikhel.

"I never expected to be where I am today. When I was elected into the National Academy of Sciences I couldn't believe that this was happening to me."

When Raikhel told her scientific supervisor she was leaving, he told her she was crazy. "'You've made it here, you have a better life than most Russians, and you are dropping everything. You will be sweeping the streets of New York and your son will be selling newspapers,' he told me. I told him, 'Look, I could have been dead, and now I have a second chance at life.'" About 10 years later, Raikhel was reunited with the professor at an international cell biology meeting in Montreal, Canada. "I invited him to dinner, and he apologized for his statements."

Because there were no direct flights to Israel from the U.S.S.R., everyone went through Vienna. Once there, Raikhel and her family decided to go to America and were sent by one of the American Jewish organizations to Rome for three months while waiting for permission to enter the U.S. "Less than a year after the plane crash, we were in Italy, in the free world, celebrating our son's fourth birthday," recalls Raikhel.

While in Vienna, Raikhel had written to University of Georgia protozoologist Jerome Paulin, who had visited her Leningrad lab, asking him for advice on how to look for a job, a process about which Raikhel, having always lived behind the Iron Curtain, had no clue. She received a telegram while in Rome that Paulin had found positions for both Raikhels: for Alexander, a postdoc in the department of entomology, and for Natasha, a temporary postdoctoral position in his own laboratory.

After working in the zoology department for a year, Natasha Raikhel seized an opportunity to join a cell biology lab in the botany department as a postdoctoral fellow. "The plant biology department was very active, with people like Joe Key who were instrumental in moving the plant research community forward and full of buzz about isolating genes and doing gene transformation in plants," says Raikhel.

Raikhel's initial studies on the distribution of wheat germ agglutinin in wheat seeds and adult plants led her to study the endomembrane system of plants, which sorts proteins and other cargo to various cellular compartments. Among the most cited of plant researchers, she pioneered the use of chemical genomics to perturb protein function in order to study essential proteins.

Here, Raikhel talks about how, after studying to be a concert pianist, she turned her sights to biology; how she discovered her talent as a mentor and motivator while starting the Center for Plant Cell Biology at UC Riverside; and how she works to empower women, especially in China, to become leaders in scientific research.

RAIKHEL REACHES

A supportive childhood. Raikhel was born in Germany and moved back to Leningrad with her parents, a surgeon and an X-ray technician, when she was one and a half years old. "I had extremely good parents. They loved my sister and me dearly. I grew up very psychologically secure and happy in a loving home. I was quiet and very much into music."

Unrealized aspirations. Raikhel began to study piano at age six, attending both a music school and a regular grade school. "I was sure that piano and music would be my life. I learned to be extremely disciplined, practicing for hours." But in her final year of high school, Raikhel's conducting teacher dashed her hope of becoming a concert

NATASHA RAIKHEL

Director, Institute for Integrative Genome Biology
Former Director, Center for Plant Cell Biology
Distinguished Professor of Plant Cell Biology
Ernst and Helen Leibacher Endowed Chair
University of California, Riverside

Greatest Hits

- Identified the sorting peptide signal that directs posttranslational processing of plant proteins and their transport to vacuoles
- Identified important genes that mediate vesicular trafficking machinery in plants
- Identified genes required for biosynthesis of xyloglucan, a major component of the cell wall in plants
- Was a founding director of the Center for Plant Cell Biology at the University of California, Riverside
- Was one of the pioneers of using chemical genomics to modify and study plant protein function
- As editor in chief of *Plant Physiology* from 2000 to 2005, revamped the journal into a high-impact plant biology publication

pianist. "She asked me if I was sure I wanted to do this for the rest of my life and whether I had considered teaching piano. I looked at her incredulously, like 'How could you even ask that?' It didn't take me long to figure out that I was not of the class of musicians destined to be professional performers." Raikhel stopped attending the music school and took evening classes in physics and math to be competitive enough to get into university, hiring tutors with the money she earned as a music teacher. "I didn't want to be a physician or engineer, but I loved nature, so I thought I would study biology. From my parents, I received unquestioning support. It was always 'Natasha knows what she wants.'"

Steadfast focus. "I had to work unbelievably hard to catch up the first few years at the Leningrad State University. Most of my fellow students came from specialized biology, math, and chemistry high schools, and I came from music school," says Raikhel. She was recruited by the invertebrate biology department to work on ciliated protozoa and earned a master's degree. Raikhel met her husband at the university and both wanted to pursue PhDs. The school tried unsuccessfully to send the couple to conduct research in Vladivostok—close to the borders of China and North Korea. "We both knew that if we went there, we were finished," says Raikhel. "Alex was finally given a job at the Institute of Zoology to make scientific labels for the insect department and, because I was his wife, I was given a job also—as a technician at a water purifying center, equivalent to the USDA. It was awful but we stayed in Leningrad."

For two years, Raikhel worked there during the day and spent evenings and weekends doing research at the Institute of Cytology. Her husband maintained a similar schedule at the Institute of Zoology. "We were stubborn, and it finally became clear that no one was stopping us." Raikhel's advisor at the Institute of Cytology, Yuri Poljansky, saw how hard she was working and offered her a technician position in his laboratory. In February 1975, Raikhel defended her PhD and, as was customary at the time, was given a position as an assistant professor, working in the institute's Protozoan Karyology Group. A few months later her first son was born.

RAIKHEL RALLIES

Another move. After settling in Athens, Georgia, and switching to a plant biology laboratory at the University of Georgia, Raikhel worked with Michael Mishkind on plant lectins, abundant proteins in grains and legumes that bind carbohydrates and play an important role in plant immunity and response to stress. Together, they found that wheat germ lectin has different localization patterns in

wheat, barley, and rye embryos. Raikhel continued to characterize the distribution and expression of lectins and, after seven years at the University of Georgia, applied for an assistant professor position in the Plant Research Laboratory (PRL) at Michigan State University. "I didn't know at the time that it was the premier place in the country for plant biology." Raikhel was invited to interview and fell in love with the place. "It was not because the institution was so renowned, which I didn't even really know then, but because it was really international, and I felt at home there." In 1986, Hans Kende, then the director of the PRL, offered her the position. By then her second son had just turned two. "We drove our car with the boys and our cat and a little trailer full of monoclonal antibodies in a nitrogen tank and all of our frozen materials. It wouldn't be allowed now!"

Ahead of the curve. During her interview at the PRL, Raikhel had proposed to study cell type–specific expression of the sugar-binding protein known as wheat germ agglutinin and related lectins in monocots such as rice and barley and in some dicot plants. The project was almost impossible to pull off 20 years ago, and it is still very difficult even now in monocots like wheat, she says. "Monocots have complicated polyploid genomes, long life cycles, and are difficult to transform. The plants where this type of study was possible were the dicots, like *Arabidopsis* and tobacco plants, but the lectins we were studying were not found in *Arabidopsis*."

Finding her niche. As Raikhel continued to work on wheat germ agglutinin in Michigan, she and her students noticed that the protein was synthesized as a precursor that was longer than the mature lectin. The protein was losing more than just the N-terminal signal peptide that directs it to be inserted to the endoplasmic reticulum. The lab found the C-terminus acts as a sorting signal to the vacuole, an organelle that can serve many functions, including trapping water and holding waste materials. This C-terminal portion is removed posttranslationally to form the mature protein.

The work led to the identification of sorting signals at the C-terminus of other proteins as well. When this signal was deleted, the protein was secreted instead of being targeted to the vacuole. Raikhel's lab also added this signal to an unrelated protein that is normally secreted and was able to find it in the vacuole. "We did the study at the same time that yeast researchers found a different targeting signal for the yeast vacuole. I immediately concentrated my lab on understanding the mechanisms of trafficking to different organelles of the cell."

Raikhel's lab has since worked on the secretory system in plants and the mechanisms of protein synthesis, modification, and final delivery into the plasma membrane, cell wall, or vacuole. In the early 1990s, Raikhel's lab began to work on *Arabidopsis* when the plant first started to become a popular plant model system. "I was trained as a cell biologist, so I had to learn genetics and molecular biology."

A center of her own. In 2001, Raikhel was recruited to join the Department of Botany and Plant Sciences at UC Riverside, and to establish the Center for Plant Cell Biology. "I wanted the center to serve all science departments at the university. Infrastructure helps everyone. Science is complicated and there is no one that can do everything." Raikhel hired a bioinformatician and a microscopy specialist and recruited new plant biology faculty. "I am good at pulling people together, and have learned that I have an innate ability to talk to and inspire people, but also to be straightforward. People come to me to discuss their strengths and weaknesses, and I know they trust me."

A chemical bounty. In her new position, Raikhel began to focus on using chemical genomics to study the functions of essential plant proteins, as an alternative to using the classic genetics approach of mutagenesis to study gene and protein function. "Many genes that function in intermembrane trafficking are essential or redundant. Chemical biology allowed us to screen a library of chemicals and find ones that specifically perturb a protein of interest and study the phenotype." In 2004, her lab's first paper using the approach identified several compounds that could be used to study vacuolar sorting in *Arabidopsis*.

