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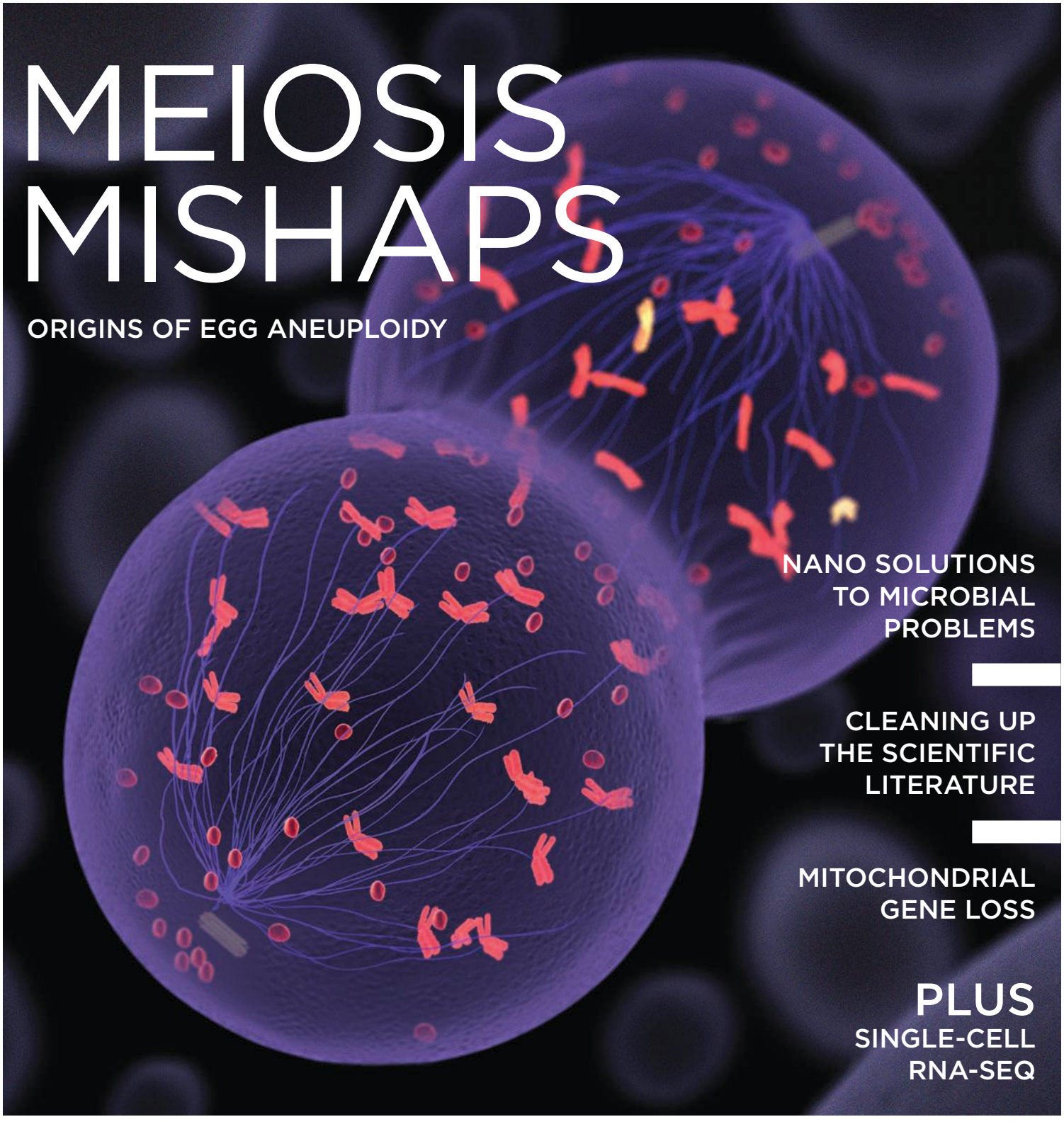

MEIOSIS MISHAPS

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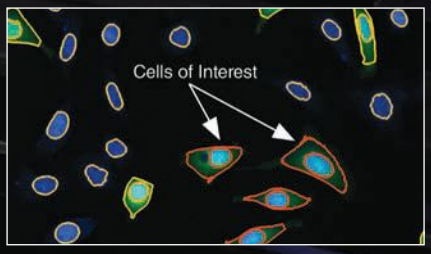

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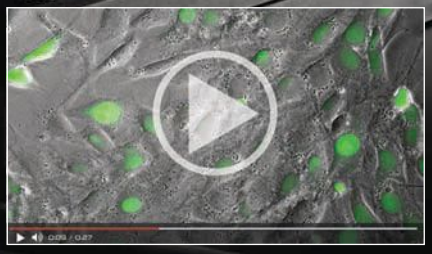

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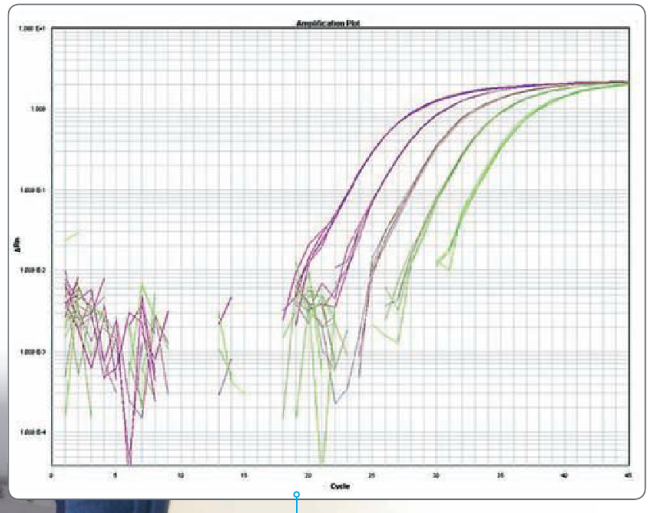

Standard Amplification Curves for COX gene. Standard cDNA samples were serially diluted 5-fold and amplified in 5 replicates (dilutions left to right: 5x, 25x, 125x, 625x and 3125x).

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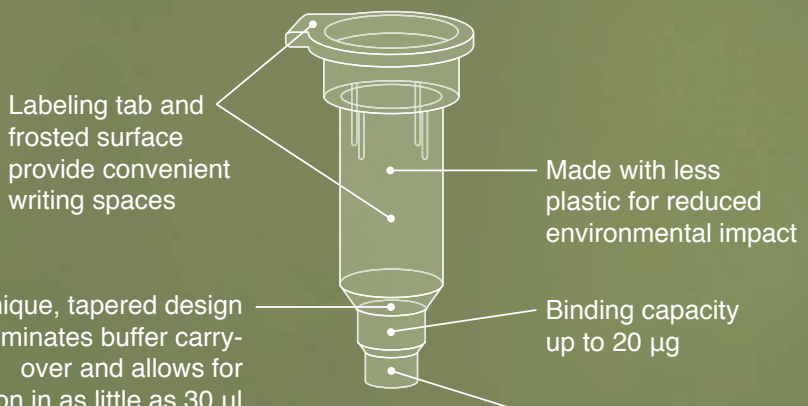

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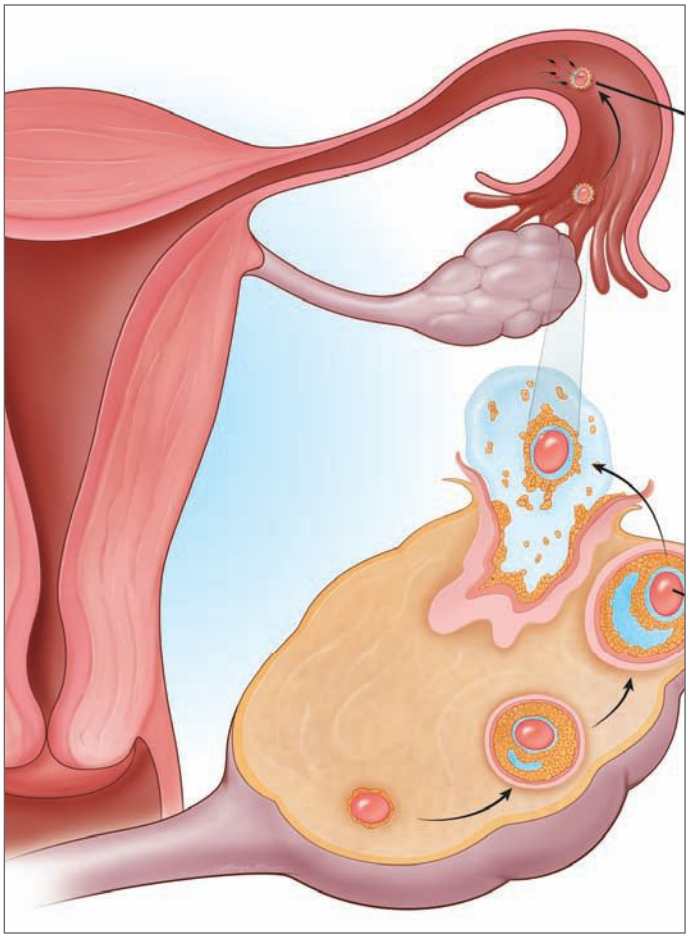

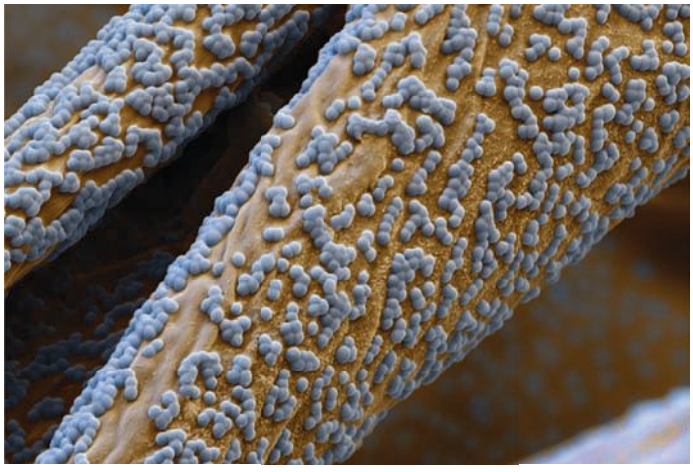

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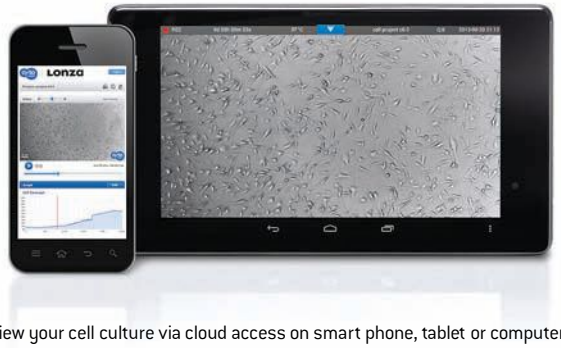

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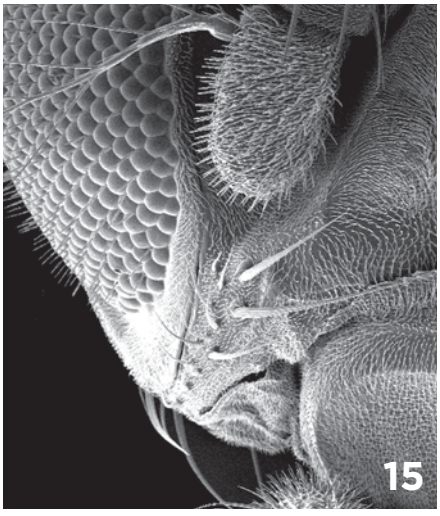

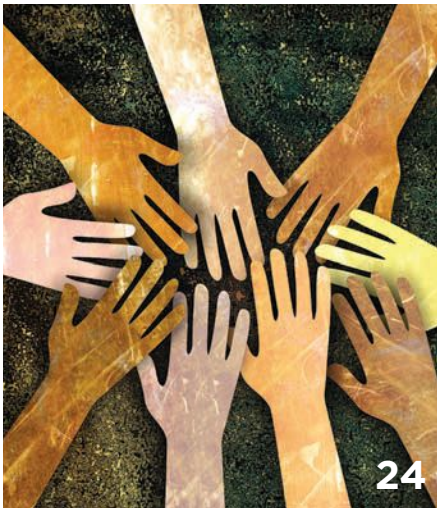

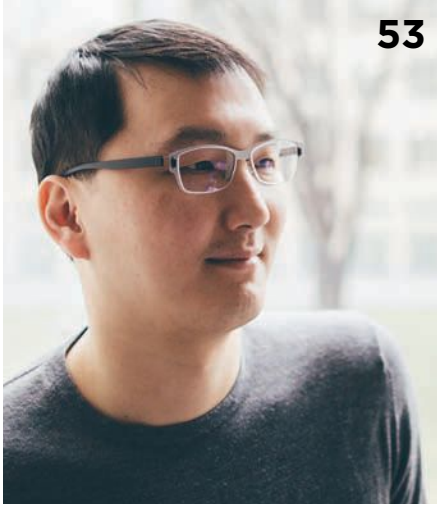

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THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Fuchs on the Future
Watch May profilee Elaine Fuchs talk about her contributions to the burgeoning field of reverse genetics.

VIDEO
Copper Stopper
See a video report on the research that found coating hospital surfaces in copper helped battle microbes and the infections they spread.

SLIDE SHOW
Monkey See, Monkey Die
What's killing howler monkeys in the jungles of Central America?

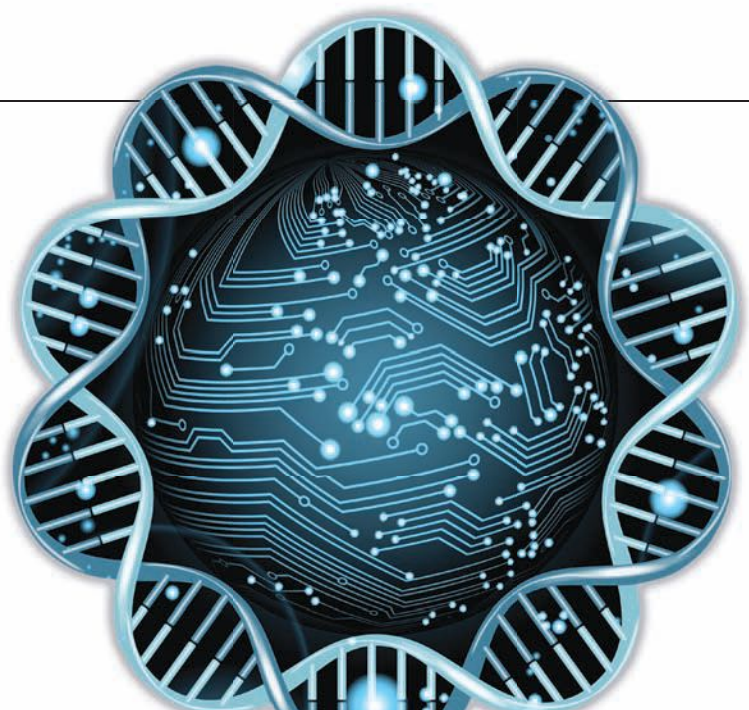
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in June

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

- Synthetic biologists create novel gene circuits.
- Getting to the root of autoimmunity
- Techniques for IDing mislabeled noncoding RNAs
- In vitro approaches to vaccine development
- The great patent debate

AND MUCH MORE



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Contributors

Graduating with a biology degree from Loyola University in Baltimore, Maryland, in 1999, **Karen Schindler** moved to Thomas Jefferson University for a PhD in biochemistry and molecular biology in the lab of Edward Winter. It was there, while working on the regulation of meiosis in budding yeast, she says, "I became really interested in how what I was studying in such a simple system related to human reproduction and fertility." Translating her experience with yeast into a mouse oocyte system, Schindler joined Richard Schultz's lab at the University of Pennsylvania as a postdoc in 2005. "He was a wonderful mentor," Schindler says. In 2011, Schindler joined the faculty at Rutgers University, where she has expanded her research to aneuploidy in human patients, while continuing her more mechanistic work in mice. "Our dream is to identify biomarkers to predict clinical outcomes and empower women to make reproductive choices based on solid genetic predictions," she says.

Schindler explores the mechanisms leading to aneuploidy in "A Scrambled Mess" (page 28).

Edward Marks encountered his first PCR machine as a New Jersey high school student. "It wasn't digital, like they are now," he says. "But I thought it was really cool how you could just take DNA and expand it." Taking advantage of a biotechnology program at nearby Rutgers University, Marks explored several areas of biotech research before graduating with a BSc in 2011. His next move was to the University of Delaware for a Master of Business and Science degree. "In some pharmas, there's a disconnect between the bench and the boardroom," he says. "I wanted to be at that interface." After graduating in 2013, Marks joined the lab of Arun Kumar at the University of Delaware to begin a PhD in nanomedicine. Now in his third year, Marks is focusing on differentiating stem cells with the aim of repairing heart tissue.

A few years ago, California-based marketing and advertising executive **Steven Smith** was ready for a career change. "I'd been doing a lot of work with demographics, and I knew I really wanted to do more with it," he says. "I wanted to leave something behind." So Smith enrolled in a master's program in the School of Hygiene and Tropical Medicine at the University of London. After obtaining an MSc in epidemiology in early 2015, Smith began working with the department of public health in California as a project coordinator for research into the 2014 pertussis outbreak in that state. "I was able to take my strength in statistical analysis and apply it," he says. He now focuses both on epidemiology and freelance writing about infectious disease. "There's a lot of cool stuff worth seeing, and I'm enjoying bringing it to the world's attention," he says.

Marks and Smith discuss nanotechnological approaches to preventing hospital-acquired infections in "Nanoscale Defenses" (page 35).

The daughter of a pair of Russianists at the University of Bristol, U.K., **Catherine Offord** considered pursuing languages for her university studies. But a steady childhood diet of nature documentaries by Sir David Attenborough established an interest in the natural sciences that Offord just couldn't shake. So she enrolled in biology at Oxford University, spending her later years in a biomechanics lab studying the effect of environmental conditions on the mechanical properties of silk fibers—initially from silkworms, later from "palm-size" spiders. For her master's, Offord headed stateside to the lab of Iain Couzin, then at Princeton University. There, she studied collective behavior in ants, and when Couzin's group moved to Germany, she remained at Princeton to continue her research, funded by an HHMI fellowship. During grad school, Offord took journalism courses and wrote articles for various newspapers; last summer, she did an internship with *BBC Focus*. "That's when I knew this was what I wanted to do," she says of writing.

In this issue, Offord describes a novel mechanism of cellular signaling ("Kissing Cousins," page 48) and a decades-long fruit fly experiment conducted in the dark ("Feeling Around in the Dark," page 20).

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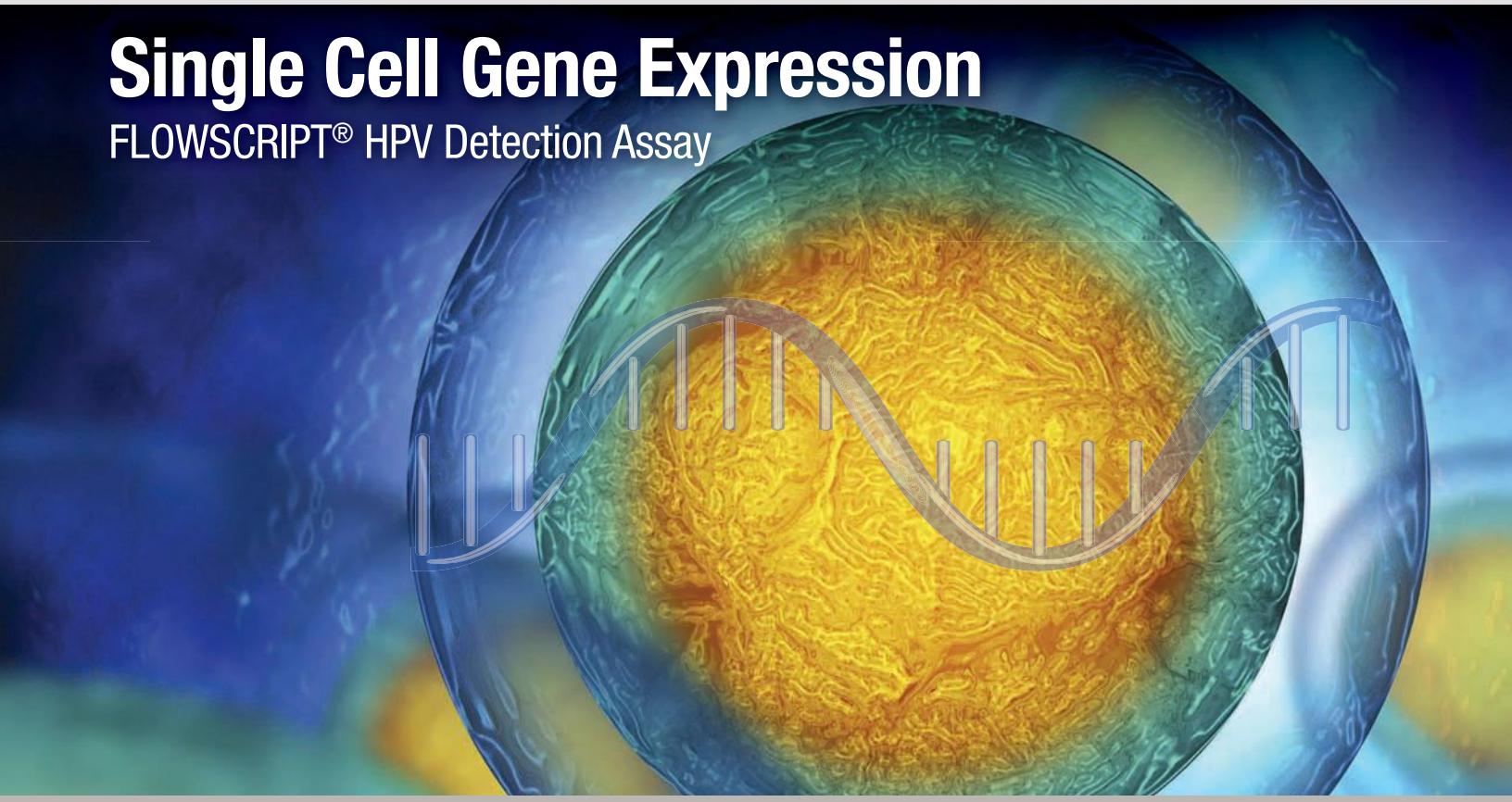

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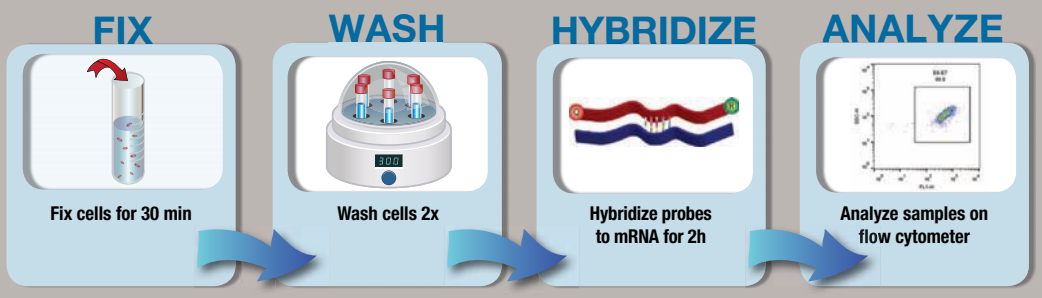

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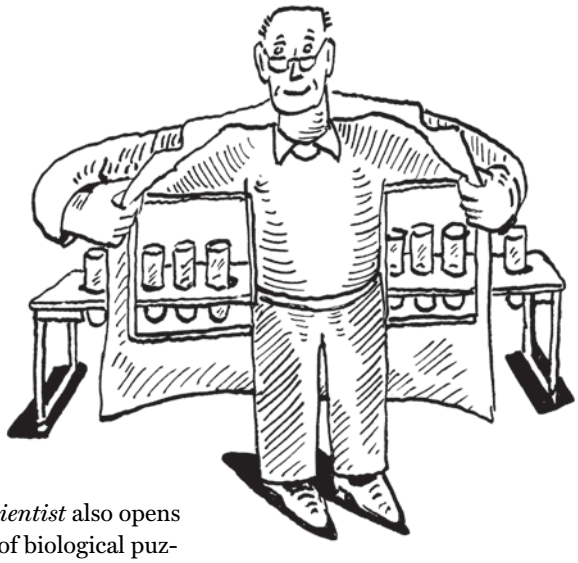


ANDRZEJ KRAUZE

At the end of March, when the prestigious Tribeca Film Festival posted its schedule, a hue and cry arose about one of the films: *Vaxxed: From Cover-Up to Catastrophe*. The film was directed and co-written by none other than discredited British gastroenterologist Andrew Wakefield, who led the infamous 1998 study purporting to have established a link between autism and childhood vaccination against measles, mumps, and rubella (MMR). That highly controversial study was retracted in 2010 (a fact only briefly mentioned in the film), and Wakefield's license to practice medicine was revoked a few months later (not mentioned in the film).

Objections to the film raised by clinicians, researchers, and others led Oscar-winning actor, producer, and festival cofounder Robert De Niro, the father of an autistic child, to cancel the Tribeca screening. But almost immediately after the cancellation, *Vaxxed* opened some five blocks north at the Manhattan art-film house Angelika, where it continues to play to both the curious and the small but vocal coterie of anti-vaxxers who still espouse the scientifically unfounded autism connection (one such believer is actively campaigning for a US presidential nomination).

It took 12 years and a chorus of saner voices decrying the paper before its retraction by *The Lancet*, even though 10 of the paper's 13 authors had published a "retraction of an interpretation" in 2004. More specifically, it took an exhaustive two-part *BMJ* series in which investigative reporter Brian Deer painstakingly pointed out the errors and misrepresentations in the paper, and research study after research study, to thoroughly debunk the conclusions. This was a high-profile outing of shoddy results.

But what about subtler errors that persist in the scientific literature? Flawed papers that will never be retracted or corrected, especially those resulting from studies that contain honest inaccuracies in experimental design or statistical analysis? In "The Zombie Literature" (page 42), Senior Editor Bob Grant reports on campaigns afoot to make all aspects of a published scientific paper more transparent. These efforts aim to allow reviewing, commenting, correcting, annotating, and revising of the original paper as new evidence arises. In essence, the original paper becomes a living document, rather than a static entity.

This issue of *The Scientist* also opens windows on a number of biological puzzles. One of these is why so many human eggs end up with the wrong number of chromosomes. In our cover story ("A Scrambled Mess," page 28), reproductive biologist Karen Schindler reviews the many ways things can go wrong during the meiotic divisions that produce female gametes, resulting in aneuploid eggs. And as a historical complement, this month's Foundations (page 72) shows the very first drawings of chromosomal missegregation, made in 1916 by Calvin Bridges, who termed the errors "nondisjunction."

Another puzzle is how to fight the increasingly antibiotic-resistant microbes responsible for the rising number of hospital-acquired infections. Researchers Edward Marks and Steven Smith describe nanoscale particles and patterns that could turn hospital rooms, patient implants, and other surfaces into microbe killers in "Nanoscale Defenses" (page 35). And in a Thought Experiment on page 22, Iain Johnston and Ben Williams address yet another biological mystery—why the mitochondrion has hung on to just a few of the thousands of genes harbored by the ancestral microbial interloper—by using statistical approaches to comb through mitochondrial gene sequences.

As scientists continue to pose and answer questions, we will continue to tell the stories of how they do it and what they've found—warts and all. Here's to open windows everywhere. ■

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

Speaking of Science

This finding can serve as a nice empirical middle-finger from vulgarians everywhere, directed at those who had, until now, been unfairly judging them for their linguistic abilities. Swearing, it seems, can be creative, smart, and even downright lyrical.

—**Piercarlo Valdesolo**, a psychologist at Claremont McKenna College, writing in a recent issue of *Scientific American* about a new study that found subjects who were more fluent with swear words tended to have larger vocabularies than those who exhibited less-robust potty-mouthing abilities (April 5)

WTF???: According to a new study, people who can spew or write more swear words in the space of a minute were more verbally fluent in general.

We are particularly concerned that misperceptions about NIH's priorities and interests may be causing investigators to submit fewer basic research applications.

—**Francis Collins**, director of the National Institutes of Health, and 39 other NIH signatories, in a recent letter to *Science* lamenting the lack of basic science grant applications being submitted to the agency (March 25)

If Greenland freshwater shuts down deepwater formation and cools the North Atlantic several degrees, the increased horizontal temperature gradient will drive superstorms, stronger than any in modern times. All hell will break loose in the North Atlantic and neighboring lands.

—Former NASA climate scientist **James Hansen**, in a YouTube video explaining the results of a recently published climate modeling study (March 21)

The preprint movement . . . may presage the need for a greater cultural shift [that] scientists have not yet been willing to make: evaluating one another based on the substance of their papers, not where they are published.