Chemicals as tools. Raikhel's lab has continued to comb through chemical libraries in search of compounds useful in the study of plant molecular biology. The team identified bioactive chemicals that block endocytosis—the process of protein delivery via vesicles—and also exocytosis, vesicular delivery to the outside of the cell. The chemicals could be useful to further elucidate these processes, says Raikhel. In another screen, Raikhel and her colleagues identified other bioactive chemicals that target endomembrane trafficking in *Arabidopsis*.

RAIKHEL RUMINATES

An independent spirit. "I didn't want to be that generation of immigrants that just came to this country because of their children. I wanted to live too, to have an independent life and not to put myself second always. But I never expected to be where I am today. When I was elected into the National Academy of Sciences I couldn't believe that this was happening to me."

Women's ambassador. Raikhel often travels to China to give research talks, but also to speak to and mentor young female scientists. "They need to see more models of successful women in high positions, and there are not many there yet, unfortunately. I am helping to create a new institute and have convinced the institute to appoint a female scientist just elected to the Chinese National Academy of Sciences to be one of the codirectors."

Music lover. "I go to concerts and the opera all the time, and I have a baby grand piano at home. I play just for myself, but maybe I will get a teacher and play more now that I am retiring."

Art as life. "Music and art have sustained me through emigration, divorce, cancer, and many problems that I have dealt with in life. It is such an important part of my life. Many scientists don't have that same exposure. I had a rule in my lab in Michigan that no one could leave my lab until they visited the Art Institute of Chicago. One of my goals is to bring science and art together for young people, to organize a workshop and bring visual artists, musicians, and scientists together." ■

Jason Holliday: Tree Tracker

Associate Professor, Virginia Tech, Department of Forest Resources and Environmental Conservation. Age: 37

BY JEF AKST

Jason Holliday earned his undergraduate degree from the University of Victoria in British Columbia, where he worked on sea urchins and chicken embryos in a developmental biology lab. He then headed to Stanford University as a research assistant in a cell imaging lab studying signal transduction in mammalian cells. But when it came time for grad school, Holliday was ready for a change. He was interested in ecological genetics, and he knew that trees are key species in many ecosystems. He also knew there was funding for tree research. So he decided to visit the University of British Columbia's Sally Aitken, who studied tree evolution.

"I was just very impressed," Aitken says of her first visit with Holliday. "He's clearly very smart and also very personable." Even before he officially joined Aitken's group, Holliday was out collecting samples for a project on how spruce trees adapt to climate variation. "I threw him into the deep end, really," Aitken recalls.

Shortly after Holliday joined the lab, the group and its collaborators landed funding from Genome Canada to expand their study of spruce genetics. Holliday looked at gene expression levels in Sitka spruce plants grown from seeds gathered from trees throughout the species' natural range to study growth patterns and the trees' ability to withstand freezing. "Local populations need to appropriately time these transitions, as they can't both grow and be substantially cold-hardy at the same time," Holliday says.

He also compared single-nucleotide polymorphisms (SNPs) in candidate genes, such as those that influence cold hardiness, and the timing and rate of growth in the spring. Holliday and his colleagues identified signals of local adaptation as well as clear signs of multiple genetic bottlenecks. It appeared that spruce had migrated out of the Seattle area in a series of postglacial jumps northward to Alaska, growing new populations from a relatively small number of seeds each time.[1] "With every dispersal and with every colonization you get a bottleneck [and] lose variation," Holliday explains.

According to Aitken, Holliday's work helped launch the $3.5 million AdapTree Project, which Aitken coleads with Andreas Hamann of the University of Alberta. The project, funded by Genome Canada, aims to document the genetic basis of climate adaptation. "The work that Jason did in my group, with others involved, that was really the proof of concept for the AdapTree project," Aitken says.

"His work was among the very best in the world at that point in time," says University of California, Davis, forest geneticist David Neale. "It was broad; it was comprehensive; it was carefully done."

In 2009, before he'd even gotten his PhD, Virginia Tech offered Holliday a faculty position. Two years later, he was awarded an Early Career Grant from the National Science Foundation to launch a genomic interrogation of the black cottonwood tree (*Populus trichocarpa*). Now, in collaboration with Neale and others, Holliday studies genetic variation along both latitudinal and altitudinal transects. His team is also examining the genetic mechanisms that underlie the tree's switch from growth to dormancy, which can be triggered by day length, nutrient limitation, and other factors.[2]

For this work, he has pioneered the use of sequence-capture technology, which employs 200,000 unique RNA probes for a total of more than 40,000 black cottonwood genes[3]—and he encouraged Aitken and her colleagues to use it in their AdapTree work. "That proved to be an excellent decision," Aitken says. "It was key to the success of that project." ■

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Marriages of Opportunity

New ideas for antibody-drug conjugate design

BY JEFFREY M. PERKEL

Until relatively recently, chemotherapies for cancer have walked a precarious line between maximizing tumor cell death and minimizing toxicity. With few exceptions, physicians simply had no way to direct these potent drugs solely to where they were needed.

Now, they do. Using antibody-drug conjugates (ADCs), oncologists can aim a chemical payload at any cell for which they can identify a specific antigen, such as the HER2 receptor on breast cancer cells.

ADCs—tripartite structures in which antibodies are covalently joined to toxins via a linker—are the biological equivalent of a smart bomb. In theory, the configuration renders poisons inert as they circulate through the body, preventing them from doing harm until they are released and activated inside the targeted cell, and pharmaceutical companies have embraced the concept: dozens of ADCs are now in clinical trials, and two have been approved by the US Food and Drug Administration.

What does it take to make a good ADC? A good antibody and a potent drug, of course, but also good design. For instance, pharmacokinetic properties can vary dramatically depending on the number of drug molecules an ADC carries, says Jonathan Drachman, chief medical officer and executive vice president of research and development at Seattle Genetics. When antibodies are loaded too heavily, he says, they tend to clear more rapidly from circulation, and also to aggregate. Drug developers thus strive to control precisely how many drug molecules each antibody molecule bears. The sweet spot is typically a drug-to-antibody ratio of two or four.

A related and critical variable is the drugs' positioning on the antibody. Avoiding an antibody's antigen-binding site is a must, but there's more to it than that. In 2012, researchers at Genentech demonstrated that an ADC can have very different properties depending on the chemical environment of the amino acids that surround a toxic molecule, a finding they attributed to the stability of the chemical linkage under those different conditions (*Nat Biochem*, 30:184-89, 2012).

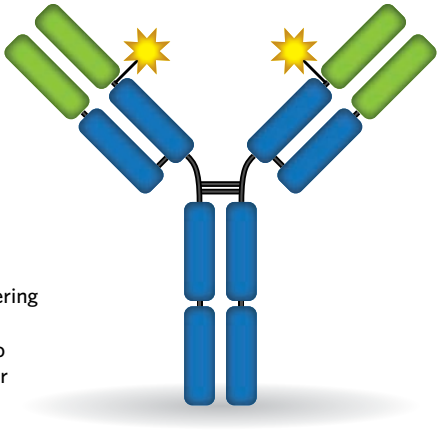
ADC developers have devised multiple strategies for ensuring their molecular cargoes end up precisely where they want them. These technologies have been commercialized by pharmaceutical companies, but they typically spring from academic labs. According to Vaughn Smider, an assistant professor of cell and molecular biology at the Scripps Research Institute in La Jolla, California, who penned a recent review on ADC conjugation strategies using unnatural amino acids (*Mol Pharmaceutics*, 12:1848-62, 2015), researchers can generally direct conjugation with little more than site-directed mutagenesis and some easily available plasmids. "It may not be plug-and-play, like getting a GFP vector; a research lab would have to do a little optimization," he says. "But it's not an enormous undertaking to get it up and running." Here are four options.

ENGINEERING CYSTEINES

The two ADCs currently approved for market, Genentech/Roche's Kadcyla and Seattle Genetics' Adcetris, couple their payloads via endogenous amino acids in the antibody sequence—lysines in the case of Kadcyla and cysteines for Adcetris. Neither approach allows researchers to precisely control drug placement and abundance, as there are more conjugation sites available than needed in both cases. Although ADCs typically carry two or four drug molecules, antibodies have eight available cysteine attachment sites and "probably 70 surface-exposed lysines, at least," according to Hans Erickson, associate director of the ADC and Therapeutics Proteins group at Genentech Research and Early Development. That results in a heterogeneous mixture: some efficacious antibodies, some antibodies with no drugs attached, and others with too many, which can be unstable and toxic.

In 2008, Genentech published a more precise approach. Called THIOMAB, the strategy allows control of exactly where drug/linker complexes are attached by engineering the antibody genes to replace one or more amino acids with cysteine, thereby providing a defined and selectable chemical handle upon which to attach another molecule (*Nat Biotechnol*, 26:925-32, 2008).

In a head-to-head comparison, THIOMAB-based conjugates containing two modification sites were more stable, more potent, and less toxic in preclinical models than ADCs prepared by linking the drug to endogenous cysteine residues. The THIOMABs also lasted longer in circulation than traditional ADCs,



CYS MARKS THE SPOT: Genentech's THIOMAB strategy involves engineering cysteine residues into the antibody backbone to provide defined points for drug conjugation.

COURTESY OF GENENTECH

Erickson says, with pharmacokinetics comparable to unmodified antibodies. "That was a huge, huge, impactful result," notes Erickson.

Other ADC developers have adopted similar strategies. For instance, Seattle Genetics's SGN-CD33A, entering Phase 3 trials in 2016, is based on what that company calls "engineered cysteine antibody," or EC-mAb technology.

REMOVING BLOCKS

Though simple in concept, THIOMABs do require some careful chemistry. When antibodies are synthesized, Erickson explains, the engineered cysteines are sometimes blocked due to modification by free cysteine or glutathione in the cell culture media, preventing them from coupling to the drug-linker complex. To get around that, researchers subject the protein to relatively gentle reducing conditions. That removes whatever might be bound to the engineered cysteines, but also breaks interchain disulfide bonds, which are not the intended drug targets. Allowing those bonds to reform yields a molecule in which the only available conjugation site is the engineered cysteine.

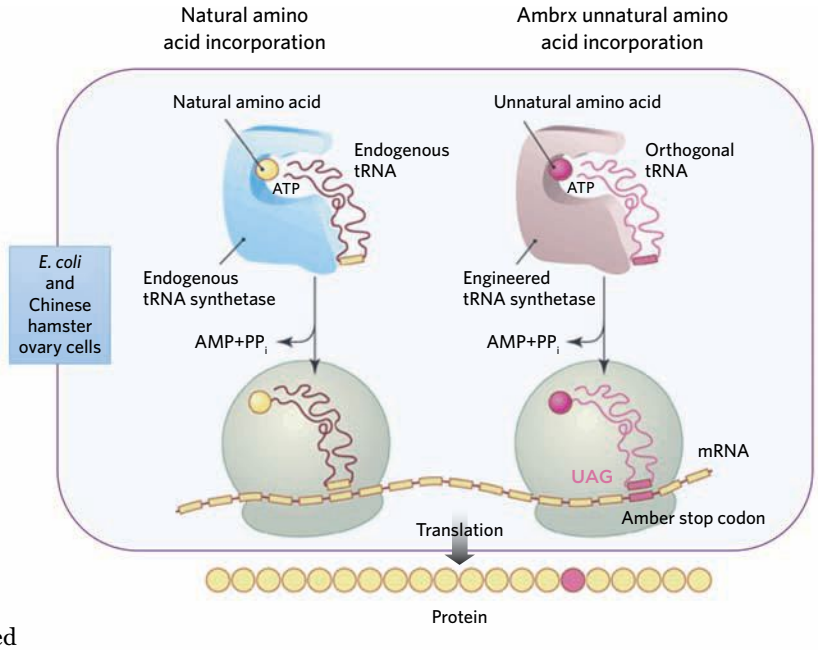
SUPPRESSING AMBER IN VIVO

Founded by Scripps Research Institute researcher and new Scripps CEO Peter Schultz, San Diego–based Ambrx takes an entirely different approach to antibody-drug conjugation.

Schultz and his team have spent years extending the genetic code beyond its normal complement of 20 amino acids, essentially teaching cells to insert nonnatural (or noncanonical) amino acids wherever and whenever the researchers want.

During translation, enzymes called aminoacyl-tRNA synthetases (aaRSs) couple specific amino acids to their cognate transfer RNAs, preparing the tRNA molecules for their role in translating the language of RNA into that of proteins. Phenylalanine aaRS, for instance, links phenylalanine to tRNAs targeting the codon UUU. Schultz engineered an aaRS to accept amino acids not normally found in nature, such as para-acetylphenylalanine, and place them onto a special tRNA that recognizes an amber codon, one of the stop codons that typically indicate the termination of protein synthesis. This series of genetic alterations directs the cell not to terminate translation when it encounters the amber stop, but to insert an unnatural amino acid instead.

Schultz then expresses the new enzyme and tRNA in a host cell from a different species (a necessary precaution to avoid confusing the host cell's translation machinery and to ensure high fidelity), feeds the cell the new amino acid, and lets nature take care of the rest. All researchers need do is modify their gene of interest with the amber codon in the desired location and their protein will be endowed with new chemical functionality.



EXTENDING THE CODE: Ambrx's technology allows researchers to precisely place "unnatural" amino acids into proteins by overriding the amber stop codon. An engineered aminoacyl tRNA synthetase couples an unnatural amino acid onto an engineered tRNA, which then inserts the new amino acid into the growing protein product at a site designated by the amber stop codon.

"This is a very precise process, and the cell will do the job for us, incorporate the nonnatural amino acid anyplace we want," says Ambrx Chief Scientific Officer Feng Tian.

To date, Schultz and others have incorporated more than 100 nonnatural amino acids into proteins, and he founded Ambrx in 2003 to commercialize the technology. One of the company's most popular choices, para-acetylphenylalanine, introduces a chemical group called a ketone that is not otherwise found in proteins. In the presence of hydroxylamine, proteins bearing the ketone group form a highly specific, irreversible bond under physiological conditions, a strategy Ambrx researchers exploited in 2014 to couple the highly potent microtubule-targeting agent auristatin to antibodies targeting both breast and colorectal cancers, producing homogeneous ADC preparations (*PNAS*, 111:1766-71, 2014).

The company has implemented this system in both bacterial and mammalian cells. The first human Phase 1 trial of an ADC built using mammalian technology, an anti-HER2 conjugate called ARX788, kicked off at the end of 2015, Tian says.

MAMMALIAN MALADIES

Those researchers wishing to try this approach with their own antibodies can obtain the necessary constructs for bacterial expression from Schultz, or from Addgene, which offers a handful of the lab's materials. But for mammalian cell work, the process is considerably more challenging. "We created a mammalian

COURTESY OF AMBRX

cell line that stably expresses the tRNA and the synthetase in the genome of CHO [Chinese hamster ovary] cells, and that is very difficult to create," Tian says.

SUPPRESSING AMBER IN VITRO

While the other companies in this roundup rely on cells to produce their antibodies, Sutro Biopharma has gone in vitro.

The company, located in South San Francisco, has taken an approach similar to Schultz's and converted it into a simple, scalable, cell-free biochemical reaction: mix cellular extract and DNA, incubate, and voilà! "Ten hours later, you've got a gram per liter of whatever protein you want to make," says CSO Trevor Hallam.

To keep translation running smoothly, the extract contains an energy-recycling system that uses glutamate and an intact citric acid cycle to drive ATP regeneration, Hallam says. "It's as if we are running a living system in a test tube."

The primary advantage of the cell-free approach, says Hallam, is flexibility. Traditionally, for every antibody version researchers want to test, they have to transfect and express that gene in a different cell clone. But cell-line development takes months, and protein production must also compete with the cell's own needs, limiting yield. A cell-free approach separates the preparation of the protein-producing extract, the raw material of this process, from that extract's use, Hallam says, making it a simple matter to test dozens of variants simultaneously and rapidly scale up the most promising hits.

In one case, he says, Sutro researchers created and tested 450 different versions of a Herceptin ADC, which targets the HER2 receptor on breast cancer cells, using a library of gene mutants, each bearing an amber stop codon in a different location. "It took us five days to find the optimal binding site [for the drug linker]," he says. "The speed for design is incredibly fast."

DOUBLE TROUBLE

Want to use multiple chemistries simultaneously? It's possible, Hallam says. Sutro has incorporated two different synthetase-tRNA pairs, which override two different stop codons, into its extracts. "You can have two different nonnatural amino acids in one protein, and conjugate that to two different linker warheads," he says. "And you still produce a homogeneous molecule." Those researchers who want to create their own extracts can find details in the work of company cofounder James Swartz of Stanford University, who first developed the cell-free protein expression technology Sutro uses today (e.g., *Nucleic Acids Research*, 41:5949-63, 2013).

A CHEMOENZYMATIC ALTERNATIVE

Catalent Pharma Solutions, based in Somerset, New Jersey, uses a chemistry called SMARTag, which it obtained as part of its 2014 acquisition of Redwood Bioscience, to endow antibodies with novel chemistries.

According to David Rabuka, founder of Redwood and now global head of research and development for Chemical Biology at Catalent, SMARTag exploits formylglycine-generating enzyme (FGE), which locates cysteine residues in the context of a specific signal sequence and converts them to formylglycine, a residue not normally found in proteins.