—*New York Times* correspondent **Amy Harmon**, on the burgeoning trend of researchers directly publishing their work on the Internet rather than going through traditional publishing channels (March 15)

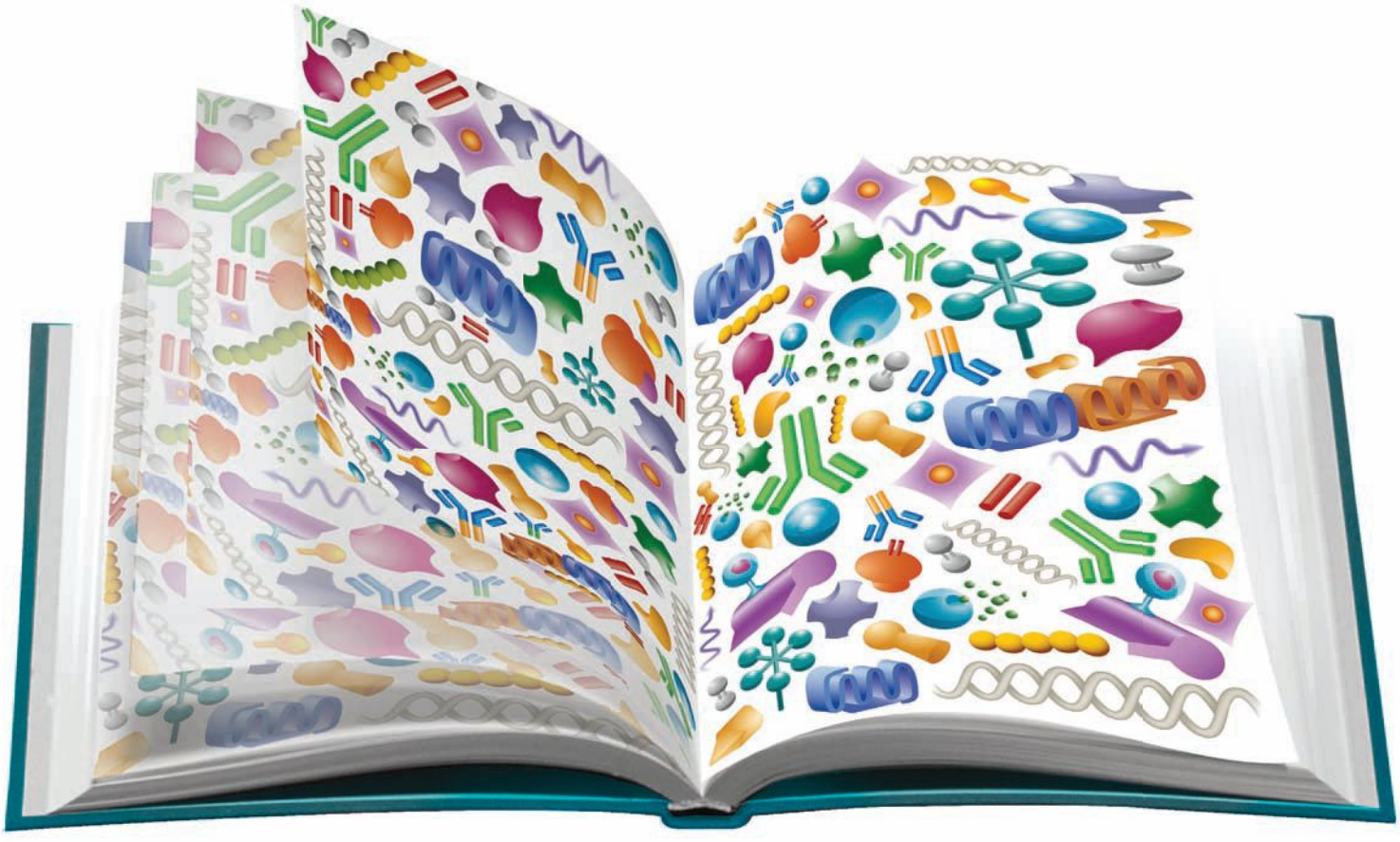
I admit that it is difficult to bring more subtle and varied political approaches to the table. Groups of researchers that have tried to do so—such as the Federation of American Scientists and Union of Concerned Scientists—have struggled to gain traction. Still, there is a fresher, grass-roots movement, exemplified by local "skeptics" groups, through which younger scientists are trying to make their work relate to society's wider concerns.

But at the top, there is paralysis: leading scientific organizations do little except chase money and reinforce the ruling nexus of politics and finance.

—Science writer **Colin Macilwain**, in a *Nature* column on the relationship between today's scientists and politicians (March 16)

The medical literature is prone to overstating results, a condition not thoroughly recognized among policymakers. . . . The state of the current medical literature makes it of utmost importance that all sections of the manuscript are read, including associated letters to the editors and information on ClinicalTrials.gov, before authors' recommendations are accepted.

—**Kevin Kavanagh and colleagues**, in a recent opinion piece published in the *Journal of Patient Safety* about the use of spin in clinical research on infectious diseases (March 24)



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Notebook

SHEKOOH BEHROOZIAN

MODELLING MEDICINE: Wet clumps of Kisameet clay (left) and a dried and ground clay sample (right)

Serious Putty

About three years ago, University of British Columbia (UBC) microbiologist Julian Davies hosted an unusual meeting in his lab in Vancouver, Canada. The visitors explained that they had recently acquired the rights to a clay deposit 250 miles north, on the edge of the Kisameet Basin, which is within territory belonging to a group of native, or First Nations, people, the Heiltsuk. The owners of the deposit planned to use the clay to create and sell cosmetics through their company, Kisameet Glacial Clay Inc., they said. They also speculated, based on scientific and medical reports about the clay published in the 1940s and '50s, that the clay had antimicrobial properties, and wondered if Davies would be willing to look into it. "This sounds like quackery, a little bit," Davies remembers thinking.

But the visitors, including the company president, Lawry Lund, told Davies that the Heiltsuk people had used the clay for healing purposes for many years and that doctors and scientists had published reports of its effectiveness for diverse medical applications in the 1950s. Davies's interest was piqued. With funding primarily from the company, he and his team began testing the clay as an antibiotic against various bacteria. "It's been almost all surprises since then," he says. Solutions of the clay can kill 16 different strains of multidrug-resistant bacteria that commonly infect hospital patients, the group reported in a recent study.

"I believe it's very interesting, because this is a kind of mineral that has activity against the majority of bugs, gram-positive and gram-negative, even the resistant ones," says Matteo Bassetti, director of the Infectious Disease Clinic at Santa Maria Misericordia University Hospital, in Udine, Italy, who was not involved in Davies's work.

The clay is effective against the so-called ESKAPE bacteria; ESKAPE is an acronym for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and various Enterobacter species,

which as a group cause most hospital infections and are resistant to current antimicrobials of choice.

Kisameet clay (KC) "displayed potent antibacterial activity in lab tests against a panel of highly antibiotic-resistant bacteria," says David Weiss, director of the Emory Antibiotic Resistance Center in Atlanta, Georgia, who was not involved in the work. "These are exciting results that highlight the potential of KC to be used to treat resistant bacteria."

Michael Mahan, who researches bacterial pathogenesis and host immune responses at the University of California, Santa Barbara, and was also not involved in Davies's work, agrees. Davies's results provide "new hope in a battle that the medical community is currently not winning," he told *The Scientist* in an email.

Research on KC dates back 70 years. In 1946, in the *Bulletin of the Vancouver Medical Association*, a pair of UBC chemists reported on the composition and curative properties of the clay (22:230-37). They mentioned that a local company was selling the clay as a product called Ray-Vite and a water-based formulation as Absor-Vite, "a smooth, creamy preparation of a bluish gray color" meant to be taken orally. In several individual cases, the researchers reported, Absor-Vite seemed to relieve symptoms of digestive-system ulcers. And solutions of Absor-Vite exhibited antibacterial activity in the lab. The report also mentioned Ray-Vite's effectiveness for external use as an anesthetic and to treat burns. In a 1952 meeting presentation, MIT chemist Ernst Hauser further described the physical properties of KC, mentioning that the Heiltsuk had used the clay for medicinal purposes for generations.

In the past, the Heiltsuk people used KC both topically and orally, according to a traditional use study on which Lund's company collaborated with the Heiltsuk Integrated Resource Management Department. "The practice seemed to have stopped, but interest is once again developing," he told *The Scientist* in an email.

Davies's team incubated different types of ESKAPE bacteria, gathered from two hospitals and a wastewater treatment plant, with an aqueous suspension of sterilized clay dust and plated out the bacteria to test viability. By 24 hours of incubation with the clay solution, the concentrations of viable bacteria had fallen below detectable limits for all of the broad ESKAPE groups save *E. faecium*, which took 48 hours to kill off. Populations of control bacteria, incubated with water only, also declined but did not die out altogether (*mBio*, 7:e01842-15, 2016).

> This is a kind of mineral that has activity against the majority of bugs, gram-positive and gram-negative, even the resistant ones.
>
> —Matteo Bassetti, Santa Maria Misericordia University Hospital

In the future, the clay could possibly be used to treat various conditions and infections, Davies says, including those of the skin and intestinal tract. Before tests of specific applications can happen, "there's one big question that remains to be answered, and that is: Does the clay have any toxicity?" Davies says he and his group are pursuing those tests, starting in animals.

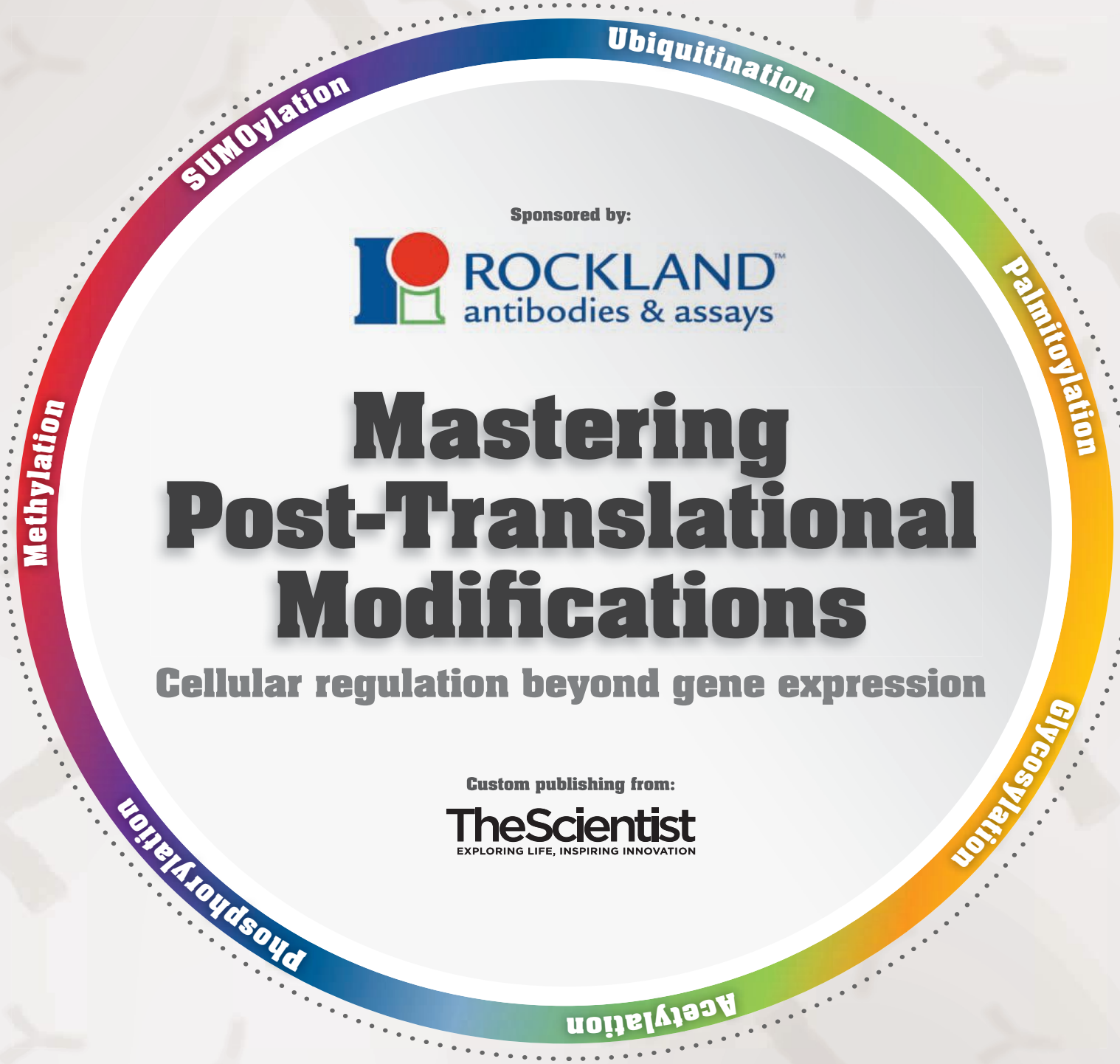
"There is a long road ahead to possibly using KC to treat resistant infections," Weiss adds.

Meanwhile, Kisameet Glacial Clay began advertising the clay as a product called Kisolite several years ago with cosmetic applications in mind. But after Davies's research revealed the clay's antibacterial promise, the company decided to hold off on selling Kisolite, Lund told *The Scientist* in mid-March; rather, it is waiting to determine its most appropriate uses.

—Ashley P. Taylor

What's in a Voice?

Joey Tribbiani was on to something. With a nod of the head and a cocky half-smile, the *Friends* character's famous



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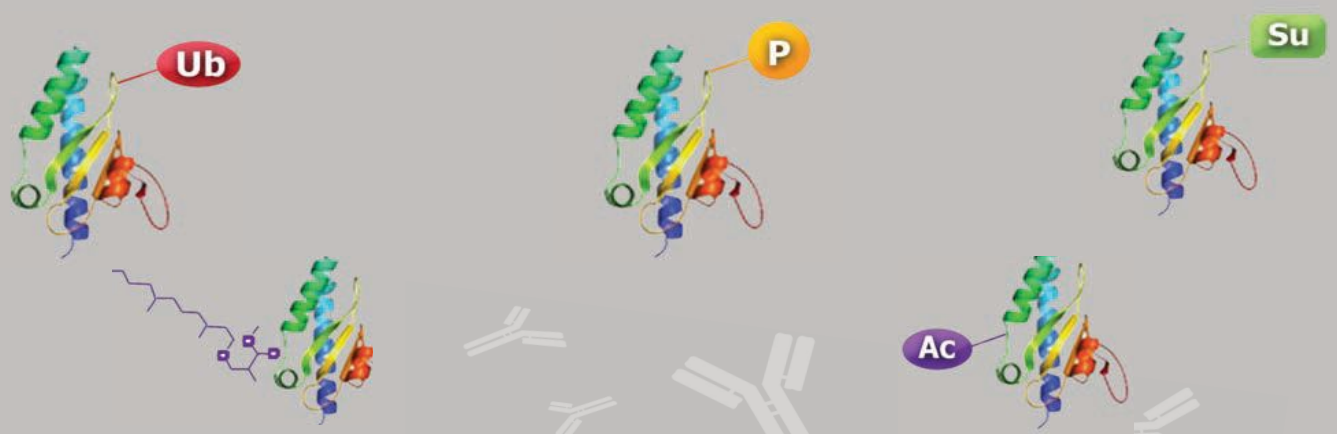

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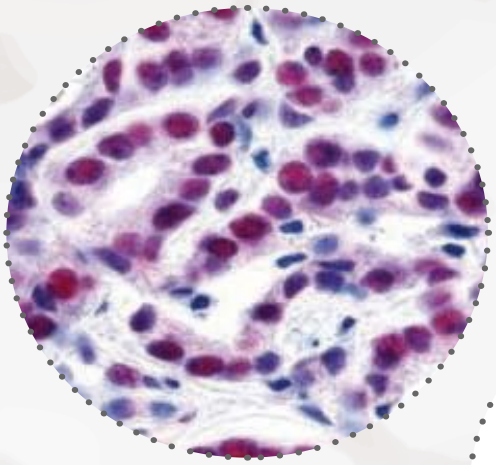
Antibody Selection Tips for PTMs

Preparation:

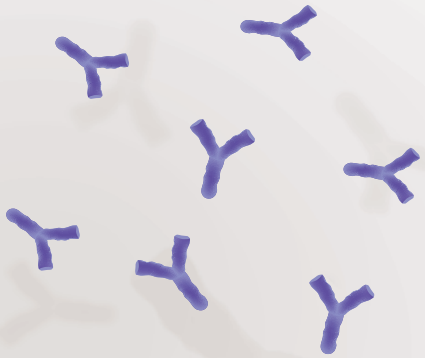
From an antibody production point of view, the differences between modified proteins can be quite small. Peptide design and immunogen quality are critical to the generation of a specific immune response to ensure to the production of high-quality antibodies.



Immunohistochemistry of rabbit anti-HDAC-1 antibody

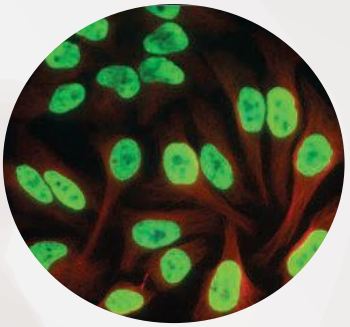
Production:

Antibodies against PTMs are generated using a short, specific region of the protein, largely eliminating the issue of specificity seen with antibodies generated using large constructs as immunogens. However, it is critical that the antibody be tested against established positive and negative controls to ensure specificity for the modification. Polyclonal antibodies can be immunodepleted during production if the sample contains antibodies that recognize other PTMs.

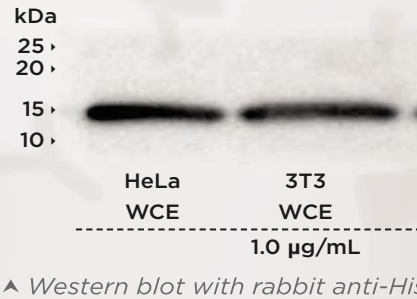


Validation:

Dot blot assays and ELISAs can be used to assess both antibody specificity and sensitivity. Keep in mind that, in addition to being specific for the required modification, the antibody must be validated for the application of choice using appropriate positive and negative controls.



Immunofluorescence of Histone H3 [Lys36ac] (green), DAPI (blue), and alpha-tubulin (red)



Western blot with rabbit anti-His

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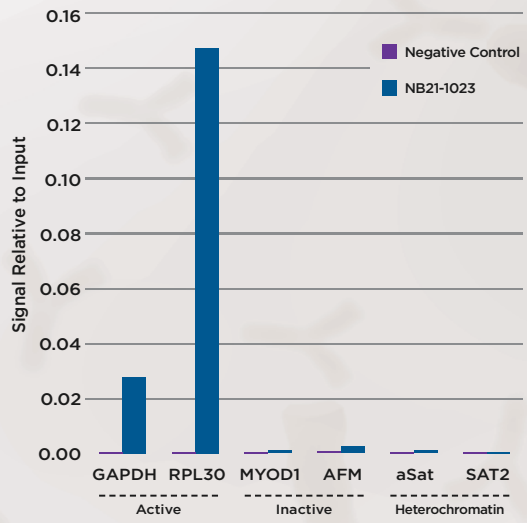
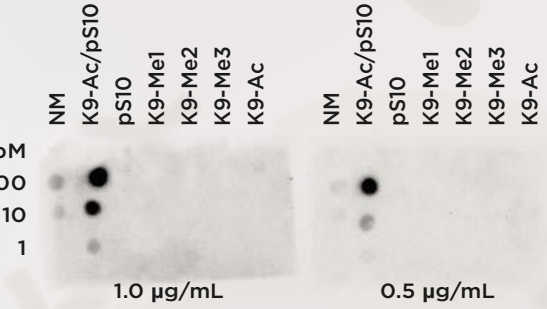


Chromatin immunoprecipitation with rabbit anti-Histone H3 K4/me3 antibody.



Dot blot with rabbit anti-Histone H3 [ac Lys9/ phospho Ser10] antibody

istone H3 [Monomethyl Lys9] antibody

curate • Dependable

SUMOylation

Methylation

Phosphorylation

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Learn how you can choose the b
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Ubiquitination

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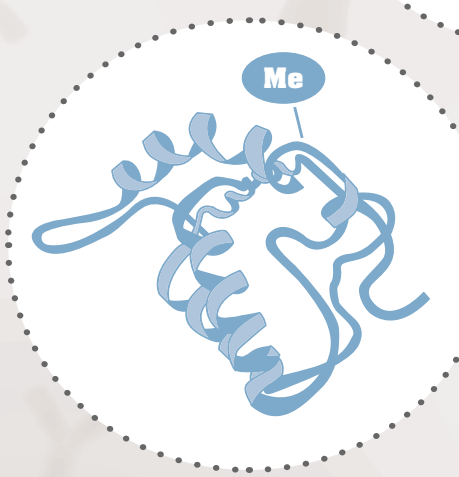
SPIRING INNOVATION

Phosphorylation:

Protein phosphorylation is controlled by kinases and phosphatases, and plays a significant role in a wide range of cellular processes, including cell growth and proliferation, metabolism, physiological regulation, and cell signaling.



Common PTMs & Their Functions

SUMOylation:

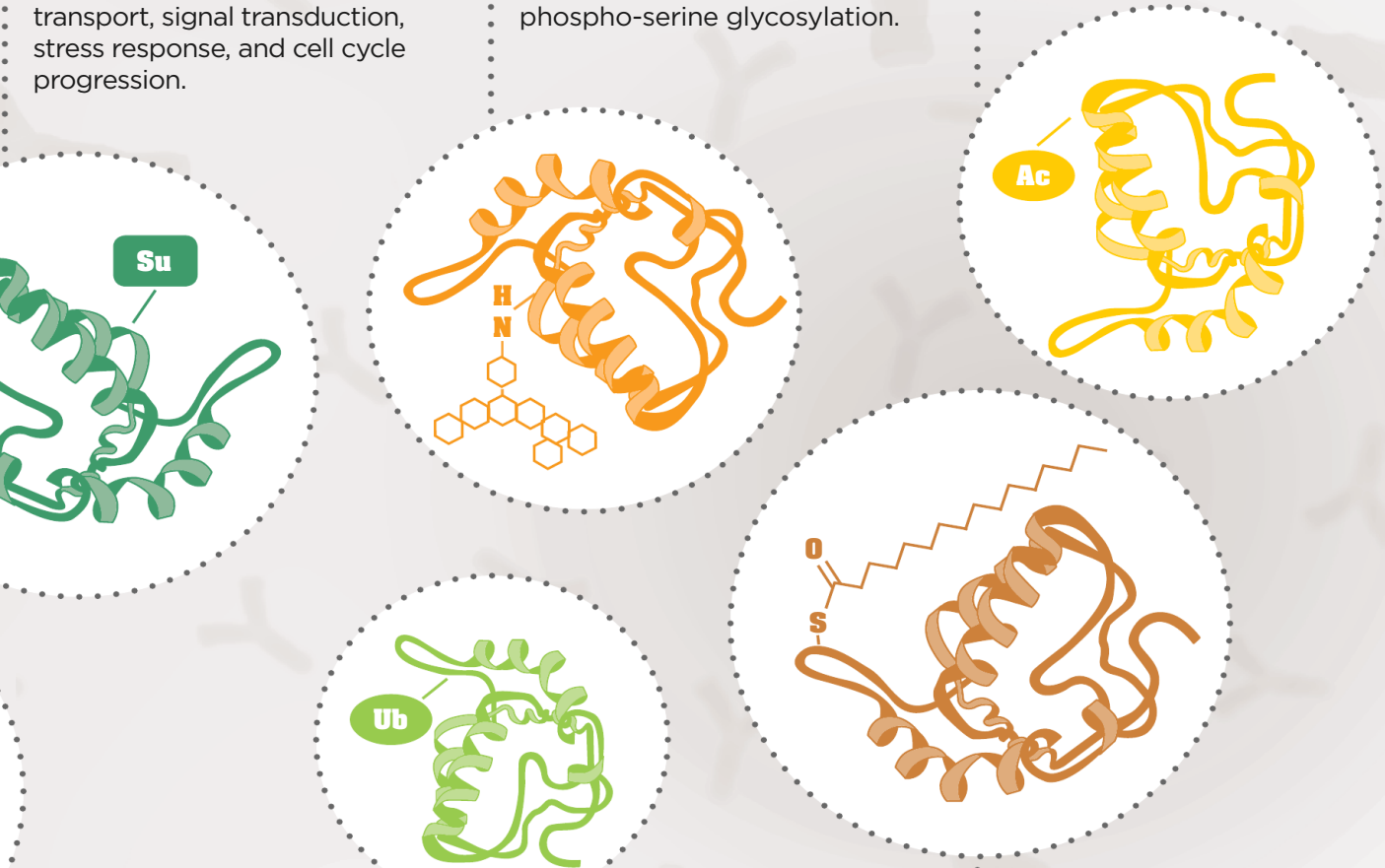
Sumoylation involves the addition of small ubiquitin-like modifiers (SUMOs) that enhance stability or modulate the subcellular compartmentalization of proteins. It has been implicated in various cellular processes, such as nuclear transport, signal transduction, stress response, and cell cycle progression.

Glycosylation:

Attachment of glycans to proteins is critical for protein folding, stability, targeting, and binding. Five types of glycosylation are observed: N- and O-linked glycosylation, C-linked mannosylation, glypiation, and phospho-serine glycosylation.

Acetylation:

Acetylation, or the addition of an acetyl group at lysine residues, is a major post-translational modification for histones, regulating gene expression and metabolism.



Methylation:

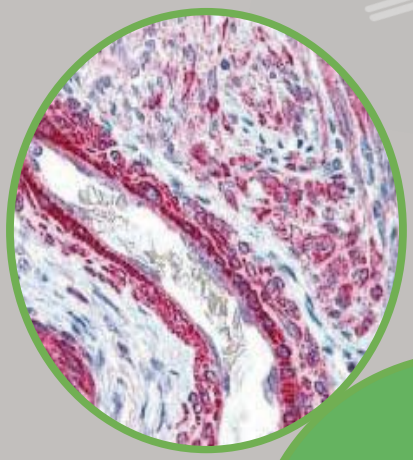
Protein methylation is a reversible process by which methyl groups are added to arginine or lysine residues, mediated by peptidylarginine or lysine methyltransferases.

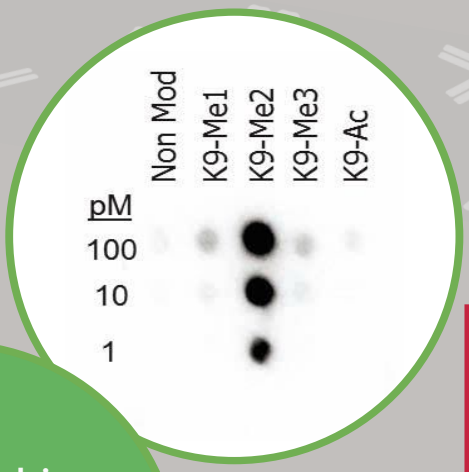
Ubiquitination:

Ubiquitination is an essential cellular process that tags abnormal, foreign, and improperly folded proteins, targeting them for degradation by the 26S proteasome.

Palmitoylation:

S-Palmitoylation involves the lipid modification of cystine residues with palmitic acid. This modification plays a role in protein localization, stability, subcellular trafficking, and protein-protein interaction.





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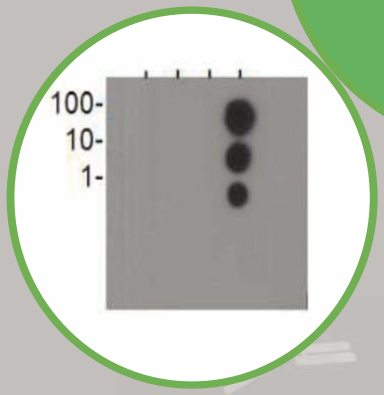

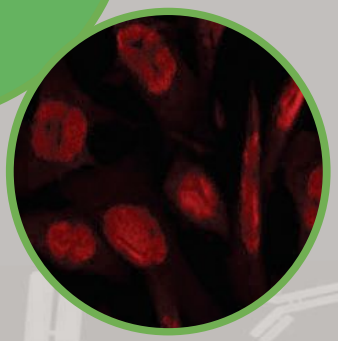

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Post-translational changes alter the structure of individual proteins, and therefore potentially affect their activity, stability, localization and/or interacting partner molecules. Antibodies are arguably the most prevalent and valuable tool for tracking these changes. Rockland has developed and perfected a process for manufacturing antibodies to detect Post-Translational Modifications (PTM) that has been in use for over a decade. In the process, modification-specific antibodies are prepared using synthetic modified peptides. The trouble is that antibodies recognizing nonphosphorylated forms must be excluded —a skill that Rockland has delicately mastered through years of experience. To ensure the integrity of these sensitive antibodies, Rockland performs quality control testing on every lot to guarantee antibodies function in the intended assays. All work is performed in Rockland's laboratories, located just outside of Philadelphia, PA.

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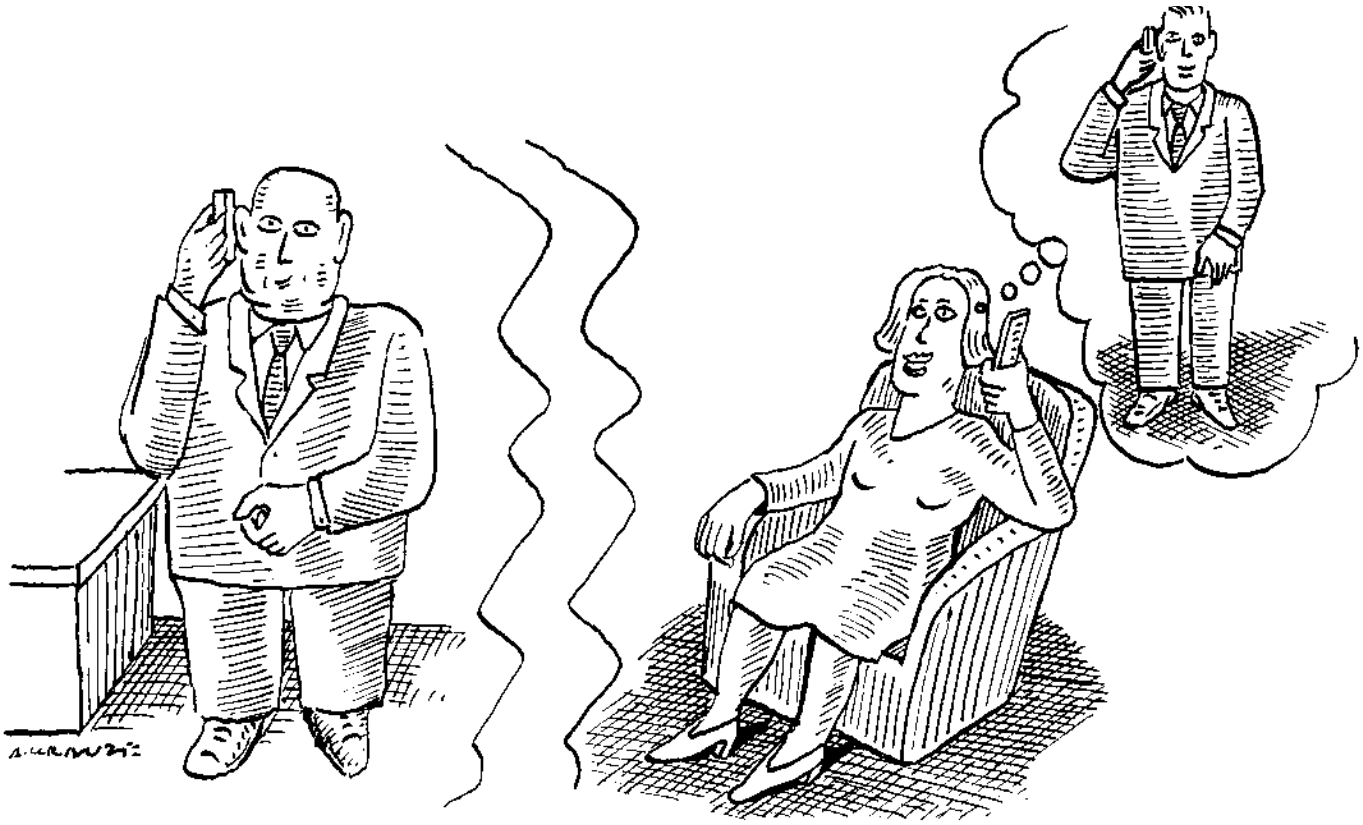


ANDRZEJ KRAUZE

"How *you* doin'?" catchphrase, intended to lure women, epitomized the attractive traits in male voices—at least according to Yi Xu, who studies speech at University College London. A 2013 study by Xu found that women rated men's voices as more attractive if they had a lower pitch, more breathiness (as opposed to a more pinched or pressed quality), and more-compact formants (which makes the voice sound deeper). "Everything reminds us of Joey from *Friends*," Xu says.