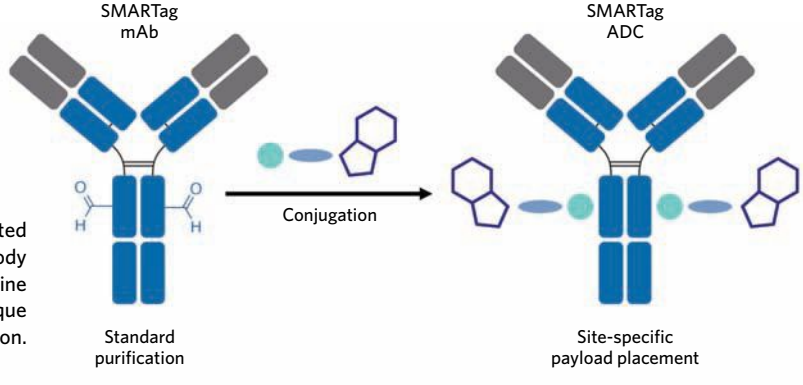
"This is a very simple chemoenzymatic method to engineer a chemical handle, a sticky bit, on the surface of a protein that can then be chemically modified," Rabuka says.

To use this system, researchers need only introduce the signal into their protein sequence and express it in eukaryotic cells that also express FGE; the purified protein will be ready to go with its cysteine-to-formylglycine conversion already complete, Rabuka says. Then, using a simple and gentle one-step chemical reaction called HIPS ligation, researchers can link anything from fluorophores to drugs in an irreversible, site-specific manner, says Rabuka.

In one proof-of-principle study, Redwood researchers used SMARTag to build several variants of Herceptin ADCs that differed only in where on the antibody the signal sequence was located (*Bioconjug Chem*, 25:1331-41, 2014). The exercise, Rabuka says, underscores the importance of drug placement in ADC design. "We see dramatic differences in efficacy."

GET SMARTAG

Stemming from work by Stanford University's Carolyn Bertozzi (then at the University of California, Berkeley), the FGE expression plasmid is available at Addgene (*Nat Chem Biol*, 3:321-22, 2007). As for the HIPS reagents, Rabuka says Catalent is partnering with a company called Click Chemistry Tools to bring those to market. "We hope they will be available in the next six months," he says. ■



A SMART ALTERNATIVE: Catalent SMARTag ADCs are created by inserting an enzyme recognition sequence into the antibody chain. The enzyme, called FGE, converts a specific cysteine residue in the antibody to formylglycine, providing a unique chemical handle for drug conjugation.

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Exercises for Your Abs

Companies make the antibodies, but it's up to you to make sure they work in your experiments.

BY AMBER DANCE

Stephan Lange had his figures all ready for the manuscript when he decided to double-check his antibody. The University of California, San Diego, cell biologist stained tissue samples from a mouse missing the G-protein–coupled receptor he was interested in, figuring the antibody wouldn't give him a signal.

But it did. He cut the figure based on that antibody from his manuscript. The rest of the story stood, though it was a bit less interesting for the loss, Lange said.

Lange is far from alone in his disappointment. Antibodies are some of the most commonly used and commonly flawed reagents in biology labs, and pricey, too. Lange points out that the typical commercial antibody—at, say, $200–500 for 100 micrograms, or $2,000–5,000 per gram—costs more than gold ($35.29 per gram as of Jan 12, 2016). But scientists say there's a lot of fool's gold in them thar antibody catalogs. In one study of more than 5,000 commercial antibodies, only half worked in both Western blotting and immunohistochemistry (*Mol Cell Proteomics*, 7:2019-27, 2008). Some researchers believe faulty antibodies bear the brunt of the blame for the fact that many scientific studies are irreproducible (*Nature*, 518:27-29, 2015; *Nature*, 483:531-33, 2012).

Antibodies are some of the most commonly used and commonly flawed reagents in biology labs, and pricey, too.

And more and more scientists are now advocating for higher antibody standards. In one effort, the Global Biological Standards Institute in Washington, DC, and collaborators aim to develop antibody-validation guidelines for researchers and manufacturers to apply to their reagents. But guidelines alone won't be enough, unless someone insists those standards are followed, points out the institute's president, Leonard Freedman. Journals might decline to publish papers with poorly validated antibodies, he suggests, or grant agencies could refuse to pay for suspect reagents. Starting this year, in fact, the National Institutes of Health will require that grant applicants detail efforts to validate the antibodies they use, though this will not affect scores, Freedman said.

Some companies that manufacture or sell antibodies are already combing through their catalogs, performing more-stringent validation tests to strengthen the credibility of their

reagents. For example, the clearinghouse called antibodies-online—with an inventory of more than a million primary antibodies and other reagents from 120 suppliers—has partnered with the independent validator Science Exchange so those suppliers can request that a disinterested lab test their products. Antibodies that pass receive a special "Independently Validated" badge in the clearinghouse's catalog.

The processes of developing standards and testing catalogs takes time, however, so for now the rule of the day is "Buyer beware." But even in the long run, the ultimate responsibility for antibody validation will lie with the user. "Scientists need to protect themselves by testing every antibody they use," says Andrew Bradbury, a molecular biologist at Los Alamos National Laboratory in New Mexico. Read on for tips on how to find the most sensitive, specific, and reproducible antibodies for your research.

Know Your Antibody Types

Polyclonal antibodies comprise all immunoglobulins generated by B cells in response to an antigen. To produce polyclonal antibodies, the first step is to inject an animal, often a rabbit, with the target protein. Serum collected from that animal can contain several antibodies which will bind to sites on the target protein, albeit with different affinities, plus a slew of other antibodies unrelated to that antigen. Often the presence of multiple antibodies directed at the same target boosts the chance of detecting a signal in your desired application.

To get a more consistent product, antibody makers culture individual B cells, fusing them with immortal cells to create hybridoma cell lines that should each make a monoclonal antibody directed at a specific target site. Then they identify those cultures that produce the desired antibodies, and use those cell lines to produce larger quantities of the monoclonals.

However, even in a monoclonal culture, the cells can stop making antibodies, or the antibody genes can mutate. Thus, Bradbury and others believe the best option is to go a step further, defining monoclonal antibodies not by the cell line that makes them, but by the DNA sequence that encodes them. For these so-called recombinant antibodies, the genes for the desired antibody would be cloned from B cells, sequenced, and—if Bradbury had his druthers—that sequence made publicly available. No matter what happens to the cell line, anyone could use that DNA or sequence to make an identical antibody. Scientists could even tweak the sequences to make all-new antibodies without ever injecting an animal.

It's also important to consider the antigen used to make an antibody. A synthetic peptide may generate an antibody that works great on a Western blot, where the proteins are denatured, but misses the fully folded version in intact cells or tissues. Conversely, an antibody generated to target an intact protein might not detect a denatured version.

Do Your Homework

Your first stop should be the scientific literature. Check what antibodies other researchers are using to track your target. Manufacturers and websites such as antibodypedia.com and CiteAb.com list references for antibodies.

Dig into those papers, advises Anthony Couvillon, scientific marketing project manager and former antibody development scientist at Cell Signaling Technology in Danvers, Massachusetts. Were the articles published in good journals? Did the authors show full Western blots, so you can see that there weren't other nonspecific bands? Did they do the proper controls?

Don't assume the most-published antibody is the best one out there, says Fridtjof Lund-Johansen, a proteomics scientist at Oslo University Hospital in Norway. Often the antibody everyone uses is just the first one that came out.

Shop Smart

One problem with antibody markets is that the manufacturers aren't always the sellers, says Jason Li, founder and CEO of Proteintech in Chicago, whose company only sells antibodies produced in-house. Antibody clearinghouses offer such huge catalogs that they have little motivation to ensure every product is a winner, Li laments.

To make good purchases, carefully review the data sheet provided by the seller. Do they show convincing data that the antibody works for your desired application? If you're looking for an antibody specific for a modified protein—such as an acetylated version—check to see if the manufacturer did a peptide array, testing the antibody against several different modifications to that protein.

HARDER EVIDENCE

By some measures, an antibody's performance comes down to a matter of opinion—does the band on a Western or stain on a tissue look strong enough, clean enough to you? A couple of scientists are working on methods that give antibodies a quantitative score or allow side-by-side comparisons.

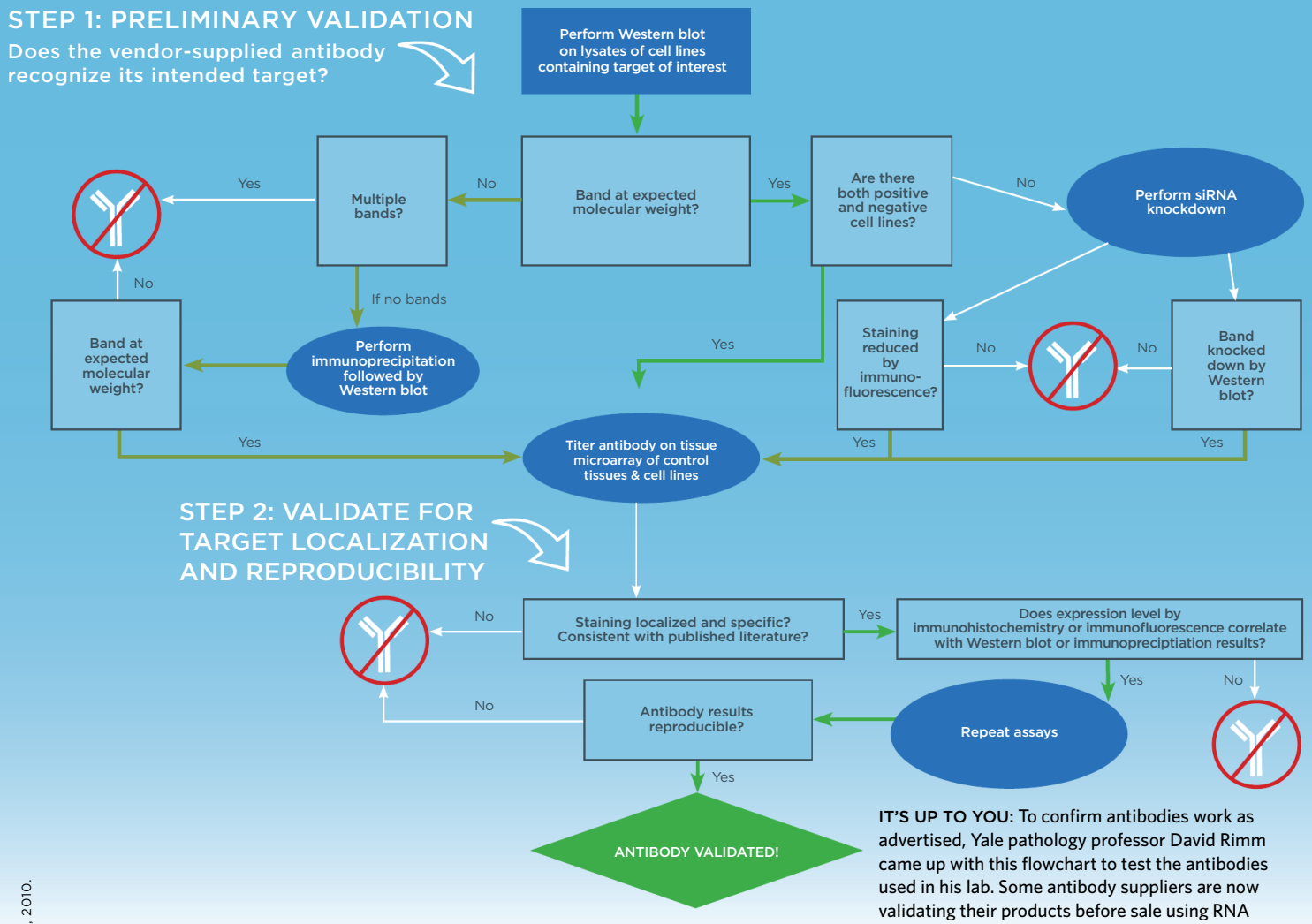
Biochemist Aled Edwards of the University of Toronto and colleagues generated 1,124 synthetic antibodies, immunoprecipitated target antigens from a cell lysate, then performed mass spectrometry on the precipitates to quantify exactly what the antibodies had pulled down. Only 354 antibodies pulled down mostly their target protein, or its known partners. (*Nat Methods*, 12:725-31, 2015).

At Oslo University Hospital in Norway, Fridtjof Lund-Johansen has developed a method that he compares to running hundreds of simultaneous immunoprecipitations. The researchers fractionate cell lysates by subcellular location and size and biotinylate the lysate's proteins for a labeling step later on. By mixing the biotinylated proteins with a pool of various antibodies, each type attached to a different color of bead, the researchers can immunoprecipitate the proteins and use fluorescent streptavidin, which binds biotin, to label them. Running the beads through a flow cytometer, they identify each antibody by the bead's color and the amount of protein bound by the streptavidin signal (*Mol Cell Proteomics*, 8:245-57, 2009). Comparing the results to mass spectrometry data from each fraction tells the researchers if their antibodies pulled something down from the fraction with the target protein or an undesirable antigen of a different size or cellular location. Lund-Johansen is also working on a method that will use denatured proteins, comparable to running numerous Westerns in parallel.

Both methods evaluate antibodies for capabilities in immunoprecipitation, which may not indicate success in other applications. However, it could at least help narrow down a panel of antibodies to a few top candidates, Edwards suggests.

Many labs won't have mass-spec capabilities, and being able to compare antibodies with either method requires precise protocols so that each antibody gets the same treatment. Edwards envisions a core facility that would compare antibodies under strict quality control. Lund-Johansen, who invented his method to identify the best antibodies for his proteomics work, hopes to partner with small antibody manufacturers to compare their products en masse.

ANTIBODY VALIDATION
Rimm Lab Verification Procedure



IT'S UP TO YOU: To confirm antibodies work as advertised, Yale pathology professor David Rimm came up with this flowchart to test the antibodies used in his lab. Some antibody suppliers are now validating their products before sale using RNA interference or knockout cell lines.

ADAPTED WITH PERMISSION OF DAVID RIMM FROM *BIOTECHNIQUES*, 48:197-209, 2010.

Couvillon suggests checking all the products a vendor offers for the same target, making sure the promises are similar. If the molecular weight or cellular location varies between antibodies that allegedly bind the same antigen, be suspicious. Pick a few antibodies to try—but he warns that if you find identical data sheets at different sellers, most likely it's the same antibody being sold under a different banner.

Check It Yourself

David Rimm, a professor of pathology at Yale School of Medicine in New Haven, Connecticut, even tests antibodies from companies he trusts. Having been badly burned by antibody issues that invalidated a clinical cancer test he had designed, Rimm developed a detailed algorithm to verify antibodies. (See figure on this page.)

One thing he looks for is sensitivity, and a high signal-to-noise ratio. On a Western blot, the antibody should label a nice clear band at the target's molecular weight, and not much else. With immunohistochemistry, it should label the tissues where that protein resides, and not others. Using immunofluorescence, it should identify the part of the cell where that protein is known to hang out. Cells or tissues that express varying amounts of the target protein should yield results that reflect those levels. If you have access to more than one antibody, look for those that give matching results.

Additionally, the antibody should find little to bind in a negative control. The gold standard is to apply the antibody to cells or tissues lacking the target protein. In 2014, Proteintech began to validate their antibodies using RNA interference (RNAi) to knock down genes in cell lines, making those its negative controls. By last fall, the company had tested nearly 800 antibodies of the 12,000-plus in its catalog. In December, Proteintech announced it will step up its rate, with a goal of RNAi validation of their complete antibody line as quickly as possible. Abcam in Cambridge, U.K., uses knockout cell lines created by CRISPR gene editing to validate its antibodies. Having started in late 2015, Abcam is working through its catalog of just under 100,000 antibodies at a planned rate of 300–500 per year, said

CEO Alan Hirzel, adding that the company has already stopped selling a handful that failed testing.

And Check Again

Found an antibody you like? Make sure it's reproducible, on a different day or by a different scientist. And check again, every time you order. Lot-to-lot variation is how Rimm got in trouble. He developed his clinical test with a panel of antibodies, but when he ordered the same products a few years later, two of them gave him different results.

This is particularly an issue with polyclonals: different bleeds or different rabbits yield different antibody cocktails. This can be controlled somewhat by strict control of the animals, Li says, such as using genetically similar rabbits of the same gender that are housed under the same conditions.

Monoclonals should be one antibody, but even they can't guarantee identical results from lot to lot. Part of the reason is that not all so-called monoclonal cultures result from a single antibody-producing blood cell, says Bradbury. Some are really oligoclonals, originating from multiple antibody-producing cells, and the dominant antibody can vary.

Recombinant antibodies offer an additional layer of protection against lot variation, but they aren't perfect, either. For example, different host cell lines might make different post-translational modifications to the recombinant antibodies, changing their affinities. Even the pH or oxygen level in the media can affect the final product. Plus, no matter what class of antibody you order, it's always possible the tube thawed at some point in transit, damaging the protein.

The processes of developing standards and testing catalogs takes time, and even in the long run, the ultimate responsibility for antibody validation will always lie with the user.

Share Your Findings

If you don't get good results on the first try, don't give up. Call the supplier's tech support. For all you know, you got a bad lot, and the company will provide a new tube. Or you might just need some help with the protocol. For example, certain antibodies are only compatible with specific methods for blocking a blot or for fixing tissues.

When you find a good antibody and publish your results, include the details, suggests Nicole Perfito of Science Exchange. By providing the manufacturer, product, and lot numbers, you'll make it easier for others to reproduce your findings. ■

Getting the Word Out

In a shifting media landscape with a growing public interest in science, some researchers are doing their own PR.