The results of Xu's study suggest that the content of speech isn't everything (I mean, really, how many women are turned on by the words Joey says?)—the voice itself carries information about the attractiveness of the speaker. Earlier studies pointed to as much. Susan Hughes of Albright College in Reading, Pennsylvania, and colleagues reported more than a decade ago that appealing voices correlate with a more V-shape upper body in men and a more hourglass shape in women.

"These ideal sex-specific body configurations are revealing of the influence of sex hormones that shape features that signal our reproductive maturity and potential," Hughes says via email. "Likewise, raters were able to decipher these sex-specific body configurations of others simply by hearing one's voice," she adds, referring to the results of a later study that asked listeners to estimate a speaker's body proportions.

While there have been quite a few studies on voice attractiveness, says Katarzyna Pisanski, a postdoc at the University of Sussex in the U.K., less attention has been paid to body size and specific acoustic parameters—that is, what are the components of the voice that communicate our appearance?

To probe this question, Pisanki and her collaborators recorded the voices of 700 people from Germany, Canada, or the U.K. as they spoke a series of vowels. Ditching words allowed the researchers to isolate the voices' acoustics, including pitch, formants, and elements of unevenness in the voice called jitter and shimmer (the more of either, the rougher or raspier the voice). Then they observed how well these vocal aspects correlated with height, waist-to-hip and chest-to-hip ratios, body mass index, and other indicators of appearance. The result, says Pisanski, "is a more complex story than what we would have thought."

Certain vocal features predicted body size and shape. Formants—measurements of resonance—are determined by the length of a speaker's vocal tract, and Pisanski found they were also the best correlates of a person's height and weight. That is, longer vocal tract lengths—which lead to a more resonant voice—were more likely among taller and heavier individuals.

A lower waist-to-hip ratio among women as well as a higher chest-to-hip ratio among men were also linked with higher levels of vocal perturbation or roughness (jitter and shimmer). This means that women with more-masculine body shapes have smoother voices, Pisanski says, "which could hypothetically be due to relatively higher levels of testosterone among these women."

Overall, there were more vocal correlates of women's body proportions than men's. Given the nature of the study, it's impossible to explain why that is, but Pisanski suspects it has to do with natural selection and communicating reproductive fitness. "In terms of what we know about the importance of women's body shape, it would make more sense to have more information on height in men's

voices and body shape in women's voices," says Pisanski.

David Puts of Penn State University says that, while there's no evidence of that just yet, Pisanski's suspicion makes sense, and Puts believes that hormones may underlie these vocal-physical correlates. Higher testosterone, for instance, is known to affect vocal cord development during puberty, and it's also related to lower pitch in men's voices. It also appears that women's monthly hormonal cycles can influence their voices. A study by Puts revealed that listeners rated the voices of women who were in the fertile periods of their monthly cycles as more attractive. "But we didn't find those [increases in attractiveness] are mediated by the usual suspects in terms of acoustic parameters," such as pitch, formants, jitter, or shimmer, Puts says.

That lack of correlation brings up the question of perception: even if Pisanski's study found acoustic traits correlated with certain physical aspects, can listeners detect those subtle variations and accurately predict what a speaker looks like? Pisanski's current postdoc advisor David Reby of the University of Sussex says the correlations Pisanski's team observed, while interesting, aren't particularly revealing. "So that means that it is difficult to gauge someone's size by their voice," says Reby, who was not involved in the study. "Overall, the relationship with body size exists and is significant, but it's not very strong."

Xu says the trouble with trying to predict body size or shape from voice is that "everybody is trying to cheat." That is, we are all trying to project the ideal physique—and, mostly unintentionally, our voices can lie. A short man may have a deep voice, while a woman with a boxy torso may be high-pitched and breathy. Xu says some of this may be influenced by society and how we learn to speak, but "cheating" is mainly due to physiology—such as the short man getting a heavy dose of testosterone during puberty, which would help to lengthen his vocal tract and deepen his pitch. "That's why the correlation [between body shape and vocal parameters], even for the best ones, is so low," he says.

"The relationships are there, but they're relatively weak," agrees Puts. Despite that, people often make inferences about appearance from voices—the height and pitch connection, for instance, being one most people generally agree upon. "It [raises the] question why we've evolved to pay attention to these things as strong indicators of body size even though they're weak indicators."

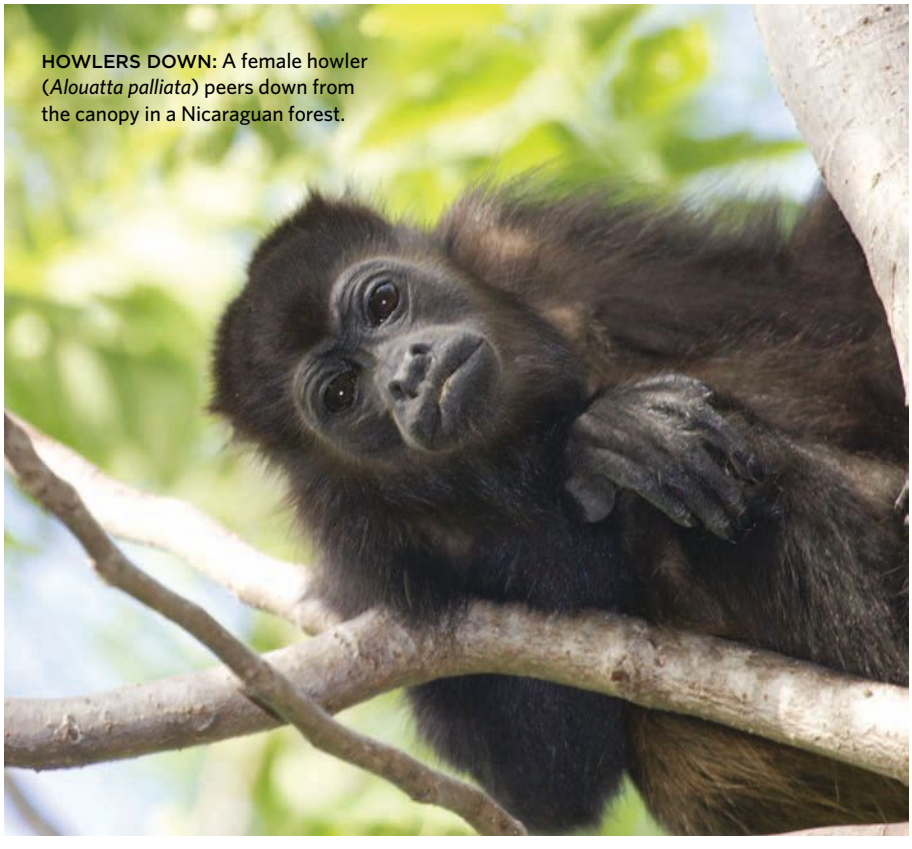
"I would say they put their finger onto something that requires more investigation," says Reby. —Kerry Grens

Silent Canopies

In late September, Kimberly Williams-Guillén, an assistant professor at the University of Washington Bothell and a conservation scientist for the Nicaraguan environmental NGO Paso Pacífico, received a report that a handful of howler monkeys (genus *Alouatta*) had been found dead at an ecoresort in Nicaragua. Bizarrely, the monkeys showed no signs of trauma or disease. "They seemed to be in fairly good condition," she recalls.

Over the next couple of months, Williams-Guillén and her colleagues continued to receive news that howler monkeys were dying. Then, around mid-January, the reports really started to flood in. Landowners, farmers, and other members of local communities in southwestern Nicaragua were all finding dead howler monkeys. Soon, the researchers began hearing of howler monkeys dying in certain areas of Ecuador and Panama as well. Williams-Guillén estimates that, in Nicaragua alone, at least 280 howler monkeys spread over an 800-square-kilometer area died in the first three months of 2016, and some Nicaraguan locals speculate that this may be an underestimate.

When it became clear that the die-offs were not just an isolated incident, Williams-Guillén hopped on a plane to Nicaragua to see for herself. "I saw many healthy-looking monkeys, but I also saw



HOWLERS DOWN: A female howler (*Alouatta palliata*) peers down from the canopy in a Nicaraguan forest.

HAL BRINDLEY

many visibly unwell monkeys," she says. "They were thin and very lethargic, often solitary and nonresponsive. There were several that I was able to walk up to and grab them out of a bush." Some witnesses reported seeing monkeys convulsing as if they were having seizures.

Although the mortality rate seems to have slowed since mid-February, the researchers are anxious to understand what's going on. Williams-Guillén speculates that the deaths may be linked to the drought that has struck Nicaragua and other areas of Central and South America this year. "The deaths are all really concentrated in the areas worst hit by drought," she says. "Even just going to the other side of the mountain, where it's slightly more humid, there's a lot fewer deaths, and there's visibly more potential howler monkey food, whereas the areas that have the highest rates of mortality, the trees are just bare—there's hardly a leaf or flower to be eaten."

They were thin and very lethargic, often solitary and nonresponsive. There were several that I was able to walk up to and grab them out of a bush.

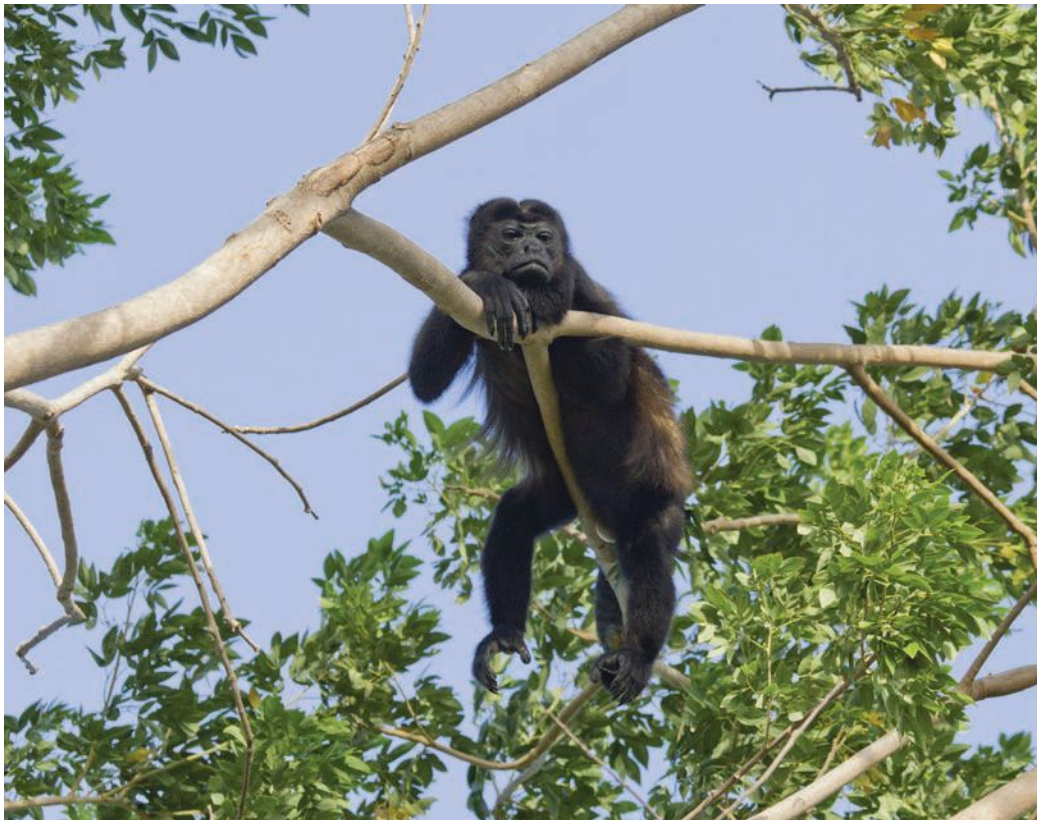
—Kimberly Williams-Guillén, University of Washington Bothell

Kenneth Glander of Duke University agrees that limited food availability is likely a contributing factor, though the cause of death may not be starvation per se. Rather, the lack of food may drive the monkeys to consume plants with high levels of certain toxins that aren't part of their normal diet. In the 1970s, Glander witnessed a handful of dead or dying howler monkeys in Costa Rica, including two that displayed convulsions similar to those reported in Nicaraguan monkeys this year. "When we did autopsies on them, I was able to determine [that] their stomachs were full of leaves that they'd never eaten before." He then collected a sample of those leaves from trees at his study site and brought them back to Duke for analysis, finding that they were chock-full of toxic alkaloids.

A good test of this hypothesis would be to see how other animals in the areas are faring, says Pedro Américo Dias of the University of Veracruz in Mexico. "If there are no reports of deaths in other frugivorous primates and other frugivorous animals, perhaps [food availability] doesn't have to do with it," he says. There haven't been reports of unusual deaths in other monkey species so far, says Katharine Milton of the University of California, Berkeley, but because howler monkeys are by far the most abundant monkey species at many sites in Central America, die-offs in their populations might be most obvious. "Death in howlers might be noticed in particular, especially if other monkey species were scarce and low in number."

Another possible cause for the howler monkey die-offs is disease. Although Nicaragua is currently believed to be free of yellow fever, Dias points out that outbreaks of the viral disease devastated howler monkey populations in the late 1940s and into the 1950s. Some researchers even speculate that yellow fever may be a cause of the relatively low genetic diversity among Central American howler monkeys, Dias says. "Yellow fever in the past could have caused important bottlenecks."

Williams-Guillén thinks that disease is an unlikely cause of the recent monkey deaths, however. While she and her colleagues are still waiting to export blood and tissue samples to US labs for further analysis, she notes that a Nicaraguan researcher has done virus diagnostics on some of the samples. And so far none have tested positive for yellow fever, Zika, chikungunya, or dengue viruses.



HANGING IN THERE: Researchers are struggling to determine why members of howler monkey species, such as *Alouatta palliata* (above), are dying in such great numbers in Nicaragua.

HAL BRINDLEY

"Between that and the lack of any necrosis of the liver in the dead animals, [disease] is unlikely," Williams-Guillén says.

In addition to monitoring animals in the affected areas and continuing to collect samples when possible, the team is reaching out to primatologists in unaffected areas within the monkeys' range that might serve as good control sites, and to establish protocols for collecting data so that the information is comparable across sites. "I think this is a really critical component," says Williams-Guillén's colleague Liliana Cortés Ortiz, an evolutionary primatologist at the University of Michigan. Dias, for one, has agreed to share data from his study sites in Mexico, where no howler monkey deaths have been reported.

For now, however, the cause of the recent howler monkey deaths remains a mystery. Williams-Guillén suspects that no one hypothesis will be correct. "There's probably an interaction of factors," she says. "Animals that might have had some clinical or secondary infections that normally aren't that problematic . . . got into a situation where they were extremely food- and water-stressed, and that might have been enough to tip them into mortality." —Jef Akst

Feeling Around in the Dark

Every couple of weeks, Naoyuki Fuse steps into a darkroom carrying a handful of milk bottles. In the reddish gloom, he transfers the contents of existing bottles into the new ones he's brought in, one after the other, until all the old bottles are empty. He's been doing this for eight years now—for the previous five decades, it was someone else's job. But carrying out this chore regularly and carefully is critical for the bottles' contents: members of a population of very special fruit flies that have spent the last 62 years evolving in the dark.

"It's tough work, especially over a long time," admits Fuse, a molecular developmental biologist who's part of an ongoing project at Kyoto University using these flies to identify genes involved in adaptation to life without light.

You can get a complete "fossil record" of evolution.

— Jeffrey Barrick, University of Texas at Austin

Dark-fly, as the *Drosophila melanogaster* line has become known, has cycled through more than 1,500 generations since being plunged into darkness in 1954 by Japanese ecologist Syuiti Mori, making the project one of the longest lab-based evolution experiments ever. The original design aimed to compare fly populations reared in the dark with control populations evolving in normal conditions. After generations without light, Mori presumed, Dark-fly would gradually adapt to darkness—rather the way "cave fish evolving in the dark famously lose their eyes and their pigmentation," Fuse says. Comparing Dark-fly to control flies might reveal morphological—and with newer technologies, genetic—adaptations to this unusual environment.

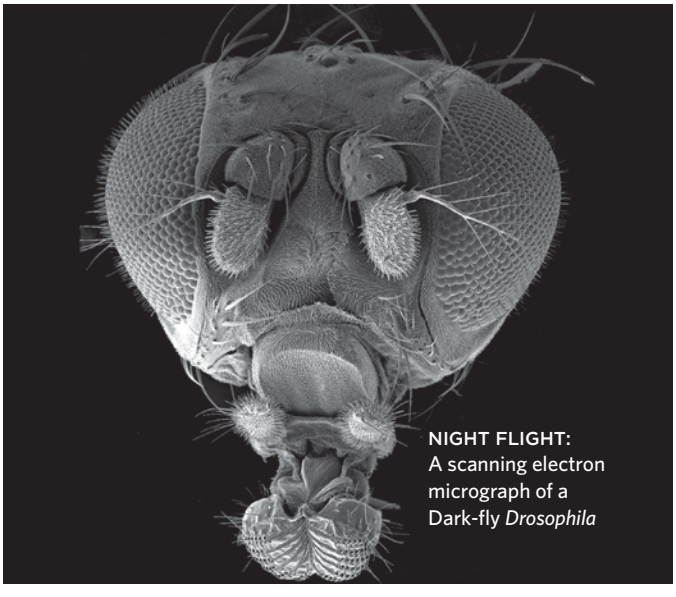
Done right, says molecular biologist Jeffrey Barrick of the University of Texas at Austin, this sort of approach can be extremely powerful. Keeping track of populations evolving in controlled lab conditions, "you can get a complete 'fossil record' of evolution," he says. "There are no missing links if it's done very carefully from the beginning."

But the Dark-fly study was not originally envisioned as a decades-long project, meaning that various precautions that are common practice in long-running evolution experiments—such as keeping populations large and avoiding inbreeding—were not established at the outset.

And, vexingly, only a fraction of the original fly lines have survived. "In some cases, the incubator broke, and the temperature collapsed," says Fuse. "Another time, fungi grew on the fly food." All three original control lines were lost, and only one of the three Dark-fly lines lived beyond 2002, eliminating the possibility of making direct comparisons between populations evolving in parallel.

"That was very unfortunate for the experiment," notes Barrick, who collaborates with Michigan State University's Richard Lenski on a now 28-year evolution project in *E. coli*. Microbes can be stored frozen, Barrick adds, so that "if something goes wrong, you can always go back to the freezer. The game is saved, so to speak." Flies, by contrast, don't freeze well—they're either living and breeding, or unusable.

Despite these setbacks, after joining the project in 2008, Fuse and his colleagues set to work searching for signatures of adaptation in Dark-fly. "Dark-fly looks normal," says Fuse, but close examination reveals tiny differences—such as slightly longer sensory head bristles—compared with normal flies. Dark-fly also produces more progeny in constant darkness



NIGHT FLIGHT: A scanning electron micrograph of a Dark-fly *Drosophila*

NAOYUKI FUSE

compared with a separate, wild-type fruit fly strain—a trait that could reflect environmental adaptation.

To get at the genetic factors underlying these differences, the researchers sequenced Dark-fly's genome, identifying 220,000 single-nucleotide polymorphisms, or SNPs (*PLOS ONE*, 7: e33288, 2012). Approximately 2 percent of those SNPs, in more than 4,300 genes, were nonsynonymous—i.e., they caused changes in amino acid sequences.

But the interpretation of these data in light of Dark-fly's small, unreplicated population proved difficult. "In any one population, in fruit flies or any lab organisms, you're always going to have accidents of genetic drift which will fix deleterious alleles," explains Michael Rose, an evolutionary biologist at the University of California, Irvine. Without replicates to confirm the consistent appearance of supposedly adaptive mutations, "you don't know which features of genomic differentiation are due to genetic drift versus due to the effect of selection."

So the Kyoto researchers tried another approach. They interbred multiple populations of wild-type flies with Dark-fly, and kept the resulting mixed populations in one of two conditions: normal light, or total darkness. After 0, 22, and 49 generations, the team analyzed the genomes represented in each population, and identified mutations in 84 genes that repeatedly cropped up in dark-kept offspring (*G3*, 6:365-75, 2016).

"Experimental breeding gives a clearer answer of what genes are involved in selection," explains Anna Kukekova, a molecular geneticist at the University of Illinois at Urbana-Champaign who studies evolution in experimental, five-decade-old populations of silver foxes. Although some loci may never be fixed completely, she adds, identifying alleles common to offspring from parallel experiments in selective environments may highlight particular mutations that confer an advantage.

Fuse and his colleagues have already linked some of the identified genes to chemoreception and pheromone synthesis, hinting at potentially adaptive shifts in communication by Dark-fly. But many of the 84 candidate genes have never been studied—not an uncommon result in evolution experiments, Barrick notes. "You learn something about genes that are clearly important—that you would never learn by doing a genetic screen—by letting evolution last longer in these interesting environments."

The goal now will be to start homing in on the role of these genes in adaptation, says Fuse, adding that he hopes that the project, despite its limitations, will continue well into the future. "Dark-fly is a kind of heritage," he says. "It's special."

—Catherine Offord

The Shrinking Mitochondrion

Scanning the mitochondrial genomes of thousands of species is beginning to shed light on why some genes were lost while others were retained.

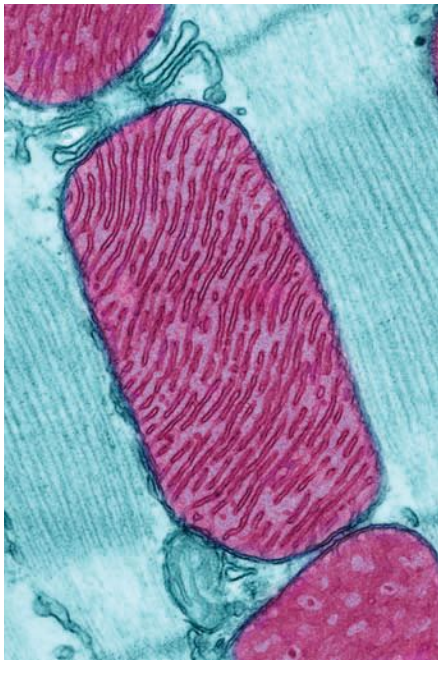
BY IAIN JOHNSTON AND BEN WILLIAMS

Billions of years ago, one cell—the ancestral cell of modern eukaryotes—engulfed another, a microbe that gave rise to today's mitochondria. Over evolutionary history, the relationship between our cells and these squatters has become a close one; mitochondria provide us with energy and enjoy protection from the outside environment in return. As a result of this interdependence, our mitochondria, which once possessed their own complete genome, have lost most of their genes: while the microbe that was engulfed so many years ago is estimated to have contained thousands of genes, humans have just 13 remaining genes in their mitochondrial DNA (mtDNA).

Some mitochondrial genes have disappeared completely; others have been transferred to our cells' nuclei for safekeeping, away from the chemically harsh environment of the mitochondrion. This is akin to storing books in a nice, dry, central library, instead of a leaky shed where they could get damaged. In humans, damage to mitochondrial genes can result in devastating genetic diseases, so why keep any books at all in the leaky shed?

Researchers have proposed diverse hypotheses to explain mitochondrial gene retention. Perhaps the products of some genes are hard to introduce into the mitochondrion once they've been made elsewhere. (Mitochondria have their own ribosomes and are capable of translating their retained genes in-house.) Or perhaps keeping some mitochondrial genes allows the cell to control each organelle individually. Historically, it has been hard to gather quantitative support for any of these ideas, but in the world of big (and growing) biological data we now have the power to shed light on this question. The mtDNA sequences of thousands of organisms as diverse as plants, worms, yeasts, protists, and humans have now been sequenced, yielding information on the patterns of gene loss and on the gene properties that may have governed this loss.

Modern statistical approaches give us ways to allow this wealth of information to speak for itself, for or against different hypotheses, without (as much) human preconception entering into the process. Such approaches often involve building models to describe how the natural world could have given rise to our observations. Sometimes we do this without realizing it: assuming that the errors on a quantity are normally distributed, for example, invokes a particular (and sometimes inappropriate!) model of the biological and experimental details underlying that measurement. So, in order to analyze the 2,000+ mitochondrial genomes available (*Cell Systems,* 2:101-11, 2016), we needed a general and unbiased way of accounting for the observed sequences.



To this end, we developed a mathematical description including all possible combinations of the mitochondrial genes we see today, and the different ways organisms could evolve from having a complete ancestral genome to having no genes at all. To avoid any personal preconceptions about possible mechanisms, we first codified our assumption, before seeing the data, that every way of getting from a full set of genes to an empty one could be equally likely; all existing genes are equally likely to be lost at any time. We then used the sequence data to perform calculations determining the probabilities of the different evolutionary paths actually having occurred.

Not surprisingly, we observed similar patterns of gene loss across different lineages, indicating that some genes are more likely to be lost than others. Some genes tend to be lost early on and are missing from mtDNA in most species, while others are retained by almost all organisms. This consistency speaks to a certain predictability of evolution; guiding trends appear to shape different species in the same way.

We then used another statistical approach called model selection to explore the mechanisms that are responsible for dictating these patterns of gene loss and address the long-debated hypotheses about mitochondrial evolution. We considered a set of possible models for how likely a given gene was to be lost based on different hypotheses, from length to sequence to chemical properties. Again, we initially assumed that all

possibilities were equally likely and let the data speak for themselves. In the end, we identified three features that together predict whether a gene is likely to be retained in the mitochondrion, rather than transferred to the nucleus: 1) it encodes a protein that forms the center of a complex, 2) it encodes hydrophobic (water-repelling) proteins, and 3) it contains many Gs and Cs in the DNA sequence.

So what do these results mean? Can we now settle the age-old debate of how and why mitochondrial genes are lost? In a way, yes, because these three features suggest that a combination of hypotheses is on the mark. Proteins that are central to complexes are important for the correct assembly of those complexes, so the first feature supports the idea that mitochondria need to keep some genes to assemble their own machinery locally. That genes encoding hydrophobic proteins are more likely to be retained in mtDNA supports the hypothesis that some proteins won't end up in the mitochondrion if they are made elsewhere, because hydrophobic proteins made in the cytoplasm tend to be shuttled to other regions of the cell. As for the third feature, we think that the numbers of Gs and Cs may be important in keeping DNA stable in the damaging environment of mitochondria, perhaps like a waterproof coating to protect the contents of the leaky shed.

Of course, these hypotheses still need to be put to the test, but preliminary work from the synthetic biology field supports our findings. Specifically, scientists have tried to transfer genes from the mitochondrial genome to the nuclear genome in yeast, mimicking the process that has occurred in evolution. While some of these experiments produced healthy, normal yeast, others did not. We found that the features we identified in our model selection predicted the genes that could not be viably transferred to the nucleus.

It is becoming clear that we need a combination of mechanisms to explain mtDNA gene loss. It is an odd feature of scientific discussion that researchers tend to develop a single explanation for the phenomena we observe in the very complex biological world; the fact that several hypotheses contribute to the full story helps explain and reconcile the heated historical debate on this topic. Moreover, our work supports the use of unbiased statistical and modelling approaches to interrogate many other biological problems, from crop design to disease infection and progression. Such approaches can help provide us with a genuinely open mind to tackle debated scientific questions and seek the underlying truth. ■

Iain Johnston is a Birmingham Fellow at the University of Birmingham, U.K. Ben Williams is a postdoc at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts.

The Global Science Era

As international collaboration becomes increasingly common, researchers must work to limit their own biases and let cultural diversity enhance their work.

BY EPHRAIM M. GOVERE

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The Earth's 195 sovereign states are becoming one scientific global village, where a scientist's success depends on their willingness to carry out collaborative research with others from around the world. By the mid-1990s, scientific collaboration at institutional, national, and international levels, as indicated by coauthorship of published manuscripts, was doubling every 15 years. And by 2008, the number of internationally coauthored articles was increasing exponentially. In 2013, researchers from The Netherlands, the U.S., South Korea, and the U.K. constructed a global collaboration map that revealed international collaborations involving all the nations in the world and estimated that 25 percent of all scientific papers include authors from multiple countries.

Some international collaborations are complex and massive. For example, the etiologic agent of severe acute respiratory syndrome (SARS) was identified with unprecedented speed in 2003 after the World Health Organization (WHO) assigned the task to a network of researchers from 11 laboratories in nine countries. The Human Genome Project involved the contributions of researchers at 20 institutions in six countries. While such large-scale projects take careful planning and coordination between international team members, analysis has shown that the more countries involved in a scientific collaboration, the greater its impact (*J Am Soc Inf Sci Tec,* 64:392-404, 2013). As Alice Gast, current president of Imperial College London, wrote in *Scientific American* in 2012: "While scientists become more specialized as they proceed through their studies, broadening and collaborative experiences make them better able to 'think differently' and 'connect the dots' to discover new things. Ultimately it leads to better science."

However, when collaborative scientific projects expand across geographic boundaries, they introduce a new set of challenges, as culturally diverse individuals must share responsibilities. To navigate the increasingly global scientific landscape, researchers must maintain a level of "cultural competence," or a balance of knowledge, attitudes, and skills required to manage interactions and relationships with individuals from different ethnic, racial, religious, geographic, and social groups.

The most important step in becoming culturally competent is to be self-aware. Self-awareness reveals one's stereotypes, assumptions, values, beliefs, prejudices, and biases. As explained by Steven Spencer of the University of Waterloo and his colleagues in *Annual Review of Psychology* (67:415-37, 2016), "People experience stereotype threat when they are at risk of being judged or

treated in light of a negative stereotype about one of their social identities." The potentially negative effects of stereotype threat on the stigmatized racial and ethnic group or the individual include diminished motivation, aspirations, performance, and sense of belonging.

Once you recognize your own biases, there are many actions you can take to eliminate them. Try learning a language other than your native tongue. Learning another language affects one's thought processes and perceptions and provides a means for self-reflection. In addition, you can expand your cultural knowledge by participating in cultural competence–themed conferences, workshops, and seminars, and by accessing web-based resources.

The importance of getting to know people from different racial and ethnic backgrounds cannot be overstated. By maintaining active professional relationships with colleagues from diverse cultural backgrounds, and by working in a culturally diverse environment, you can free yourself from stereotypes you may have formed and reduce explicit and automatic expressions of racial bias.

When collaborative scientific projects expand across geographic boundaries, they introduce a new set of challenges, as culturally diverse individuals must share responsibilities.

Beyond diversifying your experiences in your professional life, you could also make an effort to visit a country with different racial and ethnic composition from your own. Short-term immersion experiences with other cultures can improve self-awareness of one's own culture, while increasing one's appreciation of other cultures and fostering cultural empathy, consciousness, and flexibility (*J Multicult Couns Devel*, 43:244-61, 2015).

As worldwide collaboration becomes the norm, the need for culturally competent scientists is greater than ever. Gast was right when she wrote: "International diversity is just as important as diversity of discipline when it comes to scientific discovery." It is the role of each scientist to advocate, encourage, and serve as a role model in cultural competence and foster a vibrant, peaceful, and mutually understanding global scientific community through connections and partnerships. The more culturally competent collaborating scientists are, the greater the outcome and impact of their research. ■

Ephraim M. Govere is the director of the Soil Research Cluster Laboratory at Pennsylvania State University.