BY KAREN ZUSI

When Turing Pharmaceuticals and its reviled CEO Martin Shkreli sparked outrage last September by jacking up the price of the toxoplasmosis-fighting drug Daraprim, Bill Sullivan had a story to tell. His parasite research lab at the Indiana University (IU) School of Medicine had just published a study showing that a drug already approved by the US Food and Drug Administration successfully treats toxoplasmosis in mice, and could soon offer an alternative to Daraprim's exorbitant cost. "The fact that Daraprim was raised from $13.50/pill to $750/pill provided a strong impetus to shout our discovery from the rooftops," Sullivan wrote in an email to *The Scientist*.

He decided his group's paper, published online in *Antimicrobial Agents and Chemotherapy* in August 2015, merited special attention. "I generally leave this in the capable hands of our university's media relations team," Sullivan wrote. "However, unique situations may arise that mandate additional efforts."

His public relations office had already written a press release about the study. With that in hand, Sullivan began contacting media outlets covering the Daraprim situation. He knew the university had sent the statement out on the wires, but he wanted to reach out directly to publications "that IU may not have sent it to," he wrote. He emailed reporters and editors, sharing the press release and offering to speak about his new research. The work paid off: Sullivan successfully landed coverage of his study online in *FierceBiotech Research* and *The Scientist*. This attention also earned his research a mention in *Forbes* in December.

Not every research project has such a timely news hook, but that doesn't mean it's not worthy of media attention. More and more researchers seem to be taking it upon themselves to garner that attention. Some do so in partnership with their press offices; others strike out on their own. These practices are a shift from the traditional method of working behind the scenes and allowing public information officers (PIOs) to serve as the initiators and mediators of contact with journalists—a change spurred by an evolving media landscape. As the Internet has provided instantaneous news and increased reporter accessibility, the public has begun to demand timely information on cutting-edge science.

Paul Shaw was a postdoctoral fellow at the Neurosciences Institute in California when the media became an important part

of his career. As a nonprofit research organization, the institute relied entirely on private donors for its funding. "We weren't allowed to apply for grants," Shaw remembers. But to reach potential donors, Shaw had to make them aware of his research, which involved developing fruit fly models to study the molecular basis of sleep deprivation. "Part of my job as a scientist is to try to get my work, that's interesting, out to the public," he says.

At the Neurosciences Institute, a dedicated PIO bore the brunt of the burden, regularly checking in with Shaw and the other researchers and then reaching out to reporters. But now that Shaw is an associate professor at Washington University in St. Louis—home to more than 3,000 research projects each year and a much busier press staff—he has at times found it more efficient to personally alert members of the media to an upcoming paper. "If you have an exciting story, it's worth highlighting where it came from."

Diamonds in the rough

Not every research paper will be interesting to the general public, of course. Many scientific achievements are incremental or theoretical in nature, with less appeal to a lay audience. "We have some stories that are very much 'inside baseball,'" says Michael Levin, director of the Tufts Center for Regenerative and Developmental Biology, who studies bioelectric cell signaling during regeneration. "There will be a segment of the scientific community that will be really interested, but an average person won't be."

Knowing what makes a good read can be difficult for a researcher who has been immersed in a field of study for years, but the difference can become clear as you try to describe your research to a nonscientist. "There are things that resonate with me that may not resonate with other people," says Shaw. "There's a lot that we think is cool and that we're excited about, but don't think the public would be jumping off their couches to hear about."

Is there context you can provide that will make it interesting, even if the study itself is complex? The papers likely to be picked up by the press are those "that have a story associated with them. That's the thing," says Richard Unsworth, a marine ecologist at Swansea University in Wales.

Pitching a home run

Once a potential subject is chosen, the real challenge begins. You or a colleague must contact a publication, and you need more than just a PDF of your manuscript—you need a message to communicate. "The story" Unsworth emphasized must be written succinctly and clearly and in a way that will catch the attention of a journalist or editor.

There's a lot that we think is cool and that we're excited about, but don't think the public would be jumping off their couches to hear about.

—Paul Shaw, Washington University in St. Louis

If a PIO has already written a press release, you may opt to send that. Others prefer to craft their own pitches. Levin compares his summaries to a cover letter for a journal. "I put it into context—trying to explain why people might care about this and what the implications are," he says. Scientists managing their own direct outreach occasionally enlist input on written pitches from postdocs or students involved in the research. "I run it past people for clarity," says Shaw.

Next, you'll need to get your message to an appropriate in-box. If you've had prior experiences with the media, you can go back to writers who've shown interest in your work and who have communicated it thoughtfully. Levin and Shaw both keep tabs on journalists in their network whom they can alert to upcoming studies. "I keep a list of science writers and reporters that I trust and who do a good job. When something comes along, I make sure to let [them] know," says Levin.

For others, finding the right people to pitch requires "what scientists do best—research," Sullivan wrote to *The Scientist*. Tina Hesman Saey, a molecular biology writer with *Science News*, advises scientists to tailor their approach to a specific reporter or outlet: "They shouldn't contact just any journalist. Really take a look at what that person has done," she says. "If you like their work, and feel that they're someone who's trustworthy, then contact them." To track down a journalist, some researchers have found social media sites such as Twitter and LinkedIn valuable resources in the hunt for contact information.

Saey says she normally receives story tips from researchers as elevator pitches. "They'll send me an email, sometimes they'll attach the manuscript, other times they'll say, 'If this sounds interesting, I can send you the manuscript,'" she describes. "It really varies."

Alternatively, researchers may choose to pitch to a publication, rather than a specific journalist. Ahmad Khalil, a geneticist at Case Western Reserve University in Ohio, successfully took this approach last year when he reached out to an editor at *The Scientist* with a story tip on long noncoding RNAs. "I read *The Scientist* magazine, and when we had this story published I thought it would be a good fit," he later told *The Scientist*. His work was featured in The Literature section of last month's issue of the magazine. (See "Managing Methylation," January 2016.)

Researchers who take such initiative generally see it pay off, especially if they can find a balance between their own legwork and the support of their institutional press offices. "It reflects my time availability," says Unsworth. "Reaching out is more effort, at the end of the day, than if you give it to a PR person to run with." (See "Know Your PIO," *The Scientist*, January 2015.)

"I get the PR people involved," echoes Shaw. "And if they sell the story, great—then I can sit back and let them do their thing." But, he adds, "I will still reach out to people I know from the past."

Timing is (almost) everything

Journals dictate how early an upcoming paper can be discussed with the news media. Many scientific publications do not allow researchers to discuss the work until it has been slated for public release. Larger journals will then offer the paper to the press under embargo; a select few studies will be highlighted in a prepublication email to journalists. Typically, these notices go out a

week or less before the study is published. "It's kind of crazy to wait, wait, wait and then hurry up," says Levin. "Some of these journals won't let you say anything until a couple of days before the story comes out."

The short turnaround time can limit how a news organization arranges to cover a given study. If there are only a few days to report and write a story, other breaking news might take precedence in that time frame, says Saey. If possible, more advance notice from a researcher is helpful. By a day or two after publication, many media outlets will already see the study as old news. "If they contact me after the paper's been published, there's not a lot I can do about that as a news story. It's already too late," Saey says, though she notes that the idea might still wind up in a different article.

Differentiating between local news media and national outlets can also make or break coverage. "I'd rather get a pitch from a local physician than from a giant PR company in New York or Chicago," says Shari Rudavsky, a health and medicine reporter with *The Indianapolis Star* to whom Sullivan successfully pitched his parasite research. "That local physician has a lot more relevance for me." At a regional outlet, Rudavsky also finds herself less bound to the daily news cycle, and able to cover work that has already been published.

Levin says he can't always predict which of his pitches will be well-received. "I've been surprised when certain papers get more publicity, and I'm not exactly sure what determines that," he says. "But I kind of roll with it." And often when a pitch gets rejected, the journalist or editor will make a point of requesting future contact. After all, media professionals benefit from relationships with scientists, gaining access to research that might be overlooked by other outlets and having a reliable source to comment on related work in the field.

"I don't always cover it," says Saey, "but I always appreciate it." ■

SELECTED SCIENCE NEWS OUTLETS

News from *Science*
Type of publication: Peer-reviewed journal with editorially independent news and podcasts
Audience: Scientific community
Coverage: The latest research or science policy developments
Contact: *Science* has a full staff list of news writers and editors available online.

Nature News & Comment
Type of publication: Peer-reviewed journal with editorially independent news and podcasts
Audience: Scientific community
Coverage: Every aspect of science except clinical medicine
Contact: Brief biosketches and email addresses of the reporters and editors for News & Comment are listed with the rest of the journal staff online.

The Scientist
Type of publication: Daily online news plus a monthly print magazine
Audience: Scientific community
Coverage: Life science–related research, trends, and events
Contact: Editors in charge of the different magazine sections are listed on the online pitch page; a full staff list is also available on the website.

Science News
Type of publication: Daily online news plus a biweekly print magazine
Audience: General public
Pitch advice: All fields and applications of science and technology
Contact: News editors and writers are listed with staff at the Society for Science & the Public.

STAT News
Type of publication: Daily online news plus a biweekly podcast
Audience: General public
Pitch advice: News and investigative reports about health and medicine
Contact: Email addresses of the writers and editors are listed on the website's staff page.

New Scientist
Type of publication: Daily online news plus a weekly print magazine
Audience: General public
Pitch advice: Applied and basic science that is of interest to a general audience
Contact: Four news editors cover different subject areas, but the pitching recommendations state to contact the general news desk with most ideas.

The Atlantic
Type of publication: Daily online news plus a monthly print magazine
Audience: General public
Pitch advice: Real-world applications that explain how things and people work
Contact: Science editors receive pitches sent to science@theatlantic.com.

Scientific American
Type of publication: Daily online news plus a monthly print magazine
Audience: General public
Pitch advice: Recent discoveries, technical innovations, and ongoing research
Contact: The magazine offers a directory of editors on its press page.

Wired
Type of publication: Daily online news plus a monthly print magazine
Audience: General public
Pitch advice: Technology, science, and business
Contact: The science editorial staff is listed on the science section home page.

Mind and Matter

Research suggests that a combination of mental power and conventional medicine may be better than either alone.

BY JO MARCHANT

There are few questions as divisive in biomedical research as whether our thoughts can heal us. For skeptics, the very idea conjures visions of quacks peddling dodgy cures to desperate patients. Within the framework of conventional medicine, personal beliefs are a distraction, and drugs and other interventions are tested in rigorous clinical trials. Appointment times are kept to a minimum, and patients typically visit physicians looking for prescriptions, not reassuring chats. Surgeons certainly aren't expected to inquire about their patients' state of mind.

At the other extreme, alternative therapists (and presumably many of the millions of people who pay for their treatments) believe physical health is intimately entwined with one's mental state. Most reject evidence-based treatments in favor of long, one-on-one consultations and insist that the mind's healing power can't be captured in impersonal trials. Western researchers and physicians are misguided, they say, for focusing so unwaveringly on drugs.

It can be hard to see any middle ground, but does the choice have to be so polarizing? In writing *Cure: A Journey into the Science of Mind over Body,* I visited researchers around the world who are investigating the role of the mind in health, and I concluded that both sides of the argument have got it wrong.

I started with the placebo effect, where someone feels better after receiving a treatment that contains no active ingredient. It's traditionally dismissed as a delusion, but neuroscientists are discovering that taking a placebo can trigger physical changes in the brain just like those caused by drugs. These changes are dependent on individual attitudes and beliefs and can be surprisingly effective, particularly for relieving chronic conditions such as pain and depression, where trials show that placebos work almost as well as the most effective drugs.

The human aspects of medical care also have potent therapeutic effects. One study of 262 people found that sham acupuncture delivered by a polite but cold practitioner relieved symptoms in 44 percent of patients with irritable bowel syndrome; if the practitioner, delivering an identical treatment, was warm and empathic, that figure jumped to 62 percent. Another trial of 24 patients with acid reflux disease found no difference between placebo and an over-the-counter homeopathic supplement, but those given a 42-minute consultation improved significantly more than those who received a standard 18-minute appointment.

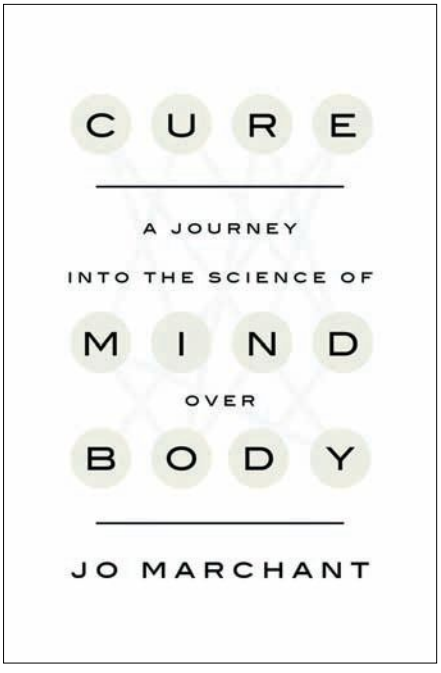
The role of the mind may go beyond improving how patients feel. Molecular studies show that a person's worldview reaches deep into their cells: social adversity boosts expression of inflammation-related genes, for example, while stress speeds cellular aging by eroding telomeres. More research is needed to work out exactly how such changes influence health, but clinical trials are finding that factors such as mood and social support can be crucial for physiological outcomes.

For example, a 2012 meta-analysis of trials involving more than 15,000 women in 16 countries found that those who received continuous one-on-one care during childbirth were significantly less likely to need an instrumental delivery or caesarean section—making this one of the only interventions known to reduce the risk of surgery during childbirth.

Meanwhile, a trial reported late last year found that patients' mood before laparoscopic procedures for vascular and kidney interventions influenced outcomes; those who felt guilty, nervous, or irritable suffered more adverse events—such as a prolonged lack of oxygen, dangerously slow heart rate, or postoperative bleeding—than those who felt neutral or positive. A series of previous trials found that talking to patients in a more empathic way during similar surgeries and encouraging them to use relaxation techniques such as visualization dramatically reduced the risk of these complications.

And in 2010, a randomized controlled trial of patients with terminal lung cancer found that those offered palliative-care sessions (in which they discussed personal issues, such as how they wished to die) were less depressed, suffered less from physical symptoms, and lived longer.

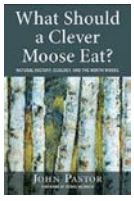
We will always need rigorous trials and physical treatments in medicine. But to best serve patients, we should also seriously investigate how we might harness the healing potential of their minds. ■



Crown, January 2016

Jo Marchant earned a PhD in genetics and medical microbiology and has written for a variety of magazines, newspapers, and websites. Read an excerpt of Cure *at the-scientist.com.*

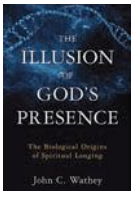
What Should a Clever Moose Eat?: Natural History, Ecology, and the North Woods
John Pastor
Island Press, February 2016



In *What Should a Clever Moose Eat?*, University of Minnesota, Duluth, ecologist John Pastor takes readers on a deep dive into the natural history of a place he clearly has a fondness for. An intellectual descendant of Aldo Leopold, Pastor chooses a domain far wider than the area of Sand County, encompassing the whole of the North Woods, which extends from the western shore of Lake Superior to the coast of Newfoundland far to the east. But his perceptive love for the ecosystem shines through just as brilliantly as Leopold's did.

As he considers the intricate natural details of the place—focusing on caterpillars, beaver ponds, and the titular moose, each in its turn—Pastor reveals an ethos that stewards of the land can (and probably should) apply on a global scale. "We are now responsible for the future of the North Woods," he writes. "Sound local, regional, and global environmental policy will need to be made on a foundation of solid understanding of the natural history of the North Woods and all biomes and ecosystems everywhere."

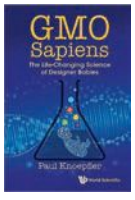
The Illusion of God's Presence: The Biological Origins of Spiritual Longing
John C. Wathey
Prometheus Books, January 2016



Could the religious experience of sensing the presence of a higher being be a holdover from the infant mind's adaptive expectation of a nurturing caregiver? Such is the argument of computational biologist John Wathey, who writes of the evolutionary origins of spirituality and religiosity in *The Illusion of God's Presence*.

Babies are hardwired to expect and respond to the presence of an almost omnipotent figure—more often than not their mothers. Wathey suggests that, as people age, this highly advantageous behavioral trait lingers, and spiritually inclined adults transfer the role of the kindly nursemaid to a more powerful, if unseen, being. In an intricately woven interpretation of the rise of religion in an evolutionary context, the author hopes to build a new model that sheds light on the doubt that some believers may experience. "Above all I have written [the book] for those who genuinely seek truth and depth of understanding," he writes in the preface, "wherever that journey may lead. If you are impressed with the achievements of science and disappointed with the unfulfilled promises of religion and spirituality, then read on."

GMO Sapiens: The Life-Changing Science of Designer Babies
Paul Knoepfler
World Scientific, December 2015

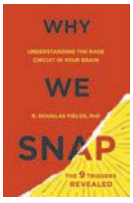


Genetically modified organisms (GMOs) are all around and in us. We genetically modified the plants we eat, the drugs we take, and the pets we cuddle long before we knew what genes or DNA were. But humanity has progressed far beyond the blunt instrument of artificial selection. Now we have razor-sharp genetic tools, and the ultimate culmination of genetic modification may be right around the corner—genetically modified humans.

In *GMO Sapiens*, cell biologist Paul Knoepfler of the University of California, Davis, explores the recent history of genetic modification, and the ethically sticky proposition of using what we've learned about how to manipulate genomes to customize our offspring. From the early days of GMO crops and in vitro fertilization to the uncertain future ushered in by the relative simplicity of the CRISPR/Cas9 technique, Knoepfler traces the explosive rise of the technology that could make genetically modified humans a reality in the very near future.