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Sensors for All

A versatile modular strategy for detecting small molecules in eukaryotes

BY RUTH WILLIAMS

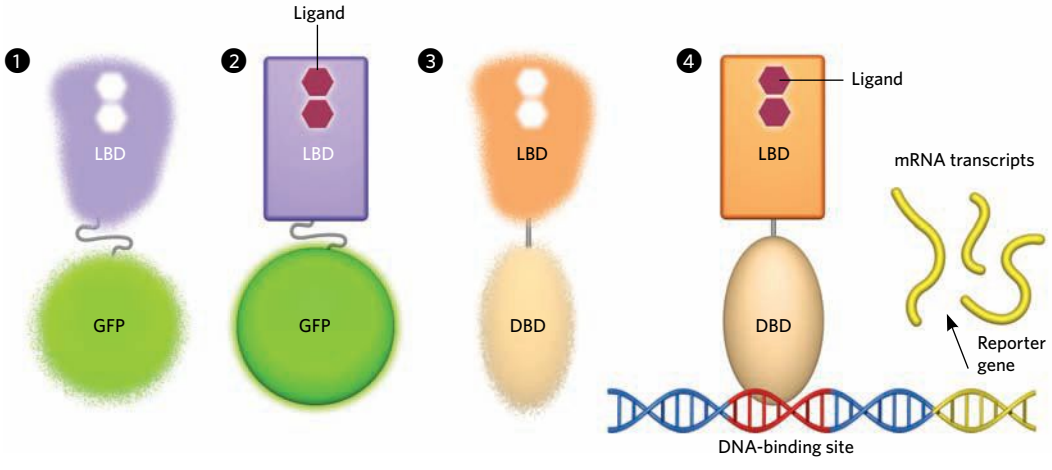
The ability to detect small molecules of interest has wide applicability in biological research, biotechnology, and especially synthetic biology. For example, turning cells into factories that produce small molecules—for use as drugs, biofuels, and more—is the goal of many synthetic biology endeavors. Just like regular factories, cellular ones require optimization. "In many cases we can create a valuable compound, but at a very low yield," says Dan Mandell, a postdoctoral researcher in George Church's Harvard University lab.

Scientists can attempt to improve production, but there is often no fast way to know whether they've succeeded. Mass spectrometry, for example, is a very sensitive and reliable way to detect small molecule production, says Mandell, but it's "somewhat cumbersome, expensive, and slow."

Specific sensors exist for only a handful of compounds. But now, Mandell and colleagues have devised a system that, in theory, could be used to make sensors for essentially any small molecule and that can be modified for use in any cell.

The key to the system is to create a conditionally stable ligand-binding domain (LBD), a peptide that tightly binds the small molecule in question, but that rapidly degrades without it. This LBD can then be fused to a range of proteins—ones that fluoresce, ones that drive transcription of reporter genes, and so on—such that the presence of the small molecule leads to the production of an easily detected signal.

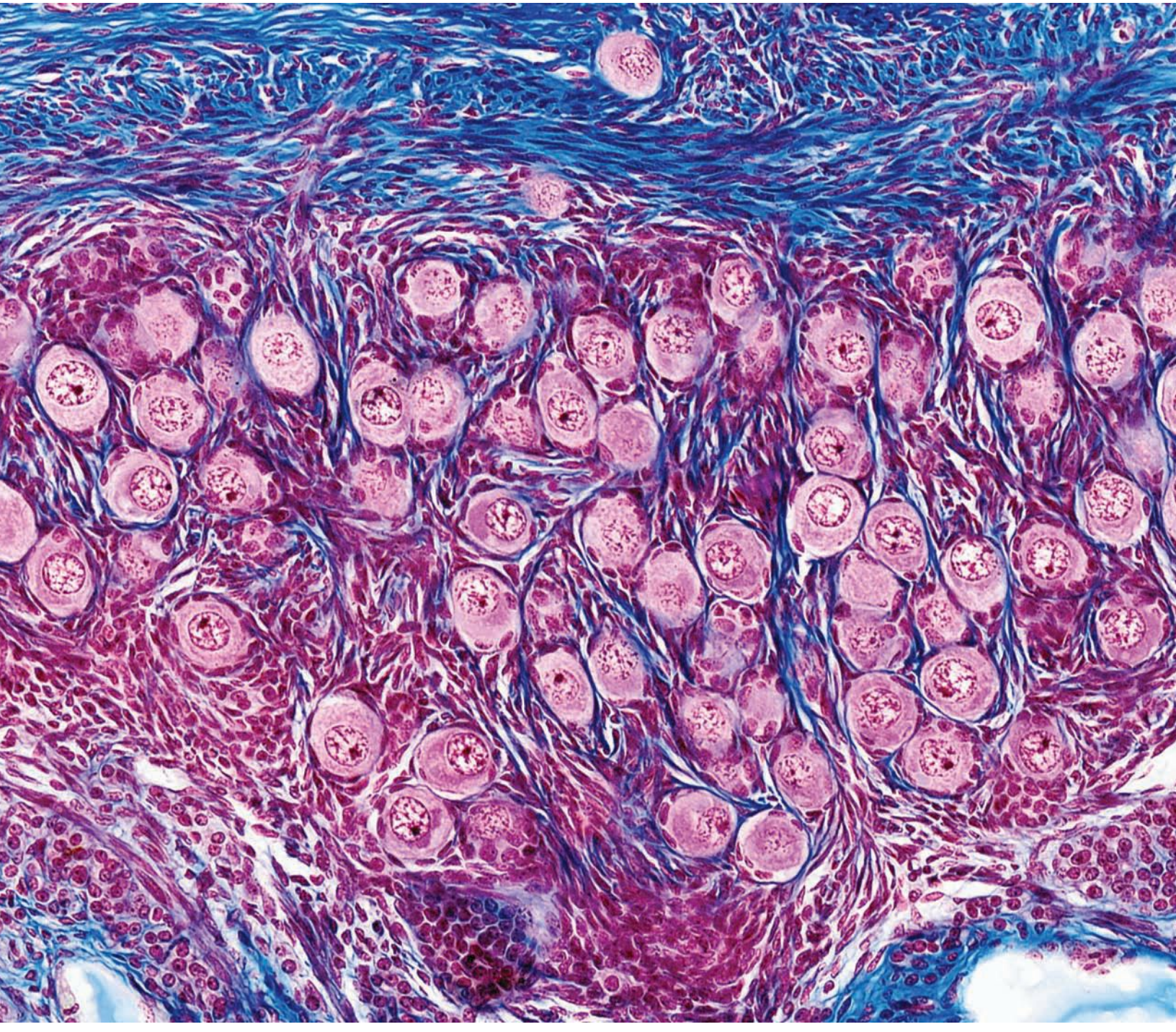
So far the team has created two LBDs—for digoxin and progesterone—and fused them to a variety of proteins to produce a range of sensors that can detect these two steroids in yeast, human cells, and even plants. "Creating sensors to detect and measure the levels of molecules inside the cell is a holy grail for synthetic biology," explains Jay Keasling of the University of California, Berkeley. (*eLife*, 4:e10606, 2015)



SENSOR SET-UP: To detect a small molecule of interest (the ligand), a conditionally stable ligand-binding domain (LBD) is fused to a reporter, such as green fluorescent protein (GFP). The complex degrades if the ligand is not present ❶, and activates the reporter when it is ❷. In another demonstration of this sensor, researchers connected the LBD to a DNA-binding domain (DBD) ❸. When the ligand is present, the DBD hooks onto to a site in the genome (red), which results in the expression of a specified reporter gene (yellow) ❹.

AT A GLANCE

SMALL MOLECULE DETECTION	HOW IT WORKS	PROS	CONS
Mass spectrometry	Contents of cells are ionized, accelerated through a mass spectrometer, and the small molecule of interest is detected and quantified.	Highly sensitive, well-established, no up-front engineering of proteins required	Very low throughput (a few dozen samples per machine per day), making optimization of production extremely slow
Conditionally stable LBD biosensors	The LBD is rapidly degraded in the absence of the molecule of interest, but is stabilized in its presence, enabling the activation of a fused reporter domain. The more abundant the molecule, the stronger the reporter signal.	Massively high throughput: billions of yeast cell variants or millions of mammalian cells can be assessed per day. Reporter domains can be changed depending on the cell system and desired readout.	A highly specific conditionally stable LBD must exist naturally or be engineered for each small molecule of interest.



A Scrambled Mess

Why do so many human eggs have the wrong number of chromosomes?

BY KAREN SCHINDLER

« A light micrograph of a section of fetal ovary shows primordial follicles (light pink ovals) with oocytes (dark pink spots) that have already begun to mature into fertilizable eggs. But the process won't be complete for decades, during which time mistakes in chromosome division can occur.

Up to a quarter of pregnancies are not carried to term; oftentimes an embryo is aborted by the body before a woman even knows she's pregnant. The most common cause of miscarriage is egg aneuploidy—the oocyte contains too many or too few chromosomes. Aneuploidy is thus the leading genetic cause of infertility, and those embryos that are not miscarried can result in children with developmental disorders, such as Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Turner syndrome (monosomy X).

For more than 80 years, the scientific community has known that the incidence of Down syndrome births increases with maternal age and that female fertility rapidly declines after the age of 35.[1] These concerns can be bypassed by the use of donor eggs from younger women, however, suggesting that the eggs of older women are the source of the reproductive decline, not the mother's reproductive system itself. Sure enough, up to 20 percent of eggs in healthy females may be aneuploid, and this number increases with age. But despite the ubiquity of egg aneuploidy, the cellular and genetic reasons for the phenomenon are poorly understood.

We now know that the multistage process of meiosis that forms a woman's eggs is highly error prone.[2] While germ-line meiosis in males initiates at puberty and provides a fresh supply of haploid sperm cells until death, the life of an oocyte begins during female fetal development but does not finish for decades, providing multiple windows of opportunity for problems that compromise egg quality. And in the past two years, clinicians and basic scientists have started conducting analyses of human oocytes to get at the molecular details of this pervasive problem. Thanks to technical advances, such as genome-wide recombination mapping and high-resolution, live-cell imaging, we now have a clearer picture of how chromosomes behave during meiosis.

Once scientists understand the basic machinery that controls meiosis, they can develop appropriate diagnostics and interventions to help women achieve pregnancies with egg cells that have properly apportioned chromosomes. Currently, one in six couples is infertile, and about half of those cases are due to abnormalities on the female side. And as the average age at which a woman experiences her first pregnancy increases in the U.S. and other developed countries—in some nations, that age has reached 30—the challenges of aneuploidy will only become more common.

Divvying up the genome

During female fetal development, the primordial germ cells that give rise to oocytes replicate their full diploid complement of DNA, with each chromosome forming two sister chromatids joined along the arms and centromeres by a protein complex known as cohesin. Homologous chromosomes then pair with each other and exchange bits of DNA through homologous recombination. The process involves breaking the chromosomes and swapping bits of DNA between nonsister chromatids of a homologous pair (homologs). During the swap, termed crossing over, linkages called chiasmata form between homologs and are maintained until the onset of anaphase I several decades later, when the chromosomes are pulled apart before division into two daughter cells. This marks the completion of the first stage of meiosis (Meiosis I). If chiasmata fail to form, the chromosomes may separate improperly, a phenomenon known as nondisjunction. (See illustration on page 32-33 and "Picturing Inheritance, 1916" on page 72.) Most cases of trisomy 21 are due to maternal nondisjunction.

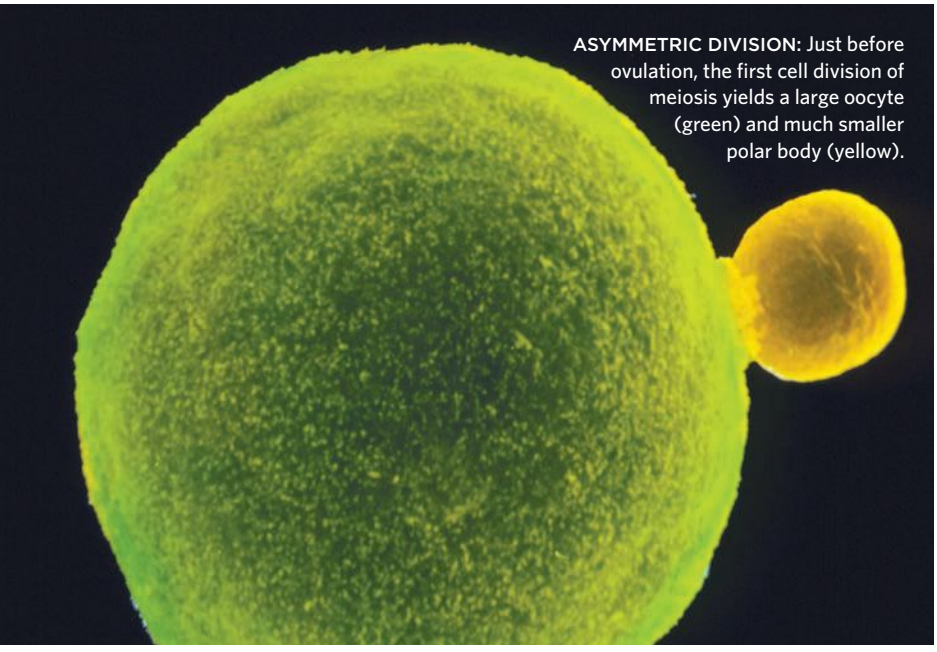
The life of an oocyte begins during female fetal development but does not finish for decades, providing multiple windows of opportunity for problems that compromise egg quality.

Last year, Christian Ottolini in Eva Hoffman's laboratory at the University of Kent in the U.K. and colleagues generated genome-wide recombination maps, dubbed "MeioMaps," and found evidence that properly functioning recombination is indeed protective against chromosome segregation errors in human oocytes. Using single nucleotide polymorphism (SNP) arrays with some 300,000 genetic markers, the researchers pinpointed the sites of recombination in 13 human oocytes and their associated polar bodies—the nonfunctioning cells produced during meiosis that do not become the mature egg—as well as 10 embryo–polar body sets from patients undergoing in vitro fertilization (IVF). Notably, this is the first time researchers have assessed all the products from a complete meiosis. In addition, the researchers performed preimplantation genetic diagnosis of 29 embryos to diagnose aneuploidy. While the number of recombination events were highly variable between samples, they tended to decrease with age. And oocytes that underwent less recombination were more likely to be aneuploid.[3]

Nondisjunction is not the only way to get eggs with an incorrect number of chromosomes. In fact, some data indicate that a more-frequent cause of aneuploidy is the premature separation of sister chromatids (PSSC).[4,5] Under normal conditions, cohesin is deposited along the length of chromosomes during premeiotic DNA replication to hold sister chromatids together. At the onset of anaphase during meiosis I, cohesin is cleaved along the chromo-

some arms, but it is protected at sister centromeres by a protein called shugoshin to ensure that sister chromatids remain together as homologs segregate. During anaphase of meiosis II, the remaining cohesin is cleaved, allowing sister chromatid separation and the formation of four fully haploid daughter cells. Therefore, to ensure proper sister chromatid associations throughout oocyte maturation, cohesin proteins laid down during fetal development must still be functional decades later.[6] If cohesin is lost or rendered dysfunctional at any point along the way, the sister chromatids can be pulled into different daughter cells prematurely.

Sure enough, as my colleagues and I as well as other groups have found, cohesin levels are reduced and sister chromatid centromeres begin to separate prematurely in oocytes from aged mice.[7,8,9] Similarly, the distance between sister chromatids in human oocytes increases with maternal age and aneuploidy rates go up.[10,11] These observations support the hypothesis that exhaustion of cohesin can lead to increased PSSC in human eggs.



ASYMMETRIC DIVISION: Just before ovulation, the first cell division of meiosis yields a large oocyte (green) and much smaller polar body (yellow).

Additionally, while in mice and other model organisms sister chromatid kinetochores—the two centromeric protein complexes that attach to the spindle microtubules extending from the cell's poles during meiosis—are fused together, recent research suggests that the same may not be true of chromosomes in human eggs. Last year, two independent groups used high-resolution imaging to examine the geometry of the sister-chromatid kinetochores in human oocytes harvested for IVF and found that they were not fused, and thus did not act as a single unit as they do in mice and other organisms, where they serve as further insurance that both chromatids end up in the same daughter cell following the first meiotic division.[12,13] The distance between sister chromatid kinetochores in human oocytes increases with maternal age, but kinetochore separation is also frequently observed in younger women, possibly contributing to the fact that even young women can have high rates of meiotic aneuploidy.[14,15] (See "In the Genes" on opposite page.)

But high rates of PSSC do not rule out a role for recombination defects in aneuploidy. In 2006, Beth Rockmill, then in Shirleen Roeder's lab at Yale University, and colleagues observed wild-type yeast strains engineered to harbor an extra copy of chromosome 3 containing selectable markers so that they could easily detect PSSC. After dissecting 1,300 tetrad spores—the equivalent of a mammalian egg and its three polar bodies—the researchers found a correlation between PSSC and crossovers that occurred close to the centromere, suggesting that where along their length homologous chromosomes recombine is important. If the crossover is too close to the centromere, it may interfere with sister chromatid cohesion, causing the sister chromatids to dissociate.[16] Ottolini and collaborators also found that some chromosomes in human eggs failed to suppress crossovers at or close to centromeres—consistent with the team's observations of elevated PSSC.

All of these missegregation scenarios are chromosome-centric. What is missing from these pictures, however, is the behavior of the microtubules that connect the chromosomes to the spindle poles on opposite sides of the cell. Even if sister chromatids do separate prematurely, they may not segregate improperly if the microtubules hook up as they would if the chromatids were still attached. But if these connections are not correct, chromosomes are at risk of ending up in the wrong daughter cell. The attachment of sister kinetochores to microtubule fibers from opposite poles during meiosis I, for example, could cause sister chromatids to split up. As the distance between sister chromatids increases with maternal age, the risk of such aberrant microtubule attachment also likely increases.

Only once scientists understand the basic machinery that controls meiosis can they develop appropriate diagnostics and interventions to help women achieve pregnancies with egg cells that have properly apportioned chromosomes.

By visualizing 100 human oocytes as they underwent spindle formation during meiosis I, Zuzana Holubcová in Melina Schuh's laboratory at the Medical Research Council in Cambridge, U.K., and colleagues observed several abnormalities in building the spindle.[17] In some cases, the spindle structure was unstable and would either lack any poles or become multipolar. The researchers also noted chro-

mosome segregation problems such as lagging chromosomes that would remain in the center of the spindle during anaphase I. They hypothesized that these lagging chromosomes resulted from errors in how the microtubules attached. Taking a snapshot of the microtubule connections, they found that 20 percent of sister chromatid kinetochores attached to both poles instead of a single pole. In mice, such attachment is a trial-and-error process in which aberrant connections are normally fixed. If human oocytes are inefficient at correcting such attachment errors, it could explain the high rate of chromosome missegregation during the formation of human eggs.

Additionally, all of the human oocytes Holubcová tracked lacked microtubule-organizing centers that help coordinate spindle assembly in mouse oocytes. Instead, chromosomes initiated microtubule growth. Moreover, the researchers discovered that human oocytes took an unusually long time to build the spindle—a whopping 16 hours, compared to just 5 hours in mouse oocytes and the 30 minutes it takes cells to build spindles for mitotic division. Such inefficient spindle formation could favor incorrect attachments that can lead to aneuploidy. Given the importance of microtubule attachments for proper chromosome segregation in human oocyte development, studying oocyte spindle biology will be critical to understanding why meiosis I is so error prone.

A closer look

Surprisingly, improper chromosome segregation doesn't always lead to aneuploid oocytes. Ottolini's team observed, for example, that some oocytes that had experienced PSSC still contained the proper number of chromosomes at the end of meiosis II. Specifically, these oocytes appeared to have completed meiosis backwards, separating sister chromatids in meiosis I and homologous chromosomes in meiosis II, as evidenced by the fact that their first polar bodies (formed during meiosis I) contained a pair of homologous chromosomes, each with just one sister chromatid. During the second meiotic division, then, the oocytes segregated those homologous chromatid pairs, resulting in a euploid cell, or one with a normal chromosome number. This phenomenon, which the authors termed "reverse segregation," brings into question how ordered chromosome segregation actually is in human oocytes.

A similar phenomenon could also result when paired homologous chromosomes, or bivalents, separate prematurely. In the 1990s, Roslyn Angell at the University of Edinburgh examined 200 discarded oocytes from patients undergoing IVF and observed 61 cases of lone homologs (univalents) that had apparently separated precociously during metaphase of meiosis I, prior to the first meiotic cell division.[18,19] Last year, using live, high-resolution confocal microscopy to track individual kinetochores, Yogo Sakakibara in Tomoya Kitajima's laboratory and colleagues at the RIKEN Center for Developmental Biology in Kobe, Japan, documented the same phenomenon in oocytes from young and old mice: homolog kinetochores were sometimes farther apart than normal, and this often led to univalent formation.[20]

The resulting univalents had one of three fates during meiosis I, two of which involve unbalanced segregation: both chromatids of one univalent could segregate into one daughter cell,

IN THE GENES

By Jacob Ohring

Although maternal age is clearly associated with the incidence of aneuploidy, it does not explain why some reproductively young women (<35 years of age) have higher than average levels of aneuploidy. Some population-based studies point to genetics as the missing link. For example, marriages between close relatives are associated with increased aneuploidy among children in specific populations. In 1970, an estimated 50 percent of all marriages among native Kuwaitis occurred between close family members, and 40 percent of non-native Kuwaitis living in the country were in familial marriages (*Clin Genet*, 27:483-86, 1985). Data from the 11,614 births that occurred that year in the Kuwait Obstetric Hospital supported the effects of increased maternal age, but also pointed to close kinship between the parents as causing an increase in the incidence of children born with Down syndrome. Bedouin Kuwaitis, who have higher rates of consanguineous marriages than urban Kuwaitis, had nearly double the risk of having a child with the disorder (3/1,000 births, compared with 1.6/1,000 births).

Analyses of Down syndrome in the U.S. between 1983 and 1990 have also linked genetics to rates of the disorder. Data from 17 state surveillance programs revealed higher rates of Down syndrome for Hispanic populations (1.8/1,000 births) than for white (0.92/1,000 births) and black populations (0.72/1,000 births), even when controlling for maternal age. The US Centers for Disease Control and Prevention blamed these discrepancies on the differential use of prenatal diagnostics, but this trend for Hispanic mothers was also identified in South American countries, where access to these services is more equal: in a remote hospital in Chile between 1997 and 2003, the prevalence of Down syndrome was 2.96/1,000 live births. These studies, and many others, support the hypothesis that some women are genetically predisposed to producing aneuploid gametes, even at a young age.

With the advent of embryo screening in IVF clinics, together with the decreasing costs of next-generation sequencing, it is easy to imagine that an evaluation of the genomes of patients who produce more or fewer aneuploid embryos could identify causal gene variants. In a genome-wide analysis of single nucleotide polymorphisms (SNPs) in 2,362 unrelated mothers, for example, researchers identified a region of chromosome 4 that is associated with a mistake in the first mitotic division after fertilization (*Science*, 348:235-38, 2015). Of the many genes contained in this region of chromosome 4, *polo-like kinase 4* (*PLK4*) stands out as possibly important for maintaining the correct chromosome number in the developing embryo, as it is known to regulate spindle formation in other cell types. This functional connection has yet to be tested, however, and until more studies are conducted, the scientific community remains largely in the dark about the genes that underlie gamete quality.

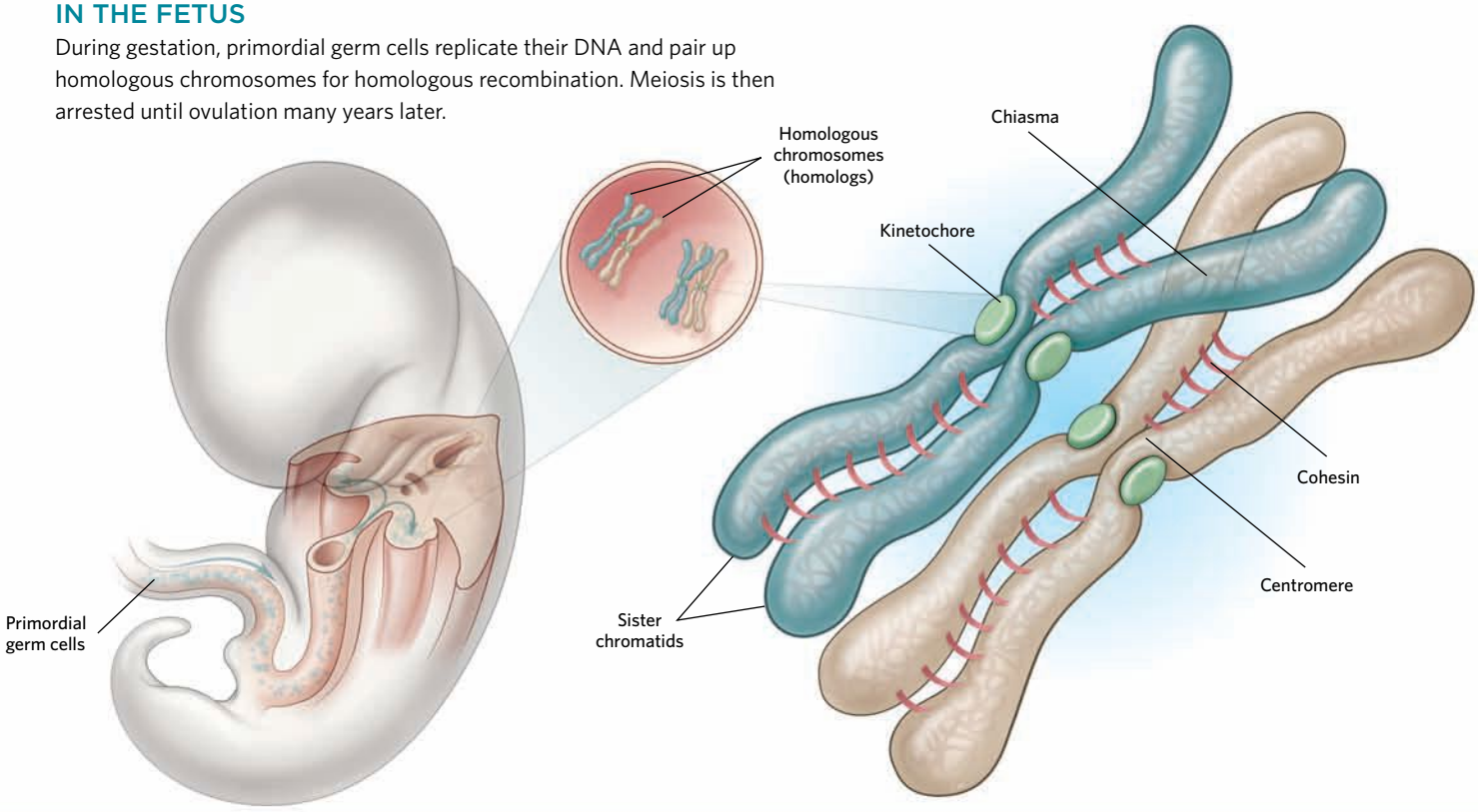
Jacob Ohring is an undergraduate genetics major at Rutgers University.

MEIOTIC MYSTERIES

Meiosis in human females takes place over decades. At any point in this process, an incorrect number of chromosomes can be transferred to daughter cells, resulting in aneuploid gametes, the most common cause of miscarriage and the root of certain developmental disorders, such as Down syndrome.

IN THE FETUS

During gestation, primordial germ cells replicate their DNA and pair up homologous chromosomes for homologous recombination. Meiosis is then arrested until ovulation many years later.

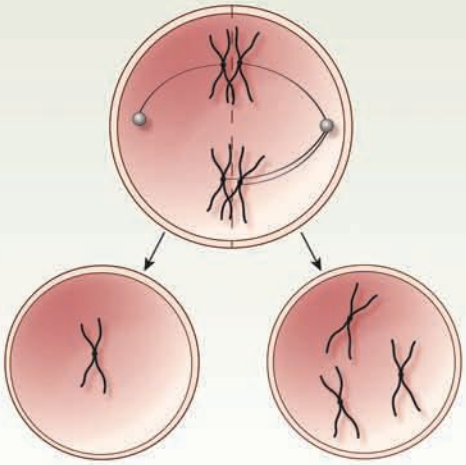


WHEN THINGS GO WRONG

There are multiple ways that the normal process of meiosis can go awry and lead to aneuploid gametes, including nondisjunction, premature separation of sister chromatids, and premature bivalent separation.

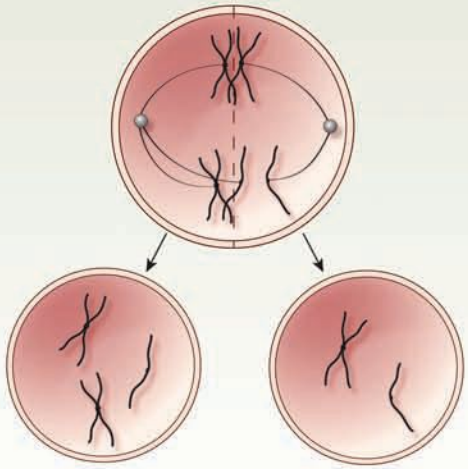
NONDISJUNCTION

Improper microtubule attachment can lead to the unequal distribution of chromosomes in the oocyte and polar body.



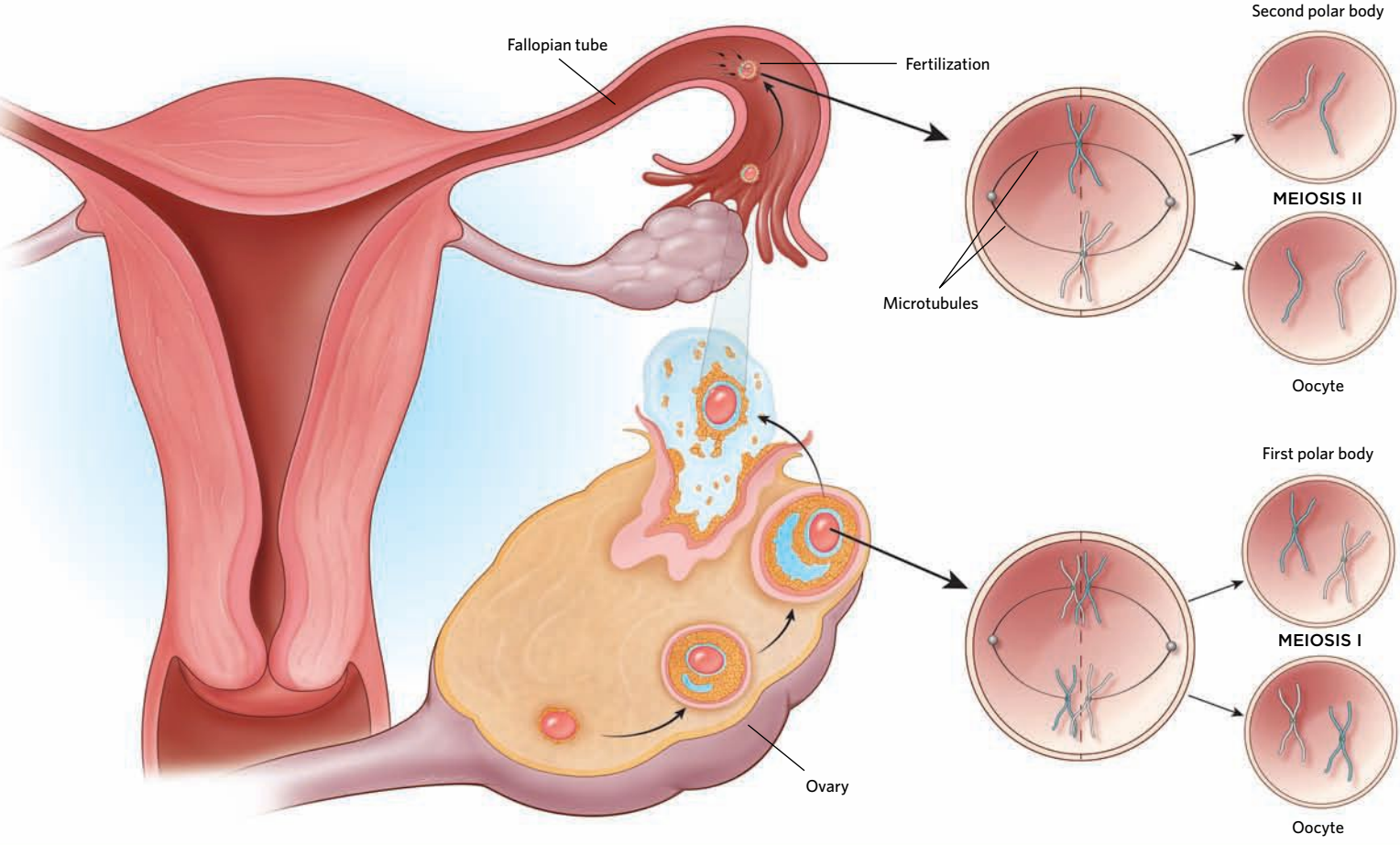
PREMATURE SEPARATION OF SISTER CHROMATIDS (PSSC)

When the cohesin that holds sister chromatids together breaks apart too early, the chromosome halves are subjected to random segregation, often getting separated prematurely.



IN THE ADULT

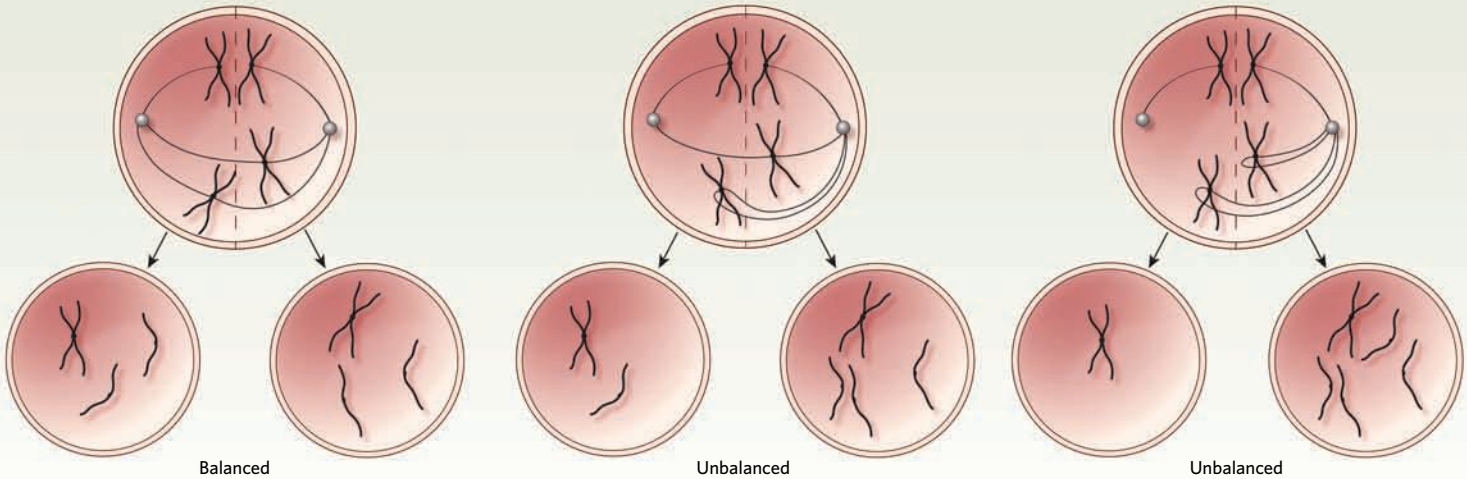
Just before ovulation, the oocyte resumes meiosis, building a meiotic spindle of microtubules to segregate homologous chromosomes. Upon fertilization, the egg undergoes a second round of division, segregating sister chromatids. (These divisions are asymmetrical, resulting in one large oocyte and two or three small polar bodies, shown here as equal size.)



PREMATURE BIVALENT SEPARATION

A third type of misfiring occurs when paired homologs (bivalents) separate prior to the first meiotic cell division. Then, during meiosis I, the cell seems to treat the resulting univalents as it would during meiosis II, with microtubules attaching to the kinetochores on either sister chromatid.

While this often results in balanced division, with the two chromatids of each homolog segregating into separate daughter cells, other times both chromatids of one or both univalents can segregate into one daughter cell.



while the chromatids of the other homolog were separated, or all four chromatids of the two univalents could end up in the same daughter cell. Most of the time, however, the segregation was balanced, where the two sister chromatids of each homolog segregated into separate daughter cells. The resulting egg was euploid but with one sister chromatid from each homolog instead of both chromatids from a single homolog—just like the reverse segregation patterns observed by Ottolini's team. (See illustration on previous page.)

Sakakibara and colleagues also examined three human oocytes from donors over the age of 35 and again observed univalents prior to meiosis I segregation, suggesting that this separation of homologs may contribute to high rates of egg aneuploidy. But because a balanced division of the resulting univalents would result in a euploid egg, a chromosome analysis without watching the chromosome behavior would fail to detect any issue. Only through the power of live imaging can researchers detect improper, yet balanced, chromosome segregation.

Because these embryos are euploid, it is not known if they are developmentally equivalent to those derived from classical meiotic segregation.[21] Perhaps selection of these euploid embryos for transfer could help explain the low success rates of IVF procedures, in which fertilized eggs are screened for aneuploidy and other chromosomal abnormalities before being transplanted into the host uterus. If such reverse segregation is detrimental to the fetus, IVF screens must sample both embryos and polar bodies after fertilization to identify all cases where meiosis may have gone awry.

A grain of salt

While the study of oocytes retrieved from IVF clinics has greatly improved our understanding of mistakes that can occur during meiosis, the results must be interpreted with caution. Most of the patients have undergone hormonal stimulation to increase the number of oocytes retrieved, possibly recruiting oocytes of poorer quality. Moreover, eggs that successfully complete meiosis I are fertilized and developed into embryos, leaving those oocytes that have not yet completed meiosis I to be used for these types of studies. Therefore, it is possible that these discarded oocytes are not representative of how a healthy oocyte would behave.

Currently, most US states and other countries do not allow financial compensation to women to donate their oocytes for research. It is therefore rare that one would volunteer to undergo an invasive process for the sake of scientific advancement, thereby limiting the oocytes used in experiments to those from women undergoing IVF.

In addition to the remaining questions about how chromosomes in normal human oocytes (mis)behave, we are also left with trying to understand why. What molecular players are deficient in human oocytes compared to other organisms such as mice that have lower rates of aneuploidy? Can methods of gamete selection that aim to fertilize only the eggs that did everything right during meiosis I be improved? And is it possible to develop interventions to correct this error-prone process when patients are undergoing IVF?

Answering these questions will be essential for improving IVF outcomes. Hopefully, by coupling these observational experiments using human oocytes with genetic and cellular biological experiments that can be conducted in model systems, researchers in the field of human reproductive biology will soon solve these mysteries. ■

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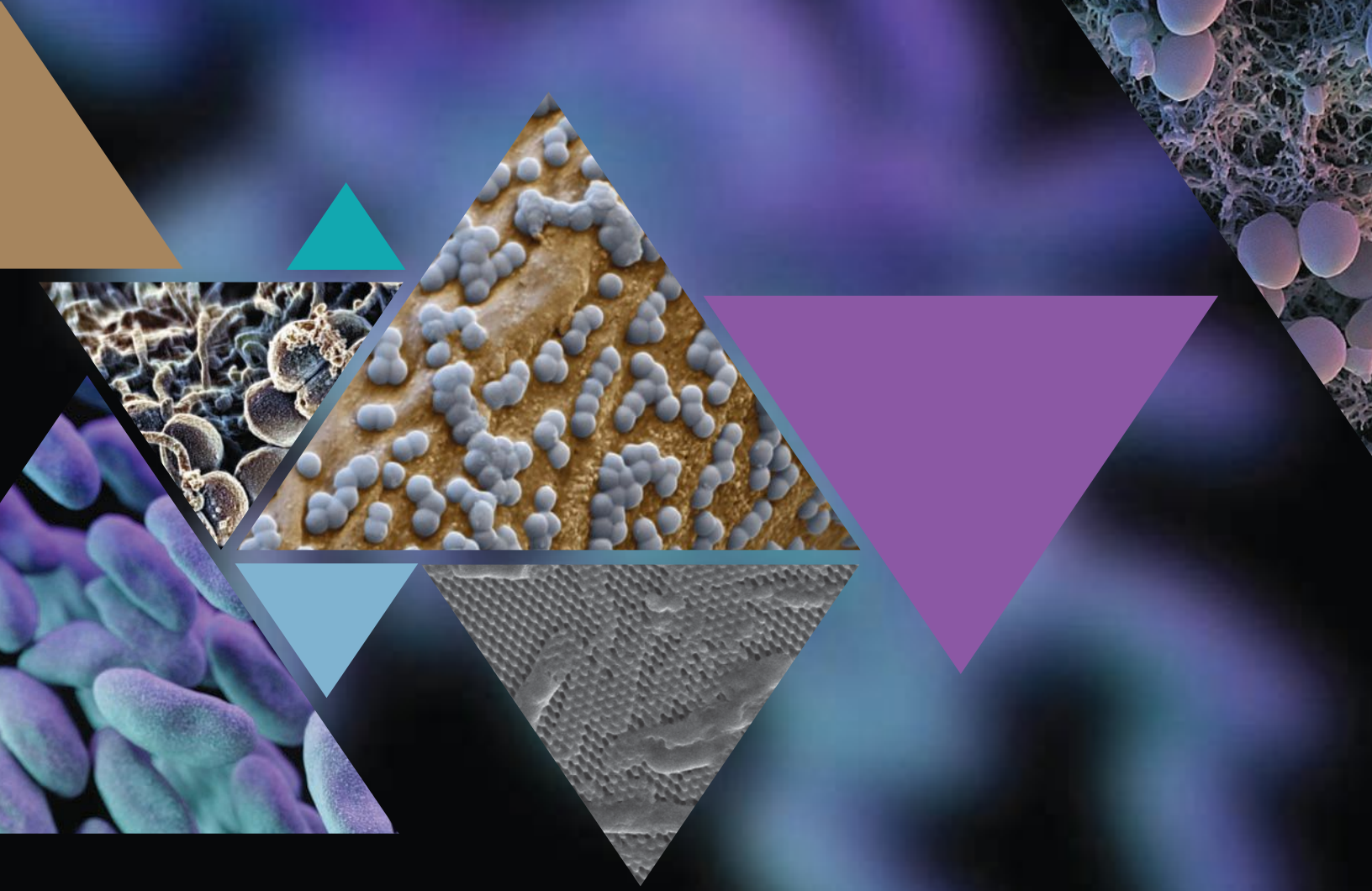
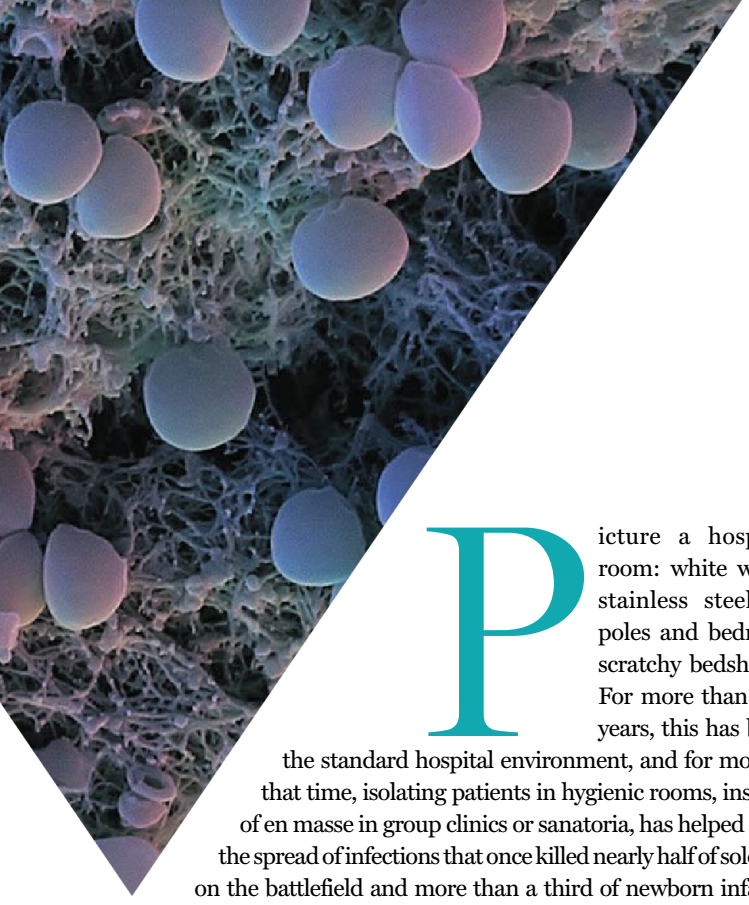


NANOSCALE DEFENSES

Coating hospital surfaces, surgical equipment, patient implants, and water-delivery systems with nanoscale patterns and particles could curb the rise of hospital-acquired infections.

BY EDWARD D. MARKS AND STEVEN SMITH



Picture a hospital room: white walls, stainless steel IV poles and bedrails, scratchy bedsheets. For more than 100 years, this has been the standard hospital environment, and for most of that time, isolating patients in hygienic rooms, instead of en masse in group clinics or sanatoria, has helped curb the spread of infections that once killed nearly half of soldiers on the battlefield and more than a third of newborn infants.

But with the recent rise in antibiotic-resistant pathogens, this standard is no longer sustainable. In 2011, the most recent year data are available from the US Centers for Disease Control and Prevention (CDC), some 720,000 patients acquired an infection while being treated in a health care facility; more than 75,000 of those people died.

"Imagine one full jumbo jet crashed each day, killing everyone on board," says Michael Schmidt, vice chairman of microbiology and immunology at the Medical University of South Carolina (MUSC). "This is precisely the number of people that die each day in the U.S. from a hospital-associated infection."

In the face of such hospital-acquired, or nosocomial, infections, and the impossibility of developing effective antibiotics quickly enough, researchers are looking to update that white-walled hospital room. The stainless steel IV poles and bedrails could, for example, be coated in nanoparticles of metallic copper, which has antimicrobial properties. Long, thin filaments of non-toxic, bactericidal zinc could provide a protective metallic coating to those scratchy bedsheets, as well as the curtains and paper towels. And the nurse call button and other surfaces could be etched with nanopillars that kill bacteria on contact.

Such nanoscale technologies can decrease the ability of bacteria to adhere to and grow on surfaces by increasing the permeability of bacterial membranes, disrupting protein function, and interfering with cell-cell communication. Moreover, by preventing bacteria from attaching to and colonizing surfaces, these technologies also make it impossible for the microbes to reach a critical population size (the threshold number varies by orders of magnitude depending on the strain) that triggers the formation of a biofilm, which inhibits the entry of antibiotics and disinfectants.

Preliminary in vitro studies have demonstrated that a diverse array of nanotechnologies are effective against some of today's most dangerous pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant Enterobacteriaceae (CRE), and *Klebsiella pneumoniae*. The widespread implementation of these approaches could serve as a stopgap until new therapies are available, and provide additional protection against infection to keep hospitals safe. In addition, nanopatterns designed to be harmless to host cells could be applied to synthetic implants to ward off bacterial growth. As researchers continue to refine and test these approaches against a range of pathogens, the full utility of microbe-resistant materials will become clear.

Bacteria-resistant surfaces

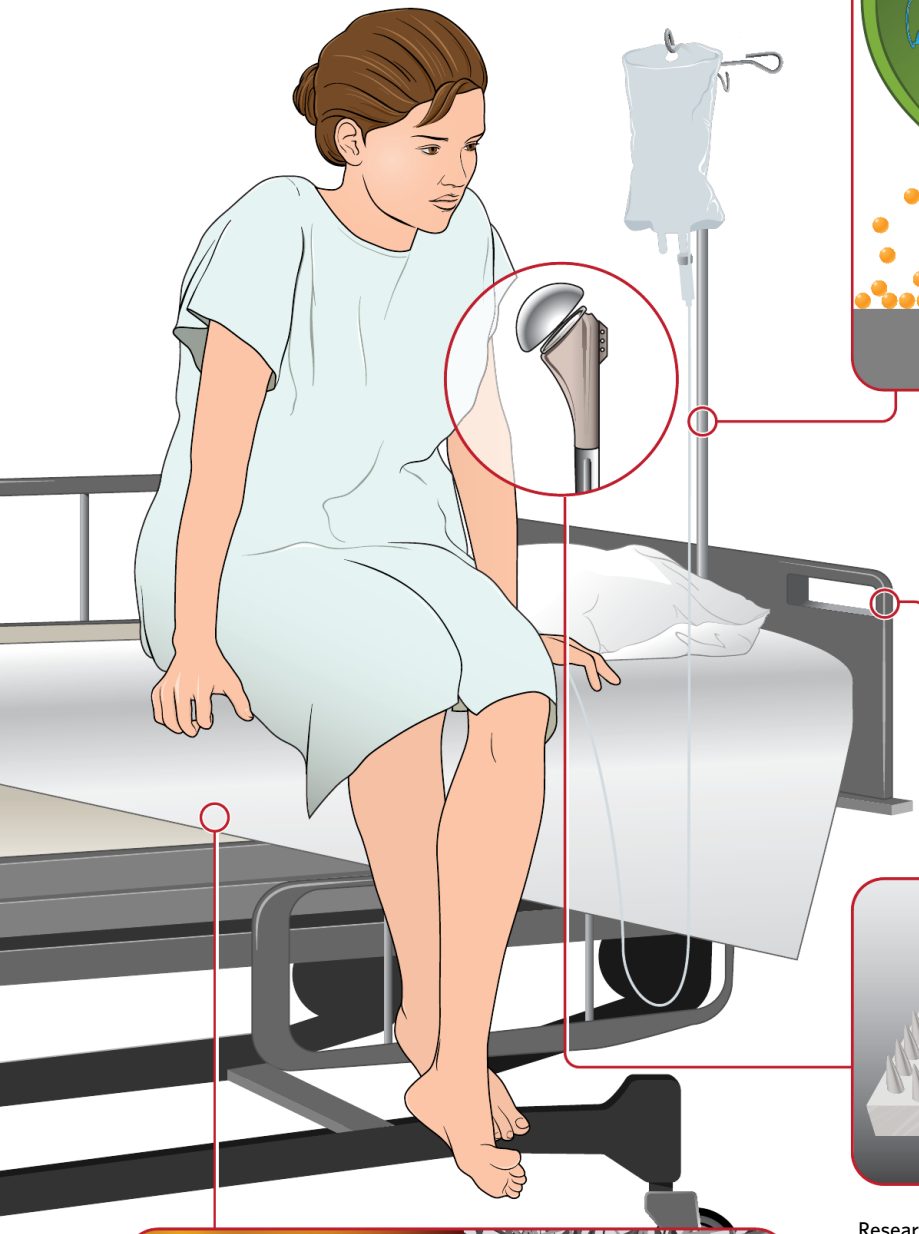
A few years ago, Schmidt and his colleagues at MUSC tested the idea that an environment coated in copper could stem the spread of infection in a hospital setting. The goal was to harness the antimicrobial power of metallic copper ions, which interact with bacterial surface proteins, damage cell membranes, and are passively or actively uptaken into the cytoplasm. Once inside the bacteria, copper ions form free radicals that damage intracellular proteins and lipids. (See illustration on opposite page.) Copper can even sometimes repel microbes from ever colonizing a surface in the first place. Bacterial membranes and cell walls are studded with proteins that initiate adhesion to surfaces; positively charged copper ions interact with these negatively charged bacterial adhesion proteins to distort protein shape and function, and can outcompete other metals such as zinc that are essential for protein function.

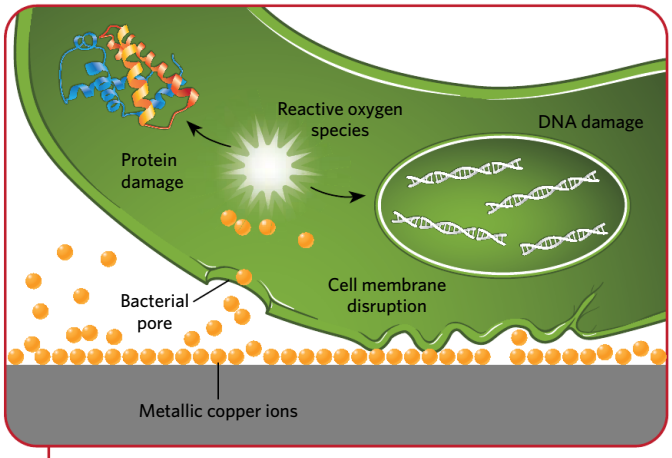
> WE CAN TURN ALMOST ANY MATERIAL INTO ONE THAT REDUCES BACTERIAL ADHESION AND GROWTH, ALL BY IMPLEMENTING NANOSCALE FEATURES.
>
> —Thomas Webster, Northeastern University

Schmidt and his colleagues refitted several hospital intensive care unit (ICU) rooms using metallic copper alloy surfacing to cover bedrails, IV poles, nurse call buttons, and visitors' chairs, then randomly assigned patients to either the copper-laden rooms or rooms disinfected using standard protocols. After one year of observation across three separate hospitals, the concentration of bacteria on the surface of the copper-covered objects was a fifth

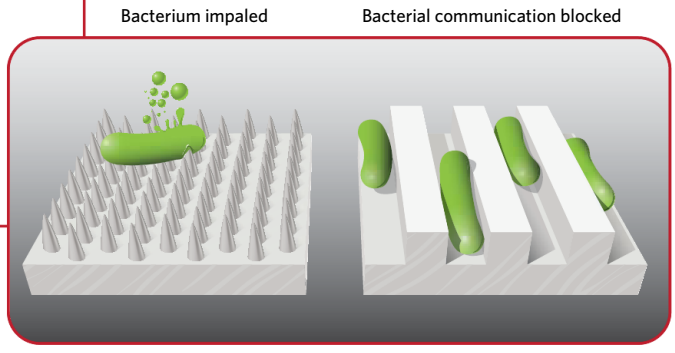
NANOSCALE SOLUTIONS

In the face of antibiotic-resistant pathogens and rising numbers of hospital-acquired infections, researchers are turning to nanotechnology to curb the spread of disease in health-care settings.

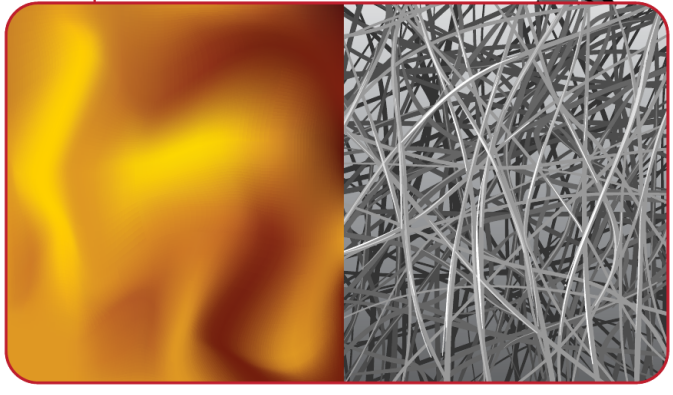




A primary goal of these nanotechnologies is to prevent the growth of bacteria on surfaces, such as bedrails and IV poles in hospital rooms. One option is to coat surfaces with metallic ions that interact with bacterial proteins, disrupting their normal function. The ions also increase cell membrane permeability and cause eventual lysis of the bacterium. Within the cell, the ions trigger the formation of reactive oxygen species that damage DNA and other cellular components.



Researchers are also using laser etching to create nanoscale patterns that are resistant to bacterial growth. Certain patterns, such as nanograting, trap bacteria and prevent the intercellular signaling that bacteria use for biofilm formation; surfaces covered in nanospikes can penetrate bacterial cell membranes, killing the microbes. Such techniques are also being applied to medical implants to prevent infection, while still allowing the much larger host cells to adhere and grow.



Metallic copper–laden fabric | Nanoscale view of electrospun fabric

Other items in the hospital room that can harbor bacteria are fabrics, such as bedsheets and curtains. To produce large sheets of antimicrobial nanofibers, researchers use a process called electrospinning. This method can also add antimicrobial nanoparticles and antibiotics to common materials such as silk and cotton to increase microbicidal properties.

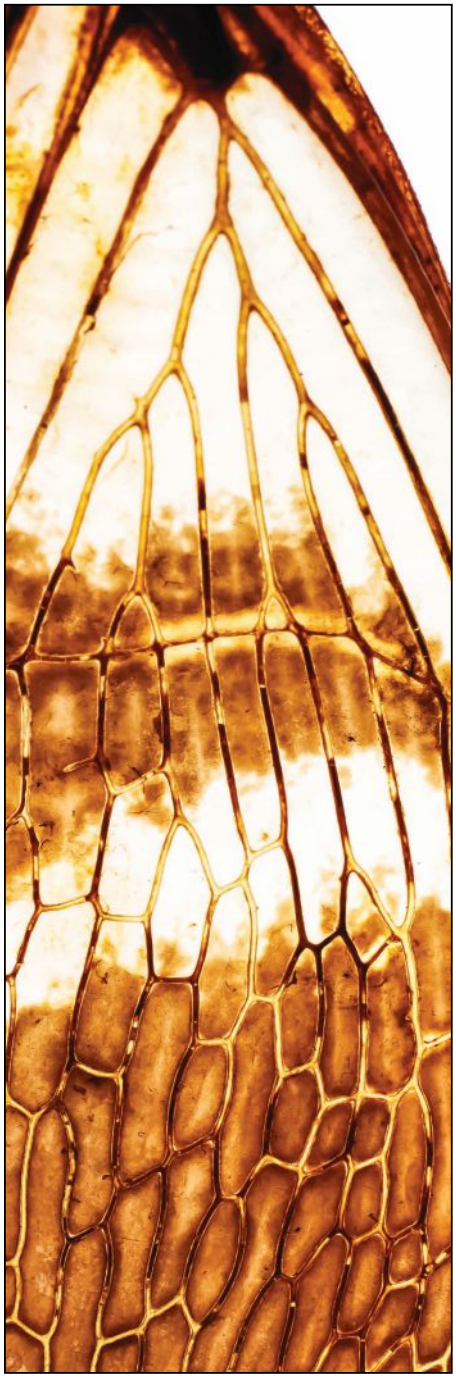
of that on objects in standard ICU rooms, and the rates of nosocomial infection among patients assigned to "copper rooms" were almost 60 percent lower than those in the control rooms.[1]

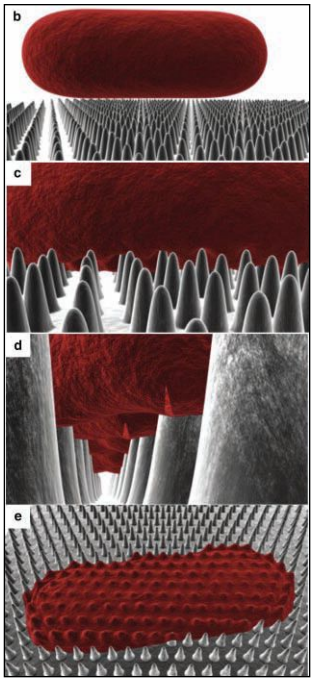
However, the MUSC team's method required that the copper coatings be constructed as full pieces of hardware, which can be prohibitively expensive and time-consuming to install in most modern health care settings. As an alternative, researchers may simply be able to apply metallic nanoparticles to an existing surface to achieve similar antibacterial effects. In 2013, Northeastern University chemical engineer Thomas Webster, president of the US Society for Biomaterials, teamed up with one of his former graduate students to create a selenium nanoparticle spray that can be applied to any surface to cut down on microbial numbers.[2] The spray dries within minutes, leaving a layer of antimicrobial nanoparticles behind, and was shown to be nonhazardous in small-animal toxicity studies. Testing the spray on common hospital items, including chairs, bedsheets, and even paper towels, the researchers found that it decreased the overall microbial burden.[3] Crucially, the nanoparticles were stable and active until the surfaces were used or washed. "We have seen we can turn almost any material into one that reduces bacterial adhesion and growth, all by implementing nanoscale features," says Webster.

Another option for preventing microbial growth is to design antibacterial nanoscale features that can be etched into a variety of synthetic materials. Nanoscale pits or troughs can trap bacteria and prevent cell-cell communication, for example. Comparing micron- to nanometer-size troughs, Joanna Verran's group at Manchester Metropolitan University in the U.K. showed that 200-nm troughs decreased adhesion of three bacterial strains and one yeast strain. As the feature sizes increased (from 500 nm to 2 μm), microbes that preferentially produced biofilms adhered to surfaces more readily: MRSA started adhering at 500 nm, *Pseudomonas aeruginosa* at 1–3 μm, and *Candida albicans* at 2 μm.[4]

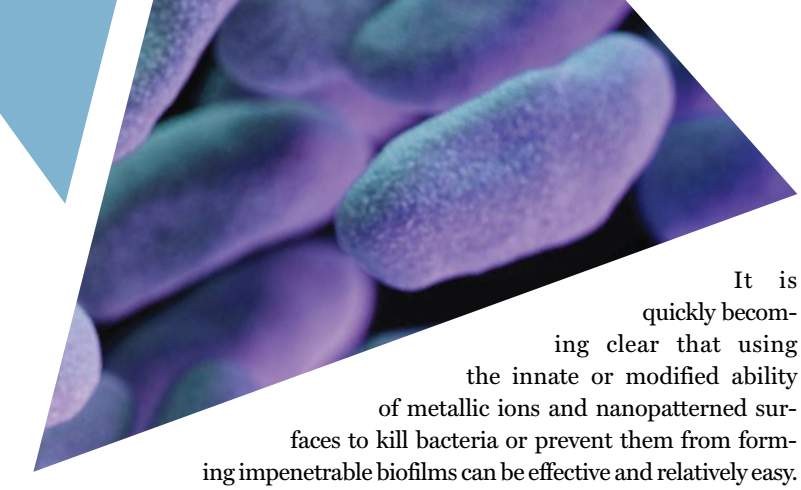
Alternatively, nanospikes can kill bacteria by penetrating their cell membranes, controlling microbial growth. In 2013, for example, Albert Yee's team at the University of California, Irvine, showed that nanopillars that mimic the texture of a cicada wing aid in killing gram-negative bacteria such as *Escherichia coli* and *Klebsiella*.[5] And in research presented at this year's American Chemical Society conference in San Diego, the team demonstrated that nanopillars of a slightly different shape modeled after the topography of a dragonfly's wing are able to kill gram-positive bacteria such as MRSA.[6]

As an added bonus, such nanopatterned surfaces can often be equipped with functional components, such as biopolymers and antibiotic side chains, that can further decrease the microbial load on a given surface. In a proof-of-concept study, Virginia Davis's lab at Auburn University in Alabama designed a sheet of antimicrobial single-walled carbon nanotubes (SWNTs). Normally used in electronics development, SWNTs are extremely stable at high temperatures, pressures, and shear stresses, and have a carbon backbone that allowed the team to attach the antibacterial protein lysozyme. In as little as 30 seconds, the material increased bacterial killing by 50 percent compared with nonlysozyme controls.[7] Davis and her colleagues formulated sheets of SWNTs as thin as 1.6 nm, allowing the nanomaterial to interact more effectively with bacteria, which have component parts as small as 0.5 nm.





SPIKY WINGS: The wings of some cicada species are covered in nanoscale pillars that provide resistance to bacterial infection. Recreating such nanopatterns on synthetic materials could inform the design of antibacterial products. In the 3-D model shown below (b-e), the outer layer of a rod-shape bacterium (red) begins to rupture against the nanopillars (gray), causing the cell to collapse.



It is quickly becoming clear that using the innate or modified ability of metallic ions and nanopatterned surfaces to kill bacteria or prevent them from forming impenetrable biofilms can be effective and relatively easy. Crucially, pathogens should be unable to evolve resistance to nanosurface strategies of fighting infection: nanoparticle surface energy can always distort protein function on a purely chemical basis, and nanotopographical features will always trap or lyse bacteria. Researchers are now working to develop scalable approaches such as high-throughput etching to tailor nanomaterials to fit large-scale antimicrobial needs.

Nanopatterned devices

Other surfaces that are prone to bacterial growth are those of medical implants, such as hip and knee replacement joints or artificial heart valves. In fact, up to half of all nosocomial infections result from implanted devices, and microbial biofilm growth is a significant cause of implant removal. Moreover, bacteria do not need to be antibiotic resistant to cause an issue, as niches within implants can shelter biofilm-forming microbes from antibiotics and host immune systems.

New nanotechnologies are poised to prevent such problems, however. The use of nanosilver as a coating on embedded medical devices has already demonstrated the ability to inhibit biofilm formation.[8] And patterning the plastic or metal surfaces of these implants with nanoscale pillars or pits could similarly decrease the growth of bacterial cells.

Nanopatterned surfaces can also improve host tolerance of the implant, potentially reducing healing time and pain after surgery. Various patterns of blocks less than 10 nm across prevent communication between bacteria, for example, while allowing relatively large, more flexible mammalian cells (~10–120 μm) to adhere. Christopher Bettinger of Carnegie Mellon University and his colleagues showed that long troughs called nanogratings etched into a solid surface promote the elongation of mammalian endothelial cells and eventual blood vessel formation.[9] Other research has shown that rough, 50-nm nanotroughs can increase bone formation by osteoblasts while decreasing microbial adhesion and biofilm formation. "Small, long nanofeatures do not allow the somewhat stiff bacteria to attach, yet they allow mammalian cells to function," explains Webster. These nanopatterned surfaces would be safer in the long run, as mammalian cells take over and grow into vasculature, bone, cartilage, and other tissues.

Alternatively, researchers have used nanopillars like those designed by Yee's team at UC Irvine to kill any bacteria that land on an implant surface, while leaving mammalian cells unaffected. Bacteria lack cholesterol and other large chemical groups that provide the flexibility to mammalian cells, making bacteria 5 to 20 times stiffer. Thickness of the peptidoglycan cell wall surrounding bacteria can also limit fluidity. As a result, bacteria are punctured by the nanostructures, while mammalian cells are able to "melt" into spaces between nanoscale patterns and grow across the surface.

If successfully developed as safe design changes to implanted devices, such nanopatterns may also help prevent microbial spread via surgical instruments. Troublingly, there were 157,000 surgical site infections in the U.S. in 2011 (21.8 percent of all nosocomial infections), and some of them resulted from use of improperly sterilized surgical equipment. In the last year alone, the US Food and Drug Administration (FDA) handed down warning letters to three makers of duodenoscopes for improper sterilization procedures and lack of infection reporting: several people died in numerous hospitals after physicians reused improperly sterilized scopes that passed *Pseudomonas aeruginosa* and CRE to at-risk patients. In October 2015, the FDA required the companies (Olympus, Pentax, and Fujifilm) to submit new protocols for sterilization procedures.

NANOPATTERNS DESIGNED TO BE HARMLESS TO HOST CELLS COULD BE APPLIED TO SYNTHETIC IMPLANTS TO WARD OFF BACTERIAL GROWTH.

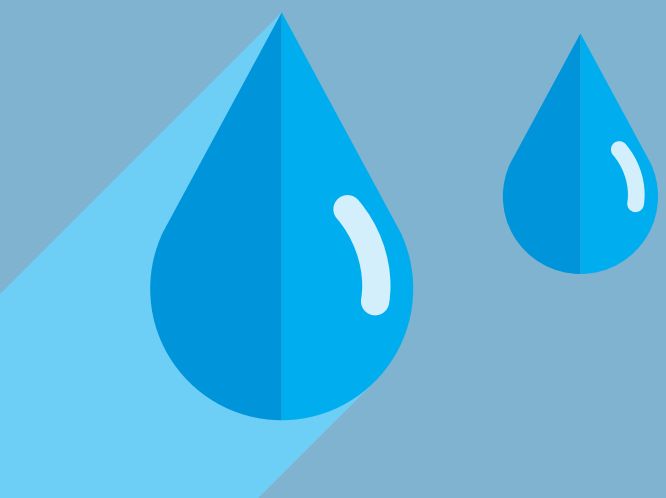
Within a couple of months, Fujifilm issued revised cleaning instructions for its duodenoscopes and received FDA approval to continue production. And in January 2016, the agency declared that Olympus had provided the necessary modifications to its duodenoscope to prevent leakage of patient fluids into a sealed area inside the device that was harboring hazardous bacteria. At time of writing, Pentax was still working with the FDA to mitigate the potential for cross-contamination due to its devices. None of these companies, however, suggested nanocoatings as a solution. If research continues to show the effectiveness of antimicrobial nanopatterns, it would behoove endoscope manufacturers to consider such technologies. As the steps required for health care personnel to thoroughly clean and sterilize endoscopes between patients are arduous and complicated, nanocoatings in

PROTECTING WATER WITH NANOTECHONOLOGY

Beyond preventing the spread of disease in health care settings, antimicrobial nanotechnologies could have much broader applications. Last summer, an outbreak of Legionnaire's disease in New York City resulted after the infectious agent *Legionella pneumophila* contaminating cooling towers left more than 100 people hospitalized and 12 dead. The outbreak brought to light how susceptible cooling towers are to biofilm formation and how the towers can broadcast the pathogen as water evaporates into a mist. Similar problems can exist within residential homes, where plumbing systems and hot water heaters can house *Legionella* colonies, as well as in hospitals, where *Legionella* colonization of water distribution systems can be aerosolized from whirlpools, nebulizers, oxygen humidifiers, spas, showerheads, and faucets. And unfortunately, once a system becomes contaminated with *Legionella*, it is virtually impossible to eliminate the pathogen.

Efforts to decrease *Legionella* contamination in both hospitals and cooling towers include thermal disinfection, ozonation, hyperchlorination, UV light sterilization, and the introduction of relatively large (colloidal) particles of copper and silver to the water. While these methods are effective in clearing *Legionella* organisms, their effects are temporary. Furthermore, the dissemination of colloidal silver particles into water delivery systems can cause birth defects, bluish discoloration of the skin, and diarrhea from gut microflora disruption.

Impregnation of fiberglass with a combination of nanometallic particles, such as silver and copper or iron, can be used as a lining within a water delivery infrastructure, especially at faucets, to achieve substantial bactericidal effects. And because the particles are not suspended within the water, they should not have any health effects. Moreover, such an approach would cost less than chemical release systems, as the particles would only need to be installed once, unlike chemicals that must be continuously added to the water. In areas of hospitals serving immunocompromised patients, where any exposure to *Legionella* could be fatal, such point-of-use filtration using nanosilver was found to eliminate *Legionella* (*BMC Inf Dis*, 14:394, 2014). Research on the use of nanosilver in filters continues to probe whether this approach will effectively prohibit the passage of virtually any *Legionella* organisms at the tap.



intricate interior chambers of these devices could ensure better microbial control.

Looking to the future

New approaches to discovering antibiotics have received much attention recently, and rightfully so. These techniques are crucial as the numbers of untreatable nosocomial infections continue to rise. But nanotechnological advances to stem such infections are fast becoming a viable strategy to supplement such drug-based approaches. Unfortunately, searches for "nano" and "surface" in clinicaltrials.gov turns up only seven ongoing trials, none of which are related to antimicrobial nanosurfaces. It's now critical to promote the advancement of nanotechnologies into the clinical setting. ■

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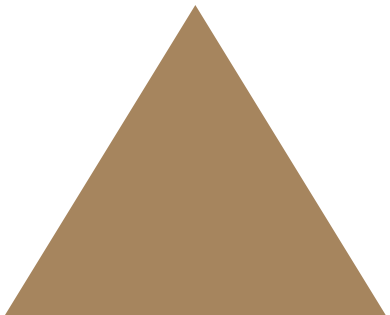

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The Zombie Literature

Retractions are on the rise. But reams of flawed research papers persist in the scientific literature. Is it time to change the way papers are published?

BY BOB GRANT

An unfortunate story has become all too common: a researcher is suspected of having manipulated data, an investigation is launched, the paper is retracted by a scientific journal, and the offending scientist is punished. But while cases of misconduct and subsequent retractions headline a growing reproducibility problem in the sciences, they actually represent a relatively small number of the flawed studies out there. The vast majority of publications that reported inaccurate results, used impure cell cultures, relied on faulty antibodies, or analyzed contaminated DNA are not the result of wrongdoing, but of honest mistakes, and many such papers persist in the scientific literature uncorrected.

"I think there is a continuum between fraud and errors, and I think people are all too willing to go easy on something if there is no fraud," says Columbia University statistician Andrew Gelman, who blogs about retractions and reproducibility problems in the scientific literature.

Are these "zombie papers" (to repurpose a term coined by academic publishing watchdog Leonid Schneider) benign—relics of antiquated methodologies or poor reagents that serve as a historical record for the field of inquiry? Or are they worrisome enough to be hunted down and excised from the body of the scientific literature altogether, in the same way that intentionally falsified reports are?

Many researchers argue for the latter. Flawed papers, especially those that become highly cited, run the danger of perpetuating faulty methods or conclusions, sending funding and effort in fruitless directions, and building layers of theory upon shaky conceptual foundations. In this way, zombie papers can spawn more zombie publications, and the damage can be amplified and spread in an infectious pattern.

"It is a big problem, and it is a pervasive problem," says Brian Nosek, a University of Virginia psychologist and cofounder/executive director of the Center for Open Science. Just how big remains unclear, but Gelman estimates that flawed publications may outnumber the good ones. "I think there are journals and years where I would guess more than half the papers have essentially fatal errors," he says.

And the zombie horde will only continue to grow as ever more journals churn out reams of scientific papers at an increasing rate. Nosek and Gelman are critical of traditional scientific publishing, which has remained essentially unchanged for centuries. They and others say it's time to modernize the process. Over the past couple of years, researchers have begun to implement new mechanisms and avenues to review, flag, correct, and annotate the scientific literature. In the future, some hope, the way that researchers and publishers interact with each other and the body of work they generate could be radically transformed.

"There is certainly evolution in how people are thinking about these issues," Nosek says, "and what role publishers then would play if there was more responsivity to evidence as it accumulates rather than just the static record of what was thought at that particular time."

A zombie mummy

In the early 1980s, Svante Pääbo was a PhD student at the University of Uppsala in Sweden studying how an adenovirus can block a human histocompatibility antigen and so conceal itself

from its host's immune system. But the young Pääbo, now director of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, had a surreptitious side project up his sleeve. "I had studied Egyptology before I went to medical school, so I knew there were all these hundreds and thousands of mummies in the museums," he says. "I thought I should try to see if DNA might be preserved in them." Pääbo obtained samples from 23 mummies and scoured them for traces of usable genetic material. And in a few of the samples he found some. He stained the mummy cells, located the nuclei, and cloned the DNA from one of the samples, taken from a child who died 2,400 years ago, using a plasmid vector, as was the era's go-to DNA sequencing protocol. In a 1985 letter to *Nature*, Pääbo reported that he had extracted and sequenced DNA from the millennia-old relic.

The publication helped launch the now red-hot field of ancient DNA research. Pääbo would become known as a pioneer of the discipline, and he would go on to extract ancient DNA from a variety of long-dead organisms, extinct mammoths and Neanderthals among them. There was just one problem. That mummy DNA Pääbo sequenced was not from the mummy at all. As Pääbo himself determined nearly a decade later, using the newer method of PCR amplification that became widely used around 1986, the genetic material he had isolated was actually from a modern-day human, likely from the antigen research that he was also conducting. "In hindsight, that clone that's presented there is surely a contaminant," Pääbo says.

In 1994, after Pääbo revisited his original mummy data and realized the error, he and colleagues briefly admitted to the mistake in a *Nature* paper describing their sequencing of ancient mammoth DNA (using methods to ensure contamination was avoided). We "believe that [contamination] represents a great danger to the field of molecular archaeology," Pääbo and his coauthors wrote, adding that sequences retrieved by molecular cloning are particularly susceptible and "are therefore of only limited scientific value." More than 20 years later, however, Pääbo's 1985 mummy DNA paper still stands without a correction or erratum.

While Pääbo is candid about the mistake he made as a PhD student, he contends that the paper doesn't need formal correction, much less retraction, for three reasons. First, the methodologies it showcased were so rapidly overtaken by advancing technology—PCR and, later, targeted sequencing library preparation and direct DNA capture—that there was no danger of anyone using plasmid cloning and obtaining similarly misleading results, he says. Second, the histological staining results he presented in that paper remain valid. "In general, I do not think I would call the 1985 paper a 'zombie paper' in the sense that if it is cited today it is to say that DNA from ancient tissues can survive and be studied," he wrote in an email to *The Scientist*. "That conclusion is right even if the actual DNA sequence shown is wrong." And third, the 1985 paper was more a proof of concept, and was not meant to form a foundation for future research, he says. "It's not that that sequence leads to any conclusions, any inference about Egyptian history or something."

Nature seems to agree that the paper, which has been cited more than 560 times since its publication, according to Google Scholar, should be viewed as more of a historical relic than a blemish in the literature. "As technology evolves, so too does science, and new technologies, techniques, and evidence may lead to the reinterpretation or refining of a finding," Sowmya Swaminathan, head of editorial policy at *Nature*, wrote in an email to *The Scientist*. "Researchers accept this as a part of science evolving."

Leonid Schneider, an erstwhile molecular biologist who now bills himself as an independent science journalist and frequently writes about science publishing and researcher misconduct, also concurs that the 1985 paper has value, but he suggests that action be taken, more on principle than because of any chance of extreme scientific damage. "I still recommend that [Pääbo] issue a statement to go with this article," he says, "so that whenever somebody clicks on this article from the original publisher, they should also see a statement explaining which part of it is not reliable anymore. So I think it is his duty, even if it's 30 years old."

> I think there is a continuum between fraud and errors, and I think people are all too willing to go easy on something if there is no fraud.
>
> —Andrew Gelman, Columbia University

The state of publishing

In today's era of digital publishing, flawed studies are much more likely to attract immediate criticism than did Pääbo's 1985 mummy DNA paper. In December 2010, for example, then NASA research fellow Felisa Wolfe-Simon and her colleagues published a paper showing that a gammaproteobacterium collected from Mono Lake in California was capable of replacing the essential element phosphorus with arsenic, so that it could grow in an arsenic-rich medium devoid of phosphorus. But after a NASA press conference about the findings and the online posting of the manuscript on *Science*'s website, critics descended on the paper.

Dozens of researchers wrote on blogs, in online forums, and directly to *Science* claiming that they spied problems with the study's experimental design and the authors' interpretations of the results. The journal published much of the debate, including the authors' responses and a news story detailing the controversy, and when users pull up the paper on the journal's website, they will find a list of links to these resources. "The scholarly record associated with this paper was significantly amended to reflect the seriousness and volume of questions raised by the scientific community," Marcia McNutt, editor-in-chief of *Science*, wrote in an email to *The Scientist*. "*Science* published an unprecedented number of technical responses and comments, as a package." That said, the paper remains, uncorrected and unretracted, largely because its authors maintain the voracity and robustness of its findings.

Just how many flawed papers like the arsenic-life study, as it has come to be known, continue to stand in the literature is

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anyone's guess. But it's likely a very large number, especially if one goes beyond just those papers with identifiable errors to include any study whose methodologies or conclusions have been replaced with new knowledge or understanding. "Any paper has errors. This is part of how science works, right?" says Nosek. "We don't understand the phenomena we're investigating, and so we do some research, we identify some things, we learn a little bit more, and we're a little bit less wrong in how we understand that phenomenon."

Of course, correcting or retracting the vast numbers of flawed papers isn't exactly practical. Obesity researcher David Allison of the University of Alabama at Birmingham recently got a taste of the challenges involved in taking on the zombie horde: last year, he and a few collaborators began searching for and trying to correct errors in published papers. For 18 months, the researchers pored over the literature in their fields of obesity, energetics, and nutrition, finding dozens of errors that warranted corrections. But they also found that trying to correct those errors or to retract the papers containing them was a difficult proposition. "After attempting to address more than 25 of these errors with letters to authors or journals, . . . we had to stop—the work took too much of our time," Allison and his coauthors wrote in a *Nature* comment published this February.

Too often, Allison says, the concerns he and his coauthors raised—which typically involved problems with the statistical analysis or design of experiments—were met with defensiveness from authors. "Nobody wants to have their errors pointed out publicly," Allison tells *The Scientist*. "We all realize they should be, but it's not fun. If it's a severe error, we really don't like it." And when Allison and his colleagues approached journal editors about the problems they had discovered, most were too consumed with the herculean task of staying on top of mountains of new manuscripts seeking publication to engage in retrospective reviews of already-printed papers. "For the editors, it's time-consuming for them to resolve this," Allison says. "So you've got all these disincentives up and down the line, and I think that's a big reason why these things aren't corrected."

A brave new world

One way to root out questionable papers is postpublication peer review and online commenting, which has become more pervasive in the form of sites such as Faculty of 1000 (F1000), PubMed Commons, PubPeer, and others, as well as commenting functions on the websites of some traditional publishers. This approach is fraught with challenges, however. "Several journals that have implemented online commenting have since discontinued it," *Science*'s McNutt wrote in an email. "For most journals, there may be a staff-power problem in terms of monitoring the commenting to keep it constructive and civil."

Another consideration is anonymity. Last year, PubPeer came under fire for allowing users to post anonymous comments. PubPeer's founders—who had retained their own anonymity but revealed themselves in response to the criticism—argued that anonymous comments on the site were not inferior to those posted by registered users, and said in an October blog post that allowing anonymous comments was "the only certain defense against legal attack or a breach of site security."

Some researchers argue that implementing new systems within the existing one will not be sufficient; policing the literature will require a new, broader approach to scientific publishing. "Our present system is an ad hoc invention that dominated science and [has] never been evaluated," says Nosek. He envisions a system that can help people assess a study's value based on all the available evidence. As highlighted by Pääbo's mistaken identification of mummy DNA in 1985 and his admission of error published in a separate paper nine years later, "there is very little direct connection between any [one] scientific contribution and any other scientific contribution," Nosek says. "The solution is to have better

curation of what is actually happening in science, which is [the] accumulation of knowledge."

Attempting to create that connection, Nosek has spent the last two years helping to launch the SHared Access Research Ecosystem (SHARE) notification service, a collaboration between his Center for Open Science, the Association of Research Libraries, the Association of American Universities, and the Association of Public and Land-grant Universities. "It's trying to create a single, open data set of all research events—so not just publications, but also grants and clinical trials and retractions and everything else that happens about research," Nosek says. Once the massive data set is compiled, he adds, "the second step is providing really good curation tools so that these different units of the research literature are linked together and [it is] much easier to search and discover these kinds of things."

In Nosek's vision, the scientific paper ceases to exist as a static snapshot of the current state of understanding. Instead, papers become dynamic entities that authors can continually update with new knowledge. "A paper is a paper, and it's a paper that way forever," Nosek says. "But really, as new research happens, we should be able to revise those papers, and then just say this is the new version. A paper is never done, because a phenomenon isn't understood at that point. So you could imagine careers built on the continuous editing of a single paper, which is what we know about a particular phenomenon."

Implementing the SHARE project—and its European correlate, the OpenAIRE project—is achievable, says Nosek. The key is to develop the technologies necessary to help researchers search, sort, and filter information about a particular paper, after gathering mountains of information about all papers into a single, searchable pipeline. Although the job of corralling not only the scientific literature but all the ancillary discussion that surrounds published papers under one roof would be a big one, Nosek concedes, there is a precedent that points to the viability of achieving the task. "This problem has already been addressed in very effective ways, and that is [by] news and media information via the Internet," he says. Search engines like Google allow users to digest a huge amount of information by providing tools that allow them to home in on and highlight specific needles among massive haystacks of information.

At least one title, open-access journal *F1000-Research*, does indeed allow authors of submitted papers to revise their original manuscripts based on comments from users made postpublication, and it posts revised versions alongside other versions, creating "living" versions of scientific studies. "I am delighted that F1000 and other groups are trying new models," says Nosek. But the real challenge lies in getting the scientific community to broadly agree to adopt such a new system. This might require both researchers and publishers to freely submit not only manuscripts, but also comments, data, and reviews of papers. "To the extent that we can move this infrastructure to be part of the publishing workflow, it's just a matter of changing our mind-set about what publishing means," Nosek says.

"If things are preprints, we know not to just believe them, right?" adds Columbia University's Gelman. "That's how published papers should be too, I think."

In addition, Allison says, the scientific community would need to overhaul its whole concept of who actually owns data and research findings. "You're in charge of it for a while, but it's really the public's data," he says. "And this [change] won't happen overnight."

So while zombie papers, such as Pääbo's mummy DNA study, the arsenic-life paper, and many others too numerous to mention here, will likely live on in the scientific literature, there is a glimmer of hope that, as science adopts a more modern model for publishing and revising results, making papers more dynamic and less static, we may see a downtick in recruitment to the zombie hordes. ■

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The Literature

CELL & MOLECULAR BIOLOGY

Kissing Cousins

THE PAPER

K. Hattermann et al., "Transmembrane chemokines act as receptors in a novel mechanism termed inverse signaling," *eLife*, 5:e10820, 2016.

Kirsten Hattermann knows a thing or two about chemokines. A researcher working with Janka Held-Feindt's lab at the University of Kiel in Germany, Hattermann has spent the last decade studying these little proteins, which bind—either as transmembrane (*tm*) proteins or as soluble (*s*) equivalents that are shed from the membrane or secreted by the cell—to complementary receptors on target cells. Binding of the *s*-chemokines can elicit several responses in target cells, including cell migration and proliferation, but scientists are still working out the consequences of *tm*-chemokine binding.

Recently, while investigating chemokine signaling in tumor cells from a variety of human cancers, Hattermann and her colleagues found something they couldn't explain. When they exposed glioma and carcinoma cells lacking known chemokine receptors to the soluble form of the chemokines CXCL16 and fractalkine, the researchers assumed there would be no binding and, hence, no signal transduction. But to their surprise, Hattermann says, "we observed intracellular signaling."

Because "it is known that chemokines are receptor-promiscuous," explains Hattermann, "at first we were searching for another receptor."

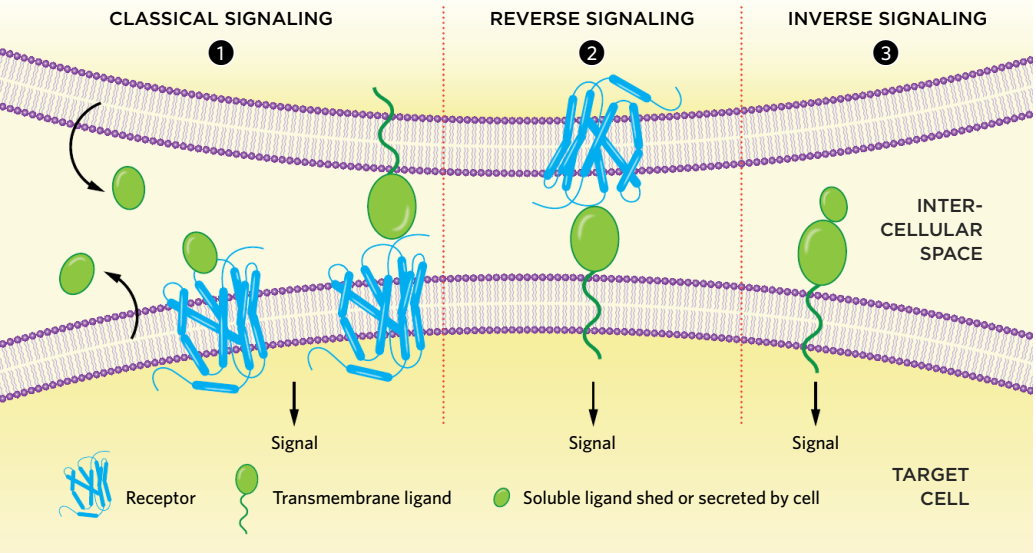
But after noticing that a line of receptor-negative melanoma cells didn't respond to the *s*-chemokines, the team began looking for differences in membrane protein composition between these cells and the responsive ones. "These [melanoma] cells lacked transmembrane chemokines," Hattermann says. "That was the first hint that the transmembrane chemokines might be critical."

Using immuno-electron microscopy, the researchers showed that *s*-CXCL16 and *s*-fractalkine directly bind to their transmembrane equivalents, implicating *tm*-chemokines as the elusive signal transducers. "If it's correct, it's paradigm-shifting in terms of the way we understand how some of these molecules work," says Gerry Graham, a professor of molecular and structural immunology at the University of Glasgow. "Binding of a soluble [chemokine] to a membrane-anchored one to transduce a signal is completely new."

Transfecting the melanoma cells with *tm*-CXCL16 and *tm*-fractalkine partly activated *s*-chemokine signal transduction, the researchers found, while silencing the *tm*-chemokines in otherwise responsive, receptor-negative tumor cells abolished the effect. This novel mode of communication, which the team has termed "inverse signaling," may fine-tune classical signaling mechanisms, Hattermann suggests.

Graham says more experiments, both in vitro and in vivo, will be essential. "I think there's a lot to be done in terms of defining [the mechanism's] breadth of applicability," he says. "Chemokines will dimerize with themselves, but also [with] other chemokines. Do you get similar signaling if you take another chemokine and attach it to these transmembrane chemokines?"

The team aims to explore this and related questions, Hattermann says, including whether other transmembrane ligands, such as tumor necrosis factors and ephrins, use similar mechanisms. The researchers also plan to investigate the prevalence of inverse signaling outside cancer, for example, during development. "We have some hints that it's not restricted to malignant tumor cells," Hattermann notes. —Catherine Offord



A NEW WAY TO TALK: In classical signaling, receptors (blue) on a target cell transduce an intracellular signal upon binding with transmembrane or soluble ligands, such as chemokines (green) 1, which can originate in another cell or the target cell itself. Signaling triggered by a transmembrane ligand binding to a receptor on another cell is known as reverse signaling 2. In a novel mechanism dubbed inverse signaling, a transmembrane chemokine transduces a signal upon binding with its soluble equivalent 3.

THE SCIENTIST STAFF

CRY IN THE EYE: The cryptochrome Cry2, involved in magnetosensing, is present in the eyes of two cockroach species, including *Blattella germanica* (above).

DOSAGE DEBATE: Analyses of gene copy number in wild strains of aneuploid yeast (*Saccharomyces cerevisiae*) have come to different conclusions.

CELL & MOLECULAR BIOLOGY

Animal Magnetism

THE PAPER

O. Bazalova et al., "Cryptochrome 2 mediates directional magnetoreception in cockroaches," *PNAS*, doi:10.1073/pnas.1518622113, 2016.

PROTEIN WITH A PURPOSE

Many animals make use of light-dependent sensitivity to magnetic fields (MFs) to navigate their environment. Researchers recently implicated cryptochrome 1 (Cry1)—a photosensitive protein involved in circadian clock function in *Drosophila*—in fruit fly magnetoreception. This led David Dolezel of the Institute of Entomology at the Czech Academy of Sciences and colleagues to ask whether Cry2, a vertebrate-type cryptochrome also present in many insects, mediates sensitivity to the presence and directionality of MFs in other animals.

RESTLESS ROACHES

Previously, the investigators found that two cockroach species with Cry2 become more restless when subjected to rotating (rather than steady) MFs. Using magnetically induced restlessness (MIR) as a measure of sensitivity to MF directionality, the team set out to test the importance of Cry2 in detecting rotation.

NO CRY

Dolezel's group found that either silencing *Cry2* or covering cockroaches' eyes with opaque paint abolished MIR when the insects were presented with rotating MFs, establishing both the protein and the eye as necessary for directional magnetoreception. "Vertebrate-type cryptochrome, which is not thought by most to have a lot of light sensitivity, actually may," says Steven Reppert of the University of Massachusetts Medical School, who was not involved in the work. "This study in the cockroach adds a lot of credibility to that prospect."

CHALLENGES AHEAD

Although the researchers located Cry2 behind the retina, "we don't know if we're hitting the magnetosensor or something downstream," says Dolezel, adding that "cryptochrome is like chewing gum—it interacts with everything." —Catherine Offord

GENETICS & GENOMICS

Aneuploid Responses

THE PAPER

A.P. Gasch et al., "Further support for aneuploidy tolerance in wild yeast and effects of dosage compensation on gene copy-number evolution," *eLife*, 5:e14409, 2016.

CHROMOSOMAL COMMOTION

Like many organisms, brewer's yeast (*Saccharomyces cerevisiae*) is intolerant of aneuploidy. "In the lab strain that's been most studied, cells with an extra copy of a chromosome have just crazy different expression across the transcriptome," says Audrey Gasch of the University of Wisconsin–Madison. But wild yeast, her team recently found, may not be so sensitive.

EXPRESSING DIFFERENCES

In 2015, Gasch and colleagues published an analysis comparing RNA levels and DNA content in aneuploid strains of wild yeast (*eLife*, 4:e05462). Doubling gene copy number ought to double RNA abundance, the team reasoned, but some genes in these strains showed lower-than-expected gene expression, or "dosage compensation."

DISTRIBUTION DILEMMA

A reanalysis of Gasch's data, published by Angelika Amon of MIT and colleagues, revealed equal proportions of genes with higher- and lower-than-expected expression (*eLife*, 5:e10996, 2016). To conclude that dosage compensation is occurring, "there should be a departure from the one-to-one correlation between gene copy number and expression levels" beyond the variation expected from statistical noise, says Amon. "When we looked at their data, there was no skewing. It was just a normal distribution, which is what you'd expect when gene expression doesn't change."

OPPOSING OPINIONS

In its latest article, Gasch's group argues that a distribution approach to dosage compensation may miss subtle differences in expression. "We're thinking about it in different ways," Gasch says. "I think this is of evolutionary importance, regardless of how many genes are subject to this effect." She adds that the team will now investigate the mechanisms driving gene expression differences between aneuploid strains of wild and lab yeast. —Catherine Offord

More Than Skin Deep

Elaine Fuchs has worked on adult stem cells since before they were so named, figuring out how multipotent epidermal cells renew or turn into skin or hair follicles.

BY ANNA AZVOLINSKY

In 1978, Elaine Fuchs was just one year into a postdoctoral fellowship at MIT when her PhD advisor, Charles Gilvarg of Princeton University, called to tell her about an available academic position at the University of Chicago. "He remembered that my family was from Chicago and that I might want to go back," says Fuchs, now a professor of molecular genetics and cell biology at Rockefeller University in New York City. "I told him that was fine but that I was still doing my postdoc, and he said that he would recommend me anyway. I could treat the interview as practice, he explained, to get a sense of what it was like, for when I was ready to get a job." Fuchs was invited for the interview and the university's biochemistry department took its time deciding, finally offering her an assistant professorship in the fall of 1979. "I was relaxed, as it never occurred to me that I would get a job offer," she says. "Possibly, the department took their time because I had told them I hadn't applied anywhere else." Fuchs requested another year to finish her postdoc in Howard Green's laboratory, where she was studying the biology of cultured human keratinocytes, the most abundant cell type found in the epidermis, the skin's protective barrier at our body's surface.

"We're learning that it is the basic mechanisms that stem cells use to make and repair tissue that become hijacked in cancer."

"I finished a full three years at MIT. What was nice in that last year was that I could plan out exactly what I wanted to do in my own lab. I wrote for and had my NIH grant before I arrived in Chicago. It was a really nice recipe to hit the ground running. Now, looking back, it was kind of a poised-to-succeed situation," says Fuchs.

Since her time in the Green lab almost four decades ago, Fuchs has been hooked on decoding and unraveling the complicated biology of epidermal cells. In her own labs at the University of Chicago and now at Rockefeller University, Fuchs has used the epidermal-cell culture system to define epithelial stem cells, extending her findings to understand basic principles of multipotent cells in general. Her research has also tackled the biology of other cell types within the epidermis, identifying the progenitor cells that give rise to sweat glands and ducts and isolating hair-follicle stem cells. Fuchs's lab was also among the first to characterize a cancer stem cell.

Here, Fuchs traces her research path from keratins to stem cells, and discusses her work ethic and her love of world travel.

FUCHS FASCINATED

Bucolic Chicago. Fuchs grew up in a suburb of Chicago that at the time, in the late 1950s and 1960s, was "less suburb and more cornfields," she says. At home, her father made furniture for the house, and her mother sewed clothing for Fuchs and her sister and also did oil painting. Her parents kept a large flower and vegetable garden during the spring and summer months. "I grew up in a very active, self-sustaining environment back in the days when we were allowed to stay out from after breakfast until it became dark outside," says Fuchs. "My mom made us butterfly nets and sent us out to the swamps and fields."

One-man show. Fuchs's family lived near Argonne National Laboratory, which is funded by the US Department of Energy. Fuchs learned about how research is conducted from her father, Louis Fuchs, who was a geochemist there, working on identifying novel minerals in meteorite samples. The only mineralogist employed at Argonne, he had discovered 8 of the 13 known extraterrestrial minerals by the time he retired. "My father was well-known in the field, but was really a one-man show. He had an electron microscope and worked largely on his own," says Fuchs.

In pursuit of science. "The progression into science in college was natural," says Fuchs, whose older sister, Jannon Fuchs, is now a neuroscientist at the University of North Texas. Her aunt, a University of Chicago alum, couldn't get into medical school because she was female. "She was a feminist and encouraged my sister and me to do something meaningful with our lives." Fuchs entered the University of Illinois in 1968 and majored in chemistry because, according to her, the university's biology program at the time was not as strong as those in chemistry and physics. She did research while in college—including at Argonne for a summer—and enjoyed performing the experiments, but didn't feel particularly adept at doing science.

Scientific control. After graduating in 1972, Fuchs began graduate work at Princeton in the biochemistry department. She gravitated towards the metabolic pathways she was learning about in Gilvarg's class and joined his lab. Fuchs worked on bacterial cell wall biosynthesis, exploring how dormant spores from *Bacillus megaterium* become activated and remodel their cell walls to accommodate a

MATTHEW SEPTIMUS

ELAINE FUCHS

Professor, Molecular Genetics and Cell Biology
Rockefeller University, New York City
Howard Hughes Medical Institute Investigator

Greatest Hits

- Showed that keratins found in the epidermis come from distinct genes and are differentially expressed in different parts of the epidermis.
- Identified mutations in several keratin genes responsible for five human skin diseases, including epidermolysis bullosa simplex.
- Developed a technique to label, track, and purify quiescent, slow-proliferating stem cells.
- Uncovered the pathways necessary for epithelial stem cells to differentiate into the epidermis, hair follicles, and sweat glands.
- Among the first to describe a cancer stem cell, characterizing how squamous cell carcinoma is initiated.

rapidly dividing state. "It took my entire graduate career to become comfortable with molecular biology and biochemistry," she says. "What I gained from my advisor was the ability to carefully design a properly controlled experiment. I realized later that that this is more critical to becoming a good scientist than anything else."

FUCHS FOCUSES

Strong cell biology footing. Next, Fuchs decided to study how human cells make tissues, joining Green's laboratory at MIT in 1977. "I wanted to pick apart the cell's biology and biochemistry and liked the idea of working with a cell-culture system," she says. Fuchs had heard a seminar by Green, who had developed the 3T3 fibroblast cell line and was also the first to culture epithelial cells, which required a layer of irradiated "feeder" fibroblast cells in order to grow in the lab. The epithelial cells Green was studying were human keratinocytes, skin cells that make up about 90 percent of the cells of the epidermis, where they occupy the basal layer of the stratified epithelium. "He didn't call them stem cells, but essentially that is what they were. These were cells that you could take from human skin, passage long term in culture, and induce them to make differentiated tissue," Fuchs says. "Green basically opened up the door to the stem cell field as we know it."

Fuchs published three *Cell* papers, one for each year spent at MIT. First, using an enzymatic protein cleavage reaction, she demonstrated that keratins—the abundant fibrous, structural proteins that protect epithelial cells from mechanical stresses—were likely distinct proteins coming from distinct genes rather than originating from one single protein that is cleaved posttranslationally. For the second paper, Fuchs fractionated RNA species, separated them on methylmercury gels, and showed that human keratins are indeed coded by distinct messenger RNAs. The third paper showed, for the first time, that keratins are differentially expressed not only during terminal differentiation within the epidermis but also in different epithelial tissues. "This is a concept we now take for granted, but at the time, it was a very important finding. The use of specific intermediate filament proteins like keratins to identify a particular cell type and stage of differentiation has been enormously useful to pathologists in the diagnosis of cancers and other human disease states," explains Fuchs. "The finding also formed the foundation of our understanding of what are now more than 20 human disorders of intermediate filament genes."

Towards independence. "It was jolting to go from physical chemistry to biochemistry and then cell biology. It took me

forever to get it. There were always far too many variables in biology. In chemistry you could always solve equations but you can't solve equations in biology. It took me my whole graduate career to feel comfortable with that notion," Fuchs says. "And then, during my postdoc, that is when I started to realize that I didn't have to rely upon my training or my lab to guide my research. When I needed to learn something, I could find another lab to learn it. Someone at MIT almost always had the expertise I needed to learn to move my research forward. This helped me develop skills to become interactive and to really run a project myself. So I was resourceful and productive, but I still didn't think I was doing exceptionally well. A *Cell* paper didn't really mean much to me at the time. I just thought this was a publication like any other. I was just pleased with what I was doing and what I was finding."

Work ethics. Fuchs started her own lab at the University of Chicago in 1980. "I pretty much knew exactly what I wanted to do when I started my lab. I didn't have a technician or graduate student. I just started doing experiments on my own after I had cleaned up the lab and office I inherited. Two months later, the department chair came down and asked if I was ever going to hire a technician. I was so naive. I knew what I wanted to do and how to do it, and I didn't want to take out time to interview or train anyone," says Fuchs. "I hired the first person I interviewed and she was good, and I realized that she was really helpful. The two of us did all the work for the first year. I was very cautious about taking people on and only taking good people, and I highly recommend that route."

Hitting the ground running. Fuchs's lab immediately began to clone and characterize the various keratins and their genes. As her lab grew, they began doing in vitro filament assembly studies with recombinant proteins, and they engineered mutations that perturbed keratin filament assembly in a test tube and in cultured keratinocytes. Protein chemists had tried unsuccessfully for years to crystallize keratins, but remained stymied by the proteins' propensity to self-aggregate. By obtaining the protein sequences through cloning and DNA sequencing, Fuchs overcame these hurdles. Using transgenic techniques, the lab made mice that expressed various keratin mutants to decipher their functions. Point mutations in one of the keratin genes resulted in mice with a disease akin to epidermolysis bullosa simplex (EBS), a human skin disease characterized by severe blistering. From skin biopsies obtained from such patients, her team verified that EBS, and other related skin disorders, stemmed from keratin mutations.

FUCHS FLOURISHES

A big move. While still at the University of Chicago, Fuchs began to isolate and characterize the cells from skin that could make new tissue or repair wounded tissue. This included identifying the signaling pathways involved and the cellular context necessary for self-renewal. Fuchs's team showed that Wnt is a critical signal for activating stem cells to make follicles. After packing up the lab—including three trucks filled with laboratory mice—and moving to Rockefeller University in New York in 2002, the team developed a way to fluorescently tag slow-proliferating cells by labeling a histone, marking stem cells by their unique quiescent property. "It was a clever technique, but also let us demonstrate, in transplantation assays, that these cells were behaving like stem cells," says Fuchs. "After that, we could monitor their behavior in normal tissue formation, wound repair, and then malignant transformation." That same year, the lab showed that these stem cells could make epidermis and hair when grafted onto the backs of nude (hairless) mice.

A delicate balance. In 2011, Fuchs's lab defined the stem cells that can initiate squamous cell carcinoma, a type of skin cancer, and characterized the signaling pathways that drive malignancy. "Stem cells in their niche are quiescent most of the time. What we've learned is that their neighboring cells dictate their behavior. So when you take stem cells out of their niche, they are faced with a new environment and they dramatically change their behavior. That notion has been really instructive in our tackling how stem cells acquire mutations that make them malignant. Malignancy involves intrinsic changes and altered signals from their new neighbors," say Fuchs.

"Our recent papers point to a better understanding of how stem cells become malignant. What fascinates me most is the parallel of cancer stem cells—the cells that make cancer—with normal stem cells—the cells that make tissue. We're learning that it is the basic mechanisms that stem cells use to make and repair tissue that become hijacked in cancer," she says.

Sweating it. "What has been a difficult nut to crack has been the sweat gland stem cell. I am very curious as to why it's only the higher primates and humans that have eccrine sweat glands that allow us to run marathons and live in extreme climates," says Fuchs. "We would like to learn how to grow sweat glands because burn patients can be treated with epidermal cell cultures to repair their skin, but the engrafted skin never makes sweat glands, so the patients can't properly regulate their body temperature. If we can grow sweat stem cells in culture, we might be able to help these patients."

Travel bug. "Ever since graduate school, I've always worked like crazy and then taken a month off to travel. I think you have to have a well-balanced life but everyone balances their life differently. I enjoy working hard and I can't sit still, so for me, if I am working, I can go 24–7 for months on end, but then I need a month to do something dramatically different. In graduate school I went to Mexico and Guatemala, India, Nepal, Turkey, Egypt, Greece, Panama, Bolivia, Peru, and Ecuador. I think the travel experiences I've had for three decades have helped me enormously in running my lab. I have an international lab and I have a real appreciation for different cultures and I think this is very helpful." ■

Timothy Lu: Niche Perfect

Associate Professor, Departments of Electrical Engineering & Computer Science and Biological Engineering, MIT. Age: 35

BY KERRY GRENS

Timothy Lu has straddled different worlds nearly his entire life, having spent the first half of his childhood in the U.S. and the second half in Taiwan. Academically, his experiences have been just as varied. Lu has studied electrical engineering, computer science, synthetic biology, and medicine. This stew of experience has made him into something of a Renaissance bioengineer—a researcher with computational skills, genetics know-how, and clinical knowledge to guide his pursuits. "He has a very unusual background," says Yoel Fink, director of MIT's Research Laboratory of Electronics. "There are not many people who have done what he's done."

As an undergraduate and master's student, Lu studied electrical engineering and computer science at MIT, but he got the sense that the big problems in those fields had already been solved. At the same time, around 2003, synthetic biology was blossoming, and Lu wanted in.

For his PhD, Lu joined the lab of synthetic biologist Jim Collins, then at Boston University. Again, Lu was bridging different worlds: his advisor's lab was at BU, but his degree-granting institutions were across town at Harvard and MIT. Among his accomplishments at the bench, Lu engineered a bacteriophage that could break up biofilms.[1] In 2009, collaborating with two fellow graduate students, he used the work to launch a biotech firm, now called Sample6, that uses customized phages to detect food contamination.

On top of that, Lu was also working toward his medical degree at Harvard. His motivation to study medicine stemmed from wanting to find applications for his research. "I was intrigued with the ability to modify cells to treat disease," Lu says. "But I didn't have insights into real problems facing people in clinical settings."

In 2010, MIT hired Lu as a faculty member straight out of medical school. Since then, his lab has swelled to about 26 members working on a variety of synthetic biology problems—from basic unknowns, such as how genetic networks control cell functions, to applied concerns, such as bacterial infections. One of the big interests Lu gained from medical school was the problem of antibiotic resistance, and among his current projects is one to develop rapid diagnostics to aid in identifying appropriate narrow-spectrum treatments.

In 2013, Lu founded another startup firm, called Synlogic, to test the ability of engineered microbes to treat infections. That same year he published work describing living cells' utility as mini tape recorders.[2] And last year, Lu's team built a high-throughput method to assess the effects of different combinations of microRNAs.[3] "I think he's got one of the most remarkable—if not *the* most remarkable—programs in synthetic biology," says Collins, now based at MIT.

Fink says Lu is a thought leader in synthetic biology, and his scientific creativity, pursuit of interesting problems, and ability to motivate and collaborate with others has helped him succeed in academia and business. "All this you put together and you find one of these amazing people that just are nearly perfect in everything they're doing." ■

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Becoming Acculturated

Techniques for deep dives into the microbial dark matter

BY JEFFREY M. PERKEL

If you take a sample of seawater and plate it on a typical petri dish, colonies of bacteria will flourish. Each of those colonies springs from a single cell; counting those colonies provides an estimate of the number and variety of organisms in the water sample. But count the cells in that same sample directly, and you'll find you've only scratched the surface.

That difference is called "The Great Plate Count Anomaly," and it is vast. By some estimates, direct cultivation captures just 0.01 percent to 1 percent of the bacterial diversity in biological samples. The rest represents a missed opportunity of sorts—organisms whose ecologic functions and metabolic potentials researchers could glimpse, perhaps by sequencing their DNA, but never directly study. This dark matter of the microbial world could be an untapped gold mine of antibiotics, biofuels, bioremediators, and more. (See "Lost Colonies," *The Scientist*, October 2015.)

In the world of microbiology, such organisms are designated uncultivable. But that label isn't quite right, says J. Cameron Thrash, a microbiologist at the Louisiana State University in Baton Rouge; after all, these organisms grow in nature, some exceptionally successfully. "I think [the term] is a failure of imagination and maybe a failure of understanding the organisms well enough," Thrash says. "I don't subscribe to the idea that anything is unculturable, though certainly there are things that are uncultivated."

Increasingly, researchers are devising methods to cope with the problem. *The Scientist* spoke with four of them about the strategies they use to cultivate the uncultivable. This is what they said.

IN SEARCH OF GROWTH FACTORS

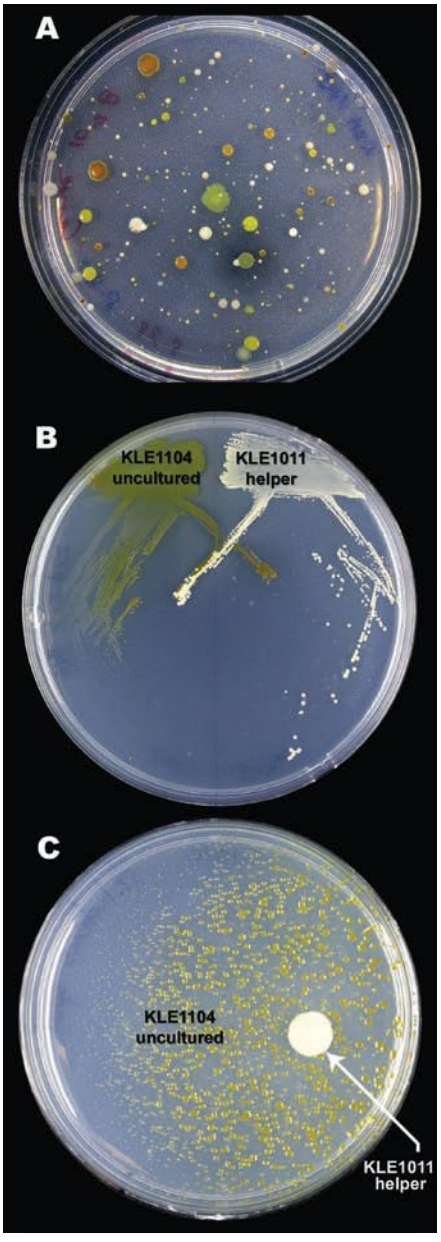
RESEARCHER: Kim Lewis, University Distinguished Professor and director of the Antimicrobial Discovery Center, Northeastern University

MICROBE: *Verrucomicrobia* sp. KLE1210

SOURCE: Intertidal coarse-sand sediment from Canoe Beach on Massachusetts Bay

SOLUTION: In attempting to culture the microbes that live on sand, Lewis and his team noticed something peculiar: when they performed serial dilutions and plated the samples onto petri dishes, they got proportionately larger counts in relatively undiluted samples than they saw at higher dilutions. In other words, organisms were growing in more-concentrated samples that would not grow when the cells were more spread out.

The team reasoned that some cells were secreting growth factors into the medium that other cells then scavenged. To find those factors, the group plated concentrated cell mixtures and selected pairs of cell types in which a small colony was found in close proximity to a large one, like a moon orbiting a planet. "The bigger [colony] is probably cultivable, so it started growing earlier," Lewis explains. "The unculturable organism is not going to make a bigger colony than the one that helps it."



A. D'ONOFRIO ET AL., *CHEMISTRY & BIOLOGY*, 17:254-64, 2010

A HELPING HAND: To identify and cultivate bacteria whose growth depends on a soluble growth factor, Lewis plated bacteria from sand grains on agar (A) and isolated and streaked candidate pairs (B). The growth of KLE1104 (green) depends on KLE1011 (white), as its colonies get smaller the farther away they are from the helper cells (C). Used media from a helper strain culture is sufficient to support KLE1104 growth, indicating a growth factor is all that's missing.

The researchers then cross-streaked both organisms on a fresh plate in an X pattern, looking for evidence that growth of the smaller colony depended upon proximity to the larger. Frequently, it did. According to Lewis, of 100 tested pairs,

about 10 percent met this criterion. "It's not a very rare event."

That approach is sufficient to grow a supposedly uncultivable organism, albeit not in isolation. So the researchers set out to determine the needed growth factor. Using *E. coli* as a substitute "helper" strain, they systematically tested mutations in genes involved in the biosynthesis of secreted molecules, zeroing in on enterobactin as the missing ingredient (*Chem Biol*, 17:254-64, 2010).

Enterobactin is a siderophore, a chelator molecule that scavenges iron from the environment. As the team discovered, many uncultivable bacteria will grow if siderophores (or soluble iron) are included in the growth media. Among those is *Verrucomicrobia*, a group of bacteria that live in the ocean and also in the human gut, and that had been known largely from their 16S rRNA gene signatures. "We would not have found [this organism] if not for this approach," Lewis says.

SIDEROPHORES "R" US: According to Lewis, researchers can easily emulate his approach to discover novel growth factors and cultivate uncultivable microbes. "That's a fairly rapid screen that one can do; you can process hundreds of pairs that way." In the meantime, researchers can purchase a variety of siderophores from Germany-based EMC Microcollections (microcollections.de).

HIGH-THROUGHPUT CULTIVATION

RESEARCHER: J. Cameron Thrash, Assistant Professor, Department of Biological Sciences, Louisiana State University (LSU)

MICROBE: SAR11

SOURCE: Coastal waters of Louisiana

SOLUTION: Before starting at LSU, Thrash was a postdoctoral researcher with microbiologist Stephen Giovannoni at Oregon State University. In 2002, Giovannoni and his team described a strategy called high-throughput cultivation and used it to grow one of the most abundant, yet persistently uncultivable, organisms in the oceans: bacteria of the SAR11 clade (*Nature*, 418:630-33, 2002).

According to Thrash, the technique involves "dilution to extinction"—diluting a microbial sample into sterilized seawater such that each well of a 96-well plate contains, on average, a single cell. Then, after a few weeks of culture in nutrient-poor media, the team measures growth by looking for a boost in cell numbers.

The point, Thrash explains, is twofold. First, seawater is nutrient-poor, and the microbes that live there seem to be adapted to that diet. Give them too much food, and they either won't grow, or they'll die. Plus, these cells are very slow-growing, so they must be isolated from other organisms that might outcompete them. The goal is to give the organisms as many opportunities as possible to grow, and then watch to see what happens. "We're essentially doing a technique where you try to isolate the organism in advance," he explains. "Then we just have to wait and see if that one cell in that well grows up."

The technique's reliance on sterilized seawater has been problematic, however, because precise nutrient conditions can vary widely, Thrash says. Cells may grow on water collected at one place and time but not another, and keeping large volumes of seawater on hand for large-scale experiments is not practical. So, more recently, his team developed a suite of "artificial seawater" formulations with precisely defined but adjustable salinity, pH, and organic carbon to better approximate collection sites. Diluting his samples in these solutions, he can capture between 2 percent and 7 percent of the organisms they contain, including members of SAR11.

GROW WITH THE FLOW: According to Thrash, seawater microbes don't grow like *E. coli*—a dense culture of *E. coli* might reach 10^9–10^{12} cells/mL; seawater bacteria average 10^5–10^7/mL—and the cells are also smaller, as tiny as 0.2 μm across. Long story short, a relatively populous culture of SAR11 looks, he says, "like clear water." So how to measure growth? Flow cytometry. The team mixes cells with a nonspecific DNA dye, then counts the cells as they flow past a laser. "We're capable of measuring as low as 10^4 cells/mL," says Thrash.

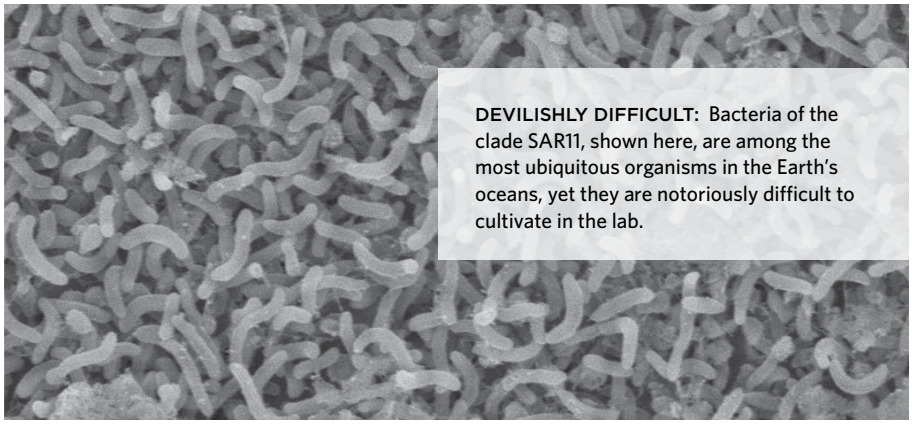
GOING INTRACELLULAR

RESEARCHER: Anders Omsland, Assistant Professor, Paul G. Allen School for Global Animal Health, Washington State University

MICROBE: *Coxiella burnetii*

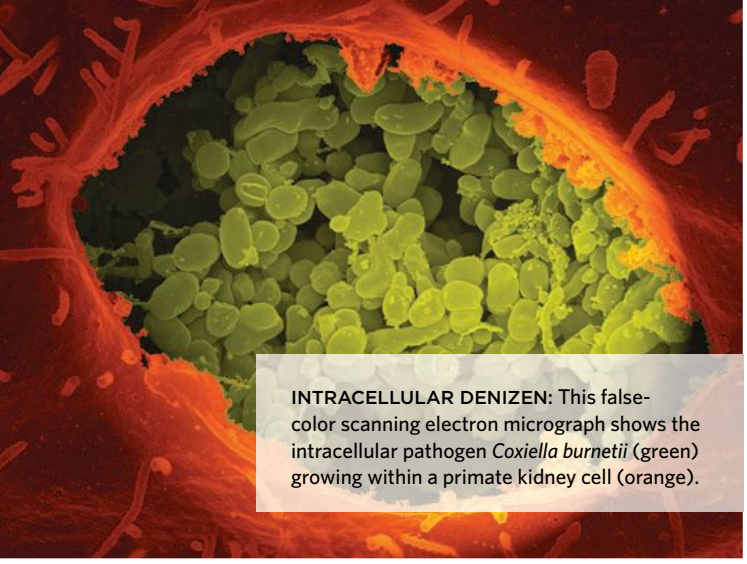
SOURCE: Human cells

SOLUTION: *C. burnetii* is the microbe responsible for the zoonotic disease Q fever. It grows inside the cells of its host, scavenging nutrients from the intracellular environment. The challenge, then, is to create a so-called "axenic" or host-cell-free culture that nonetheless replicates the conditions inside a cell. But there are an awful lot of chemicals inside the typical



DEVILISHLY DIFFICULT: Bacteria of the clade SAR11, shown here, are among the most ubiquitous organisms in the Earth's oceans, yet they are notoriously difficult to cultivate in the lab.

MICHAEL RAPPE, SOEST/UHM



INTRACELLULAR DENIZEN: This false-color scanning electron micrograph shows the intracellular pathogen *Coxiella burnetii* (green) growing within a primate kidney cell (orange).

cell, and not all of them support growth, Omsland says. "You have to go after the problem in a very systematic manner."

The first step, he says, was to identify a buffer that would support *Coxiella* metabolism for extended periods. Previous work had shown that *Coxiella* is a moderate acidophile, metabolizing optimally at about pH 4.5. By supplementing a moderately acidic citrate buffer with critical ions and nutrients, the team achieved robust protein synthesis, but not division. The question was: Why?

Using gene-expression microarrays, the team determined that ribosomal genes were underexpressed, suggesting the cells simply weren't producing sufficient protein to divide. According to Omsland, then a postdoctoral fellow with Robert Heinzen at NIAID's Rocky Mountain Laboratories in Hamilton, Montana, that's where knowledge of the *Coxiella* genome proved invaluable. Intracellular pathogens tend to evolve to fill their needs efficiently by coopting resources from the host environment. In this case, because the organism could swipe amino acids from its surroundings, *C. burnetii* dropped its amino acid biosynthetic genes in favor of amino acid transporters. Addition of exogenous amino acids and peptides to the axenic media increased protein production some 13-fold (*PNAS*, 106:4430-34, 2009).

But that still wasn't sufficient to achieve cell division. So the team once again turned to the genome, which also encodes certain oxygen-responsive cytochromes. The presence of a terminal oxidase with a particularly high affinity for oxygen suggested the cells might thrive under reduced-oxygen conditions. This makes sense, Omsland says: although breathable air contains 20 percent oxygen, the oxygen availability in deep tissue, where *Coxiella* grows, is only about 2 percent to 4 percent. "So, we tested the ability of *Coxiella* to respire under low oxygen."

That, Omsland says, did the trick. Although the cells would not grow in standard 20 percent oxygen, they would divide at 5 percent oxygen or lower, expanding about three orders of magnitude over six days. "If you can simulate the natural conditions closely enough, these organisms will eventually replicate," he concludes.

STEP BY STEP: Cultivating unculturable microbes is all about replicating growth conditions. But in this case, there was no one "silver bullet," Omsland says. "You have to consider a number of different variables to optimize the system." His advice for others looking to replicate his strategy: plan carefully and systematically, and start slow. "These projects are one step forward and two steps back."

MAKING PREDICTIONS

RESEARCHER: Matthew Oberhardt, Fellow, Insight Data Science, New York

MICROBE: 18,049 microbial strains

SOURCE: Leibniz Institute DSMZ—German Collection of Microorganisms and Cell Cultures

SOLUTION: Although the vast majority of microbes cannot be directly cultivated, there exists an enormous wealth of knowledge about strains that can be. Oberhardt, working with Uri Gophna, Raphy Zarecki, and Eytan Ruppin at Tel Aviv University in Israel, figured those data might allow them to predict promising media formulations for uncultivable varieties.

The Leibniz Institute DSMZ houses one of the largest bacterial culture collections in the world, as well as detailed records of the media required to grow them. When Oberhardt and his team realized what a data trove that represented, they got excited about the possibilities, he says. "People have been relying on intuition and trial and error to develop culture media for the last 100 years. By using this resource, we saw an opportunity to do better."

They combed through the DSMZ database, logging some 3,335 media variants, 18,049 strains, and 20,824 media-strain pairings to create the known-media database, KOMODO. Then, using the same basic algorithms Amazon uses to recommend books and Facebook uses to suggest friends, the team developed a web-based tool (GROWREC) in which users enter either a ribosomal DNA sequence or a taxonomic identifier to predict media on which those organisms might grow (*Nat Commun*, 6:8493, 2015). The tool relies on phylogenetic distances and an algorithm called collaborative filtering, which predicts media for one organism based on the media on which similar organisms are known to thrive.

According to Oberhardt, some 90 percent of predictions turned out to be accurate in their tests. But, he cautions, those tests used only organisms already in the DSMZ collection; it may be considerably more challenging to identify media for yet-uncultured microbes. But the system does allow users to predict optimal media "richness," a term that describes the amount of usable organic carbon, a key variable for microbial cultivation. "If you have a new organism, our system can predict the richness of the media that it probably prefers," he says. And, as the database fills out, it may be possible to predict other components as well, including salinity, inorganic ions, and more—features that may help in formulating new media for previously uncultivable organisms.

LOOK IT UP: The KOMODO database and GROWREC algorithm are publicly available at komodo.modelseed.org. ■

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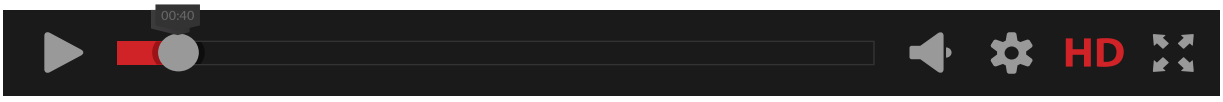

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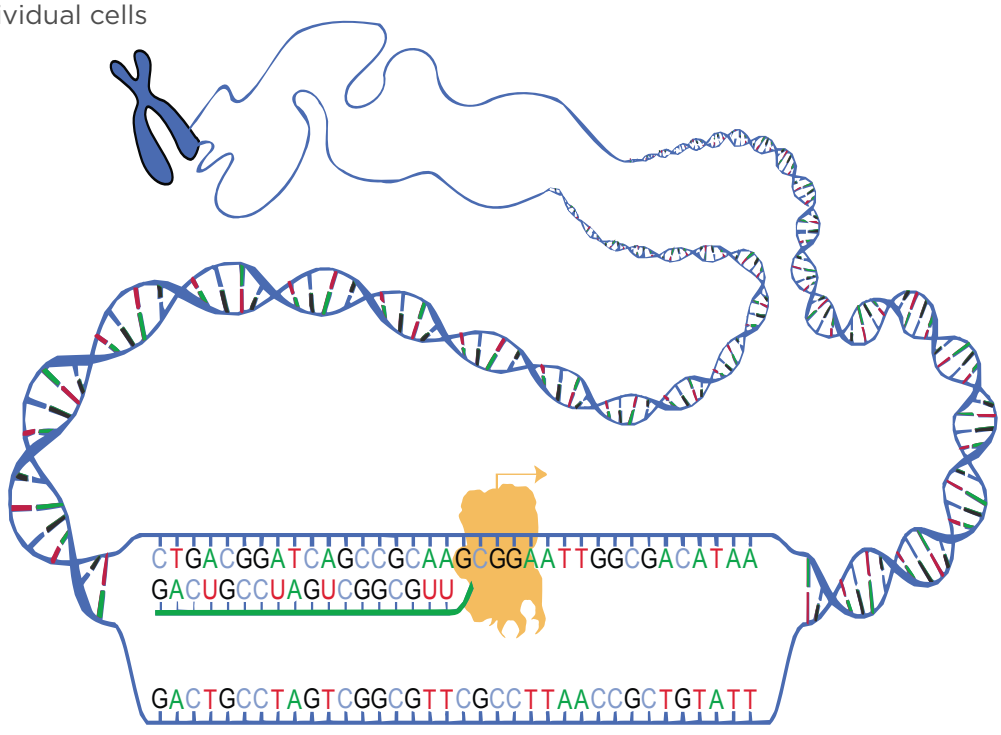
Scaling to Singles

Tips for tracing transcription in individual cells

BY KELLY RAE CHI



If there's anything the burgeoning field of single-cell biology has revealed in the past few years, it's that each cell is unique. Even cells of the same type can vary significantly in their complement of expressed genes. "We sort of knew this, but we now know it in spades," says James Eberwine, codirector of the Penn Program in Single Cell Biology at the University of Pennsylvania's Perelman School of Medicine.

That observation vindicates the monumental efforts of teams of biologists and bioinformaticians to study single cells. On the other hand, it also makes single-cell studies—especially those tackling transcriptomes—more daunting. Which differences between cells result from biological rather than technical variation? How many cells do you need to study to be able to know for certain?

Researchers are now able to answer these questions with more confidence. Single-cell RNA sequencing (scRNA-seq) is progressing on many fronts, including refining those tenuous steps of amplifying picogram amounts of RNA and generating cDNA libraries for high-throughput sequencing. New data-analysis tools, adapted for single-cell data, are coming online as well.

The Scientist asked protocol developers and experienced sequencers for advice on generating libraries and on processing and analyzing the resultant data.

ISOLATING CELLS

Isolation is still one of the toughest steps in single-cell biology. The strategies researchers use fall into a few main categories: manual picking, fluorescence-activated cell sorting, or microfluidics. *The Scientist* has covered isolation techniques in earlier Lab Tools articles. (See "Pushing the Limits," February 2015, and "Singularly Alluring," June 2014.)

PICKING A PROTOCOL

The protocol you choose for generating a cDNA library depends on your main goals. If you want to study overall variability in transcription of cells within or across different tissues, you need a large number of cells. (Hundreds, though the number depends on many factors, including the depth at which you sequence, Eberwine says.) In contrast, if you want to look at a few genes associated with a specific process, such as cell death, you can get by with fewer cells. Whether you are studying cells from multiple animals is another consideration; you need more cells to tease out individual donor effects, according to Eberwine.

Some protocols are now allowing the generation of sequencing libraries for thousands of cells, albeit at lower read depth, though sequencing costs can quickly add up even in these situations. You will probably need to sequence in greater depth to quantify the expression of low-abundance genes or to capture the overall variability of transcriptomes, Eberwine adds.

New protocols for generating sequencing libraries come out all the time, and head-to-head comparisons are hard to come by. A recent study of gene expression in mouse embryonic stem cells, led by biologist Wolfgang Enard of the Ludwig-Maximilians University Munich, compared the sensitivity, accuracy, and precision of a handful of protocols—Smart-seq, CEL-seq, SCRB-seq, and Drop-seq—and we asked others to weigh in on these as well.

Smart-seq

*S*witching *m*echanism *a*t 5′ end of the *R*NA *t*ranscript (Smart-seq) is one of the only sequencing protocols that allows you to generate full-length coverage of transcriptomes from single cells, which is important if you're studying allele-specific gene expression or splice variants.

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Fluidigm's C1 system is encased in a benchtop machine that automatically orchestrates the steps of Smart-seq, taking your cell suspension and isolating and lysing cells, reverse transcribing their mRNA, and amplifying the resulting cDNA. It requires nonreusable microfluidic array chips that analyze 96 cells. Amplified cDNA is then sequenced or detected using qPCR.

Smart-seq on the C1 was the most sensitive in Enard's comparison, but also the most costly. One upside to the technique is that you can put the arrays under the microscope to verify that healthy single cells occupy the wells, says Aleksandra Kolodziejczyk, a graduate student in the lab of Sarah Teichmann at the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI).

CEL-seq

*C*ell *e*xpression by *l*inear amplification and sequencing (CEL-seq) is a popular protocol that employs in vitro transcription, a type of linear amplification process, in the early steps as an alternative to PCR, which yields exponential amplification. One benefit of linear amplification is that it is less error-prone than PCR, though both amplification methods come with biases that depend on sequence.

Described in 2012, CEL-seq involves separating single cells (in the case of this 2012 study, manually), reverse transcribing the mRNA fragments that have poly-A tails, and giving these a barcode unique to their cell of origin (*Cell Reports,* 2:666-73). MARS-seq is another similar protocol (*Science,* 343:776-79, 2014).

Generating libraries using CEL-seq and other linear amplification protocols takes slightly longer because the in vitro amplification step is 13 hours long. On the other hand, in CEL-seq, samples are barcoded and therefore pooled early on, which cuts back on handling times. PCR is used in the final steps, but more as a means to attach the right sequencing adapters, says CEL-seq developer Itai Yanai, now at New York University.

All the reagents are readily available, and it takes about two days to generate sequencing libraries and sequence data, Yanai says. One caveat is that, like other protocols, it sequences the 3' end of transcripts. Enard, whose group did not try CEL-seq but used data from the technique in their comparison of scRNA-seq protocols, found that it is the most reproducible.

Bioinformatics tools for CEL-seq are available via GitHub. Yanai's team is working on a new version, called CEL-seq2, which will be three times more sensitive than the original.

SCRB-seq

Developed by researchers at the Broad Institute, *s*ingle-*c*ell *R*NA *b*arcoding and sequencing (SCRB-seq) uses PCR for amplification and requires access to a fluorescence-activated cell sorting (FACS) machine or another method of efficiently getting individual cells into wells.

The protocol is similar to Smart-seq, except that it incorporates cell barcodes specific to each well (which allows for early pooling of the samples) and unique molecule identifiers, or UMIs, in order to distinguish amplified molecules from the originals and thus to more accurately quantify transcripts. Unlike Smart-seq (and similar to CEL-seq), the approach enriches the 3' ends of RNA rather than generating full cDNA profiles.

The steps of SCRB-seq are found in a 2014 *BioRXiv* paper (doi.org/10.1101/003236). Following the upload of that protocol, developers updated and rebranded it as "high-throughput eukaryote 3' digital gene expression," which is still offered by Broad as a service and has been incorporated into WaferGen Biosystems's scRNA-seq platform. Fluidigm's C1 will also soon enable the SCRB-seq protocol. For DIYers, access to a FACS facility is the main barrier. SCRB-seq is Enard's current favorite, in part for its versatility: he can use the same protocol to do bulk RNA sequencing.

Drop-seq/inDrop

Two independently developed microdroplet-based methods, Drop-seq and inDrop, are newcomers to the single-cell RNA-seq toolbox. (See "Gene Expression in a Drop," *The Scientist*, August 2015.) The techniques, which isolate cells in nano- or picoliter aqueous droplets within oil, allow researchers to equip cells with barcoded primers for amplification and survey thousands of cells.

Enard finds that Drop-seq detects less than half as many genes per cell compared with Smart-seq/C1, CEL-seq, and SCRB-seq. However, in a calculation of the costs needed to detect differentially expressed genes with a specific level of statistical power, Drop-seq and SCRB-seq offered the most bang per buck, he found.

Based on user feedback, version 3.1 of the "living protocol" of Drop-seq came out in late 2015 and is available on Steve McCarroll's lab website (mccarrolllab.com/dropseq/).

Drop-seq can be up and running within six months, says Stefan Semrau of the Leiden Institute of Physics in The Netherlands, who is a coauthor on the paper describing SCRB-seq. When he moved from the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, to Leiden, he chose to set up his new lab with Drop-seq because he doesn't have easy access to FACS. The hardest part is creating the microfluidic chip and getting the oil-water emulsion just right. A postdoc in his lab who specializes in microfluidics got it running in only three weeks. The library preparation is standard for any molecular biologist. Overall, plan to spend roughly six months setting up Drop-seq and optimizing it, he says.

DEALING WITH NOISE

How do you know that the gene expression variability across cells isn't due to technical noise? Weeding out such noise is still one of the major challenges facing even the most seasoned single-cell experts. Many factors generate noise, including incomplete lysis of cells, variability in the reagents, or inefficient reverse transcription. But in general, "I don't think people will know at this point where the noise comes from," Semrau says.

FOUR SINGLE-CELL RNA-SEQ TECHNIQUES

	Smart-seq	CEL-seq	SCRB-seq	Drop-seq
PolyA-mRNA capture	With PCR primer	Oligo-dT primer with Illumina 5′ adaptor, cell barcode, UMI, and T7 promoter	Oligo-dT primer contains cell bar code and UMI	With cell barcode and UMI (primers immobilized on beads and captured along with single cells in droplets)
Reverse transcription	Reverse transcription; template switching oligo added to 5′ end of cDNA	Reverse transcription and template switching	Reverse transcription and template switching	Reverse transcription and template switching
cDNA amplification	PCR, full-length	In vitro transcription from T7 promoter	Single-primer PCR	PCR
Fragmentation/library prep	Tagmentation using Tn5 transposase; Nextera primers added to ends	Fragmentation, followed by PCR to attach Illumina adapters	Modified fragmentation-based approach enriching for 3′ ends; modified Nextera prep	Tagmentation of cDNA with Nextera XT
Unique Molecular Identifier (UMI)	No	Yes	Yes	Yes
Cell barcode	No	Yes	Yes	Yes
Transcript coverage	Full-length	3′ selection	3′ selection	3′ selection
Number of cells	96	96 (on Fluidigm's C1)	96 or 384	1,000s

TECHNICAL EVALUATION*

Sensitivity	Most sensitive			
Precision		Most precise		
Efficiency			Most efficient	Most efficient
Accuracy	Similar	Similar	Similar	Similar

*C. Ziegenhain et al., "Comparative analysis of single-cell RNA-sequencing methods," *BioRxchiv*, dx.doi.org/10.1101/035758, 2016.

One way researchers deal with this problem, from the wet-lab side, is to pick protocols that use UMIs, such as CEL-seq, SCRB-seq, and Drop-seq. Counting UMIs rather than reads can cut technical noise in half (*Nature Meth*, 11:637-40, 2014).

Another strategy is to use commercially available reference mRNAs, namely ERCC Spike-In Control Mix (Thermo Fisher Scientific). These are preformulated blends of RNA fragments of known abundances, developed by the National Institute of Standards and Technology's External RNA Controls Consortium (ERCC), an ad-hoc group of academic, private, and public organizations.

The ERCC mix allow researchers to quantify technical noise. These controls are not perfect—they are not spiked directly into the cell, and protocols may differ in their ability to efficiently lyse the cell. Dominic Grün, a quantitative biologist at the Max Planck Institute of Immunobiology and Epigenetics, has found that validating expression levels using single-molecule fluorescence in situ hybridization (FISH) has revealed some discrepancies in ERCC quantification. He does not recommend ERCCs for measuring absolute levels of gene expression, but he still thinks they are important to include for relating individual genes to transcriptomes.

THE DATA DEEP DIVE

The initial steps of processing single-cell RNA-seq data look just like those used in bulk RNA sequencing. However, you get a very large batch of data for each single cell. Most people will naturally tend to zero in on their favorite genes, but that doesn't do the data justice, Eberwine says.

If you're a molecular biologist by training, to get the most out of your data you should get somewhat familiar with the computing language R. Most of the various ways you can digest your data use R. Formal courses may not be sufficiently tailored to your needs, so Kolodziejczyk recommends just jumping in. (A molecular biologist by training, she learned R by sitting next to generous postdocs and by Googling.)

New tools come online all the time, and in the next few years some favorites will surely emerge. There's no one-size-fits-all solution: the current crop of analysis tools addresses a range of different questions in RNA-seq, such as studying single cells as they differentiate, or categorizing cell types by using various types of clustering analyses. Papers and conferences should be your first stop when shopping for the right ones, Semrau says.

GETTING HELP

Single-cell studies of transcriptomes are almost always a team effort. "There are very few labs that have all the technical expertise in the lab to create new microfluidics devices, to do the genomics, to do the informatics," Eberwine says.

Your protocol picks may well depend on what sort of equipment or expertise your neighbors have, Kolodziejczyk says. But your particular project should determine the allocation of manpower to each step. Who will generate and prepare samples, analyze data, and conduct any necessary follow-up studies pursuing function? Find a collaborator who's going to complement your gaps in training, whether that's in the wet or dry lab.

For a single study it's not worth the effort to set up single-cell RNA-seq, Enard says. Some core facilities offer the entire sequencing process as a service, or you can elect to outsource one or a few of the many steps.

Several places offer once-a-year short courses on single-cell sequencing, including Cold Spring Harbor Laboratory in New York; the European Molecular Biology Laboratory in Heidelberg, Germany; the University of Pennsylvania; and the Wellcome Trust Sanger Institute near Cambridge, U.K. ■

SOME DATA-ANALYSIS CHOICES:

- *viSNE* generates maps allowing you to see thousands of gene-expression values for a single cell.

- *scLVM* is a handy way of dissecting the sources of variability, such as the stage of cell cycle a cell is in, from technical noise. The software requires Python 2.7 and can be accessed via the repository GitHub (github.com/PMBio/scLVM).

- An as-yet unnamed machine-learning algorithm by Teichmann's group helps you exclude bad cells from your analyses in good conscience (*Genome Biol,* 17:29, 2016). The lab is working on a version of this algorithm for R.

- *RaceID* uses transcriptomic data to pick out rare cell types (*Nature,* 525:251-55, 2015). It's also on GitHub (github.com/dgrun/RaceID). (See "Looking for Loners," *The Scientist*, December 2015.)

- *Wanderlust* and *Monocle* are designed to predict developmental trajectories of a single cell by ordering single-cell transcriptomes among a trajectory of changes on the road to differentiation (*Cell*, 157:714-25, 2014).

Some downloads combine many different analysis tools. For example, *IDV* is a collection of scripts developed by Junhyong Kim, James Eberwine's bioinformatics counterpart at the University of Pennsylvania. *SINCERA* is a brand-new pipeline for single-cell RNA-seq profiling analysis, developed by scientists leading a lung cell–sequencing project.

Making the Most of School

Agencies and institutions strive to better prepare graduate students and postdocs for futures in academia and beyond.

BY VIVIANE CALLIER

When Meredith Frie started a PhD program in cell and molecular biology at Michigan State University, she, like most graduate students, focused exclusively on her research. "For the first three years of my graduate program, it did not occur to me to expand my training outside of traditional work in the lab," says Frie. "[But] when you go out and look for a job . . . having a PhD isn't enough, because everyone you'd compete against for a job would also have a PhD."

Fortunately, Frie happened to be attending one of 17 universities with a National Institutes of Health–funded Broadening Experiences in Scientific Training (BEST) grant, devoted to improving career development training for graduate students and postdocs. In her fourth year, Frie completed an MSU-BEST–sponsored internship in the campus's Innovation Center, where she learned about pathways to commercialize basic research. "I always had an interest in the pharmaceutical industry and drug development, so it has been really nice to have the opportunity to meet people in the field I might want to work in," she says.

Frie is not alone. Career development training is patchy across institutions, and most graduate students and postdocs receive few structured opportunities to gain the skills and experience that are critical for landing their first job. "It's not enough just to love your science anymore," says Patricia Labosky, program leader at NIH's Division of Program Coordination, Planning, and Strategic Initiatives who oversees the BEST grantees. "You have to be able to communicate it well. You have to be able to write grants, to manage people and budgets, all those sorts of things."

Opportunities are especially limited for those who have their sights set on nonacademic careers, which are frowned upon by some advisors. But new initiatives like MSU's BEST program are beginning to give trainees the exposure they need to succeed in whatever career they choose—and they're aiming to change attitudes about alternative careers in the life sciences.

"The research enterprise is [composed] of far more than just academic research labs," says Chris Pickett, director of the Rescuing Biomedical Research project launched last September by a group of 16 researchers from as many institutions to address the dire training and funding circumstances faced by US biomedical researchers. "Providing training for students and postdocs to successfully pursue careers outside of academia will only strengthen the enterprise as a whole."

Planning for the future

Whether trainees plan to follow a traditional academic career path or branch off to pursue alternative options, one key to success is knowing where you want to go. At the University of Massachusetts Medical School (UMassMed), a recipient of an NIH BEST grant, all third-year graduate students in biomedical sciences are required to take a course focused on completing an individual development plan (IDP) that charts their desired career trajectory.

A number of BEST programs also offer career panels to provide students the opportunity to learn about the many different science-related careers outside of academia. "One of the goals of our program is to push trainees to think about careers earlier," says Elizabeth Silva, director of the University of California, San Francisco's BEST-funded Motivating Informed Decisions (MIND) program. "In my experience in the professional development office, students would often delay thinking about these things until they were very late in their training."

Once trainees have an idea about what they might want to do, the next step is to try it out. To give students and postdocs such real-world experience, many BEST-funded programs sponsor internship/externship opportunities that allow trainees to gain experience (part-time or full-time), while building a portfolio of work and making connections with people working in a career of potential interest. Such opportunities are particularly important for those who want to pursue a nontraditional career path, according to Phil Ryan,

FACULTY SUPPORT

Many faculty members were initially wary of—or outright opposed to—the increased emphasis on career development training, which they feared would take trainees' time and focus away from the lab, says NIH's Patricia Labosky, who oversees the BEST grants. "At first I thought that would be the hugest hurdle" to building the programs, she says, "and something that we would never, ever surmount."

That's why some program directors, such as Stephanie Eberle of the School of Medicine Career Center at Stanford, coach both trainees and faculty. Eberle emphasizes to faculty members that the goal is to integrate academic and professional development, not to do one at the expense of the other. And for the students' part, Eberle has them develop a specific plan that their PI will agree to. "Students might say that they want to do an internship in biotech, but they don't know how much time it will take, and haven't planned how they are going to finish their lab project when they return," she says. "They don't come in with a plan, and this is what scares our faculty the most." Eberle even recalls one case where a student lost her research funding while doing an internship. "We learned the hard way the value of having a set plan." Now, Eberle's office has forms that help to structure the conversation between trainees and their advisors. "It's much easier this way," she says.

While some professors may still be wary of such career development efforts, the programs have a growing number of supporters among the faculty who are relieved that universities are beginning to share the responsibility of helping students and postdocs find the position they want, even if it's outside of academia. "At MSU, a large number of PIs are supportive of their students finding positions outside of universities, but they themselves don't always feel equipped to discuss this with them," says MSU-BEST program manager Julie Rojewski. "They are eager to have us partner and offer this to their students, because it's competency and knowledge they just don't have."

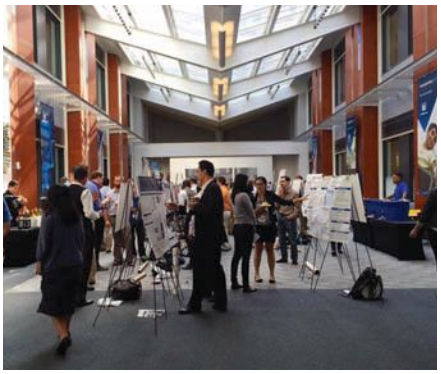
director of student services at the NIH's Office of Intramural Training and Education. "Most postdocs have no experience that makes them hirable for their first job outside of the lab," Ryan said at the American Society for Biochemistry and Molecular Biology (ASBMB) Summit on the Sustainability of the Biomedical Workforce held in Washington, DC, this past February.

At MSU's BEST program, students are asked to complete two externships in their second and third years. Program organizers help students identify opportunities and persuade resistant faculty to allow their students to take the necessary time away from the lab. (See "Faculty Support" at left.) At Spartan Innovations, part of MSU's Innovation Center that helps turn technologies developed at the university into successful businesses, Frie is currently doing a part-time, nine-month externship, working to commercialize a drug developed by Rob Abramovitch's lab to treat tuberculosis. "The experience has given me a different perspective on scientific research—why we do research," says Frie. "It [has] challenged me to think of research from a business or for-profit perspective."

Sarah Kelly, another MSU graduate student, is doing a part-time, year-long internship at the school's technology transfer office, where she has learned about markets for different kinds of inventions. "The BEST program has given me a platform to reach out to these different agencies and different kinds of people in this career field," she says.

Even universities without BEST grants are beginning to put more emphasis on experiential learning. The Johns Hopkins University School of Medicine (JHUSOM), for example, has a variety of programs designed to get trainees out of the academic setting. In addition to the Biomedical Careers Initiative, which sponsors full-time summer internships at biotech companies and scientific societies, the university recently launched the Johns Hopkins-MedImmune Scholars program for students interested in working in the biopharmaceutical industry. Students in the program must identify two thesis mentors—one at JHUSOM and one at MedImmune, the global biologics research and development arm of AstraZeneca—and then develop a project to be carried out in both laboratories.

"Access to the expertise and technologies in a major pharmaceutical company provides new opportunities that can transform your training and the research that you can



UNDERSTANDING YOUR OPTIONS: Right: Students participating in the Johns Hopkins-MedImmune Scholars program present their research, which is carried out under the guidance of a faculty member at the Johns Hopkins University School of Medicine and a research scientist at MedImmune, the global biologics research and development arm of AstraZeneca. Below: At Stanford University, graduate students and postdocs can take part in a Career Exploration Opportunities (CEO) course, where they meet with PhD-level scientists who have succeeded in biology-related fields outside of academia.

CAROLINE POUNDS; STEPHANIE EBERLE

do," says Peter Espenshade, associate dean for Graduate Biomedical Education at JHUSOM. At the end of the program, the students have the option of going to MedImmune for a 12-month practicum after completing their doctorate, which Espenshade hopes will eliminate the need to do a postdoc to get that first industry job. "The current clearinghouse for careers in biomedical science is after a postdoc," he says. "We are trying to move that decision point earlier, saving people time and increasing their earnings."

JHUSOM also organizes an equity research externship in collaboration with T. Rowe Price, a global investment management firm. Graduate students who participate in the program work in teams to pick a biotech stock that looks to be undervalued, then write up a proposal that they will pitch to T. Rowe Price executives. "Traditionally, people with MBAs or business degrees have gone into investment management, but more and more investment firms are finding that PhDs with a biomedical degree have a better ability to look at the science behind the companies," says Patricia Phelps, director of professional development at JHUSOM. Phelps also plans to kick off a similar program covering regulatory affairs, in which students will develop a new drug application to the US Food and Drug Administration and have it evaluated by regulatory-affairs professionals.

"What I'm hearing from students is that they might know what they want to do, but they don't know how to get there," says Julie Rojewski, program manager for the MSU-BEST Program. "We have a mechanism to empower the students, to introduce them to resources and connections to close that gap."

A broader reach

Outside of the 17 recipients of BEST grants and a handful of other institutions that are developing their own programs, most universities do not have the resources to create such initiatives from scratch. Although the NIH's BEST programs are the biggest investments in this area—totaling some $3.7 million across the 17 institutions—foundations and other organizations are also looking to improve biomedical career development. The Burroughs Wellcome Fund's postdoctoral enrichment program, for example, is a minority-focused program that has so far provided approximately 30 postdocs with $60,000 over three years to support their training activities. The funding can help trainees attend a Gordon Research Conference, small and focused meetings that are a great place for postdocs to network with scientists in their field; an advanced course at Cold Spring Harbor to further their expertise in a specific area; or other activities that ultimately make them more competitive in the job market. "You can't overestimate the value of having independent funding when you're a postdoc," says Victoria McGovern, senior program officer at the Burroughs Wellcome Fund.

Providing training for students and postdocs to successfully pursue careers outside of academia will only strengthen the enterprise as a whole.
— Chris Pickett, Rescuing Biomedical Research

The Burroughs Wellcome Fund also administers small institutional grants that are intended to help implement career development programs developed by offices of postdoctoral affairs or graduate-student clubs. The grants aren't as large as BEST grants—the fund offers only $30,000 to $50,000 per institution, says McGovern. "Our [program] is really aimed at getting interesting ideas and testing them out."

To help disseminate ideas for new initiatives, a working group convened at the ASBMB Summit in February proposed an online center, or repository, of resources that would help interested universities replicate successful programs. "It feels like we're at a tipping point now—there's a lot of different efforts happening, but there's no central practice or clear place to share what is happening," says working-group coleader Cynthia Fuhrmann, assistant dean of career and professional development at UMassMed. A steering committee led by Fuhrmann and the University of California, San Francisco's Bruce Alberts and supported by the ASBMB will propose mechanisms for creating and vetting the materials and certified training advisors that universities can call upon for help.

In addition to supporting the creation of more career-focused programs, Fuhrmann and Alberts hope that the center will help make career development a regular part of graduate biomedical education and overcome the stigma attached to careers outside of academia. "We're investing as a nation so much into biomedical research, which is really critical," says Fuhrmann. But when trainees are not provided adequate career development opportunities, "we are creating an inefficient system for trainees and we're not taking advantage of [that] investment."

Whether or not such programs will be successful in helping trainees land a job more quickly remains to be seen, but the NIH and its BEST grant recipients are actively evaluating the outcomes of their training services, tracking information on students' time to graduation, number of publications, and job placement, says Labosky. "We are doing a very extensive cross-site evaluation analysis of all the programs, and we'll have the baseline data soon and some initial results."

At the University of Chicago, for example, four of the eight postdocs who completed part-time, three-month internships through the BEST-sponsored myCHOICE program have received job offers, as have three of the students who did externships, says Abby Stayart, the myCHOICE program manager. "The fact that we've been getting these job offers just a few months after starting these internships to me is just jaw-dropping." Erin Adams, principal investigator of the myCHOICE program, agrees: "This is first-hand proof of how important a network is to getting a good job placement." ■

Viviane Callier is a freelance science writer living in Washington, DC. She was a postdoctoral fellow at Arizona State University from 2011 to 2013, and now serves as a contractor at the National Cancer Institute and a science fellow at the Howard Hughes Medical Institute.

To Each Animal Its Own Cognition

The study of nonhuman intelligence is coming into its own as researchers realize the unique contexts in which distinct species learn and behave.

BY FRANS DE WAAL

Much of my career has been devoted to watching and testing animals, especially some of the smartest ones, such as chimpanzees and bonobos. But I've also spent time observing human skeptics, some of them also very smart. Having devoted all of their attention to small-brained species, such as rats and pigeons, some researchers believe that animal behavior boils down to either instinct or rudimentary forms of learning. But even with regard to their favorite animals, this conclusion is probably wrong, as scientists typically put these organisms in situations that fail to stimulate their full behavioral potential.

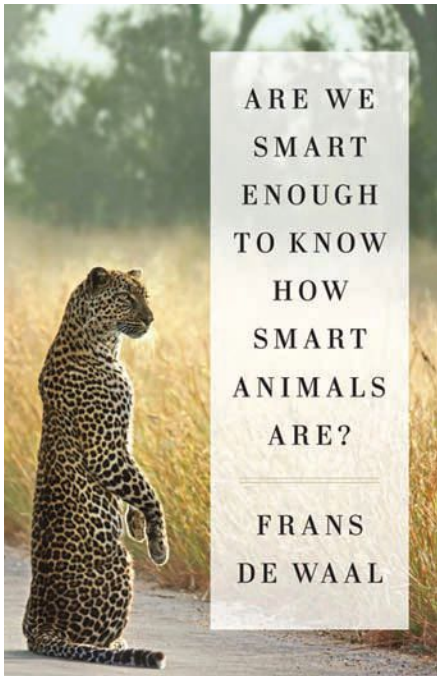
Because I greatly admire the intelligence of animals, I decided to write a book, *Are We Smart Enough To Know How Smart Animals Are?*, that both celebrates the smartness of animals and dissects the challenges facing scientists who study them. Are we innovative and open-minded enough? Nowadays, a growing number of researchers appreciate and explore animal cognition, conducting experiments to illuminate striking capacities, from planning for the future to theory-of-mind political tactics. The latter was my first interest, which I explored in my 1982 book *Chimpanzee Politics*.

The study of animal cognition predates my own interests by decades. Even during the darkest hours