Like many other researchers, Knoepfler is staunchly opposed to the idea of designer babies, but in favor of dabbling in the modification of human DNA for therapeutic purposes. "As much as I am concerned about the possibility of GMO sapiens being created in the coming years," Knoepfler writes, "I do believe that if we speak out and take action we can greatly reduce that possibility. It is also important to encourage and advocate for useful laboratory research in the areas of genetic modification of human cells and even in special cases of human embryos limited to test tube work."

Why We Snap: Understanding the Rage Circuit in Your Brain
R. Douglas Fields
Dutton, January 2016



These days people seem to snap into a state of anger and violence rather easily. Perhaps it's the expectation of instant gratification, the ceaseless bombardment of sensory stimuli, or the cultural glorification of violence and aggression that have caused such an uptick in the behavior. Whatever the external drivers, the process of losing one's cool and reverting to a primal survival mode certainly involves internal biological processes that connect modern humans with their ancient forebears. This physiological cascade constitutes the focus of neuroscientist R. Douglas Fields's new book, *Why We Snap*.

Inspired by his own experience fighting back against a gang of pickpockets who tried to steal his wallet on a dim Barcelona street, Fields, a section head at the National Institutes of Health, details the triggers that might lead otherwise amiable people to hurl themselves into fits of rage. He coins the mnemonic device, LIFEMORTS—for Life-or-limb, Insult, Family, Environment, Mate, Order in society, Resources, Tribe, and Stopped—for the nine situations in which we are most likely to snap. The impulse to do so, however, lies deep within our genes, neurons, and brain regions, shaped by the imperatives of survival. —Bob Grant

TS Webinars

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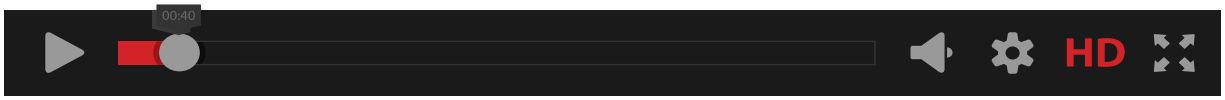
Stem cells provide an attractive, physiologically relevant cellular system for disease modeling and have the potential to play an important role in drug discovery and regenerative medicine. Innovative technologies and strategies are also being employed to assess the role of induced pluripotent stem cells and embryonic stem cells in cell therapy and translational medicine. Technical challenges persist in isolating and deriving stem cells, as well as growing them to model specific conditions for drug discovery or for understanding the repair function of stem cells. In this webinar, our panel of experts discuss the hope, the hype, and what we can realistically expect to see going forward.



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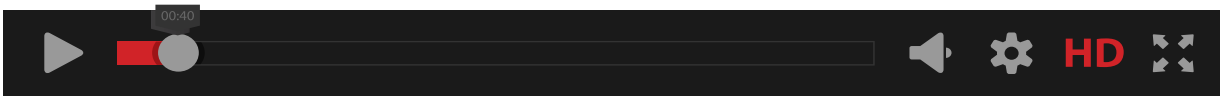

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YULING LI, PhD
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Biopharmaceutical Development
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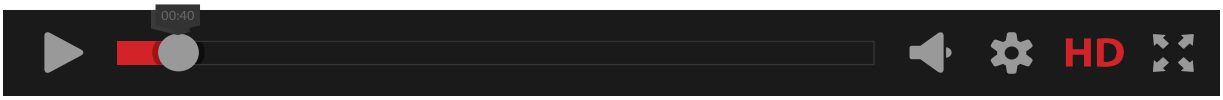
Over the last decade, the traditional toolset used to characterize RNA function has expanded. Methods exist that enable the rapid detection, localization, and quantification of RNA on a subcellular basis. *The Scientist* brings together a panel of experts to discuss emerging technologies to detect RNA and analyze its function. Topics covered include the identification and characterization of long noncoding RNAs, RNA detection with single-molecule FISH (fluorescence in situ hybridization), and single molecule RNA FISH followed by flow cytometry.



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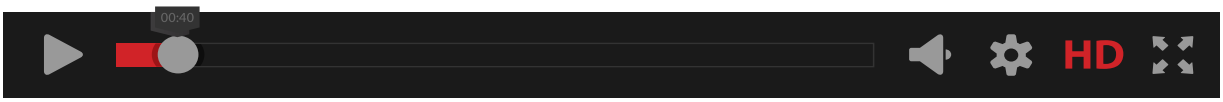

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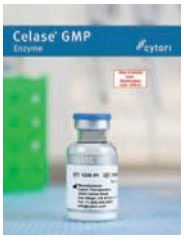

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Mendel in the Hot Seat, 1902

BY KAREN ZUSI

At the turn of the 20th century, Gregor Mendel's seminal 1866 paper on pea plants and the principles of inheritance resurfaced in the scientific community, thanks to a few intrepid botanists who had arrived at similar conclusions in their own research. Examining the findings reported in Mendel's long-buried paper, Walter Frank Raphael Weldon, an Oxford University zoologist at the time, found himself embroiled in controversy.

Weldon had excelled at statistically analyzing variations in wild populations of crabs, shrimps, and snails, and was well-equipped to take a measured look at the Mendel mania occurring among his contemporaries. "[Mendel's work] was very exciting—even potentially holding the key to a new quantitative science of inheritance," says Gregory Radick, a science historian and philosopher at the University of Leeds. "But the closer Weldon looked at it, the more skeptical he grew."

Weldon wrote to commercial pea-plant breeders asking to see samples of their crops for comparison with Mendel's descriptions. Mendel had focused on traits he designated as binary—seed color was either yellow or green, coats either round or wrinkled. Weldon, however, found much more variety. "Unless you were already convinced by Mendel, there was no reason at all to categorize the visible traits in just those two kinds of categories," says Radick, who detailed Weldon's work in *Science* last fall (350:159-60, 2015).

Weldon also took a hard statistical look at Mendel's data, and the analysis only added to his suspicion. Even if Weldon assumed Mendel's predictions were accurate, the data were just too good to be true—the equivalent of flipping a coin 100 times and getting exactly 50 heads and 50 tails.

In 1902, Weldon published his critiques in *Biometrika*, a journal he had cofounded the previous year. According to Radick, Weldon considered Mendel's data symptomatic of the wider problem with binary categories, which construct a misleading concept of genetic dominance. "Weldon's confirmed view was that biological science was just on the verge of breaking through to the next level because it was taking variation so seriously," says Radick. "But it all depended on researchers who were not satisfied with a fictional idealization of complex data—which is, in his mind, what you're dealing with when you're dealing with 'yellow.'"

For Weldon, Mendel's patterns represented special cases where humans had artificially bred out variation in domestic plants and animals. But his paper was met with harsh criticism from a colleague, William Bateson. Former schoolmates, Weldon and Bateson suffered a falling out in the mid-1890s after a series of scientific disagreements. Their rivalry continued through the early 1900s, when Weldon started to write a book detailing his thoughts on Mendel—but Weldon died unexpectedly in 1906 at the age of 46. Bateson continued to champion Mendelian principles and garnered widespread acceptance of rules still taught today in the opening chapters of genetics textbooks.

LIKE PEAS IN A POD: This photographic plate from Raphael Weldon's 1902 article displays the spectrum of pea color variation as a critique of Gregor Mendel's binary categorization of yellow and green. Images 1–6 display the color range of pea seeds of the variety Telephone as sorted by Weldon after removing their seed coats. Images 7–12 display a color scale from the pea variety Stratagem with coats removed. Images 13–18 show color variations in the two cotyledons of the same pea (Telephone variety). Images 19–24 display peas in their seed coats, which mask any color differences between cotyledons (19–20 are of the Telephone variety; 21, Telegraph; 22, Stratagem; 23, Pride of the Market; and 24, Early Morn).

"Weldon gets remembered, if at all, as the one who misguidedly obstructed the path to Mendelism," says Radick. "The winners write the history." But Weldon's ideas and his unfinished book, stored in the archives at University College London, suggest an alternative path that the early study of genetics might have taken—one that would have more closely approached a modern understanding of DNA's complexity, embracing variation and unexpected results as crucial to the system rather than exceptions. ■

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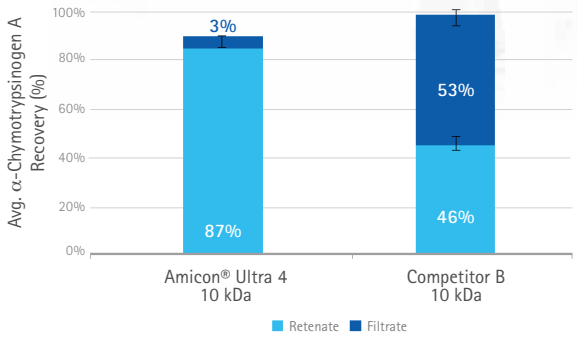

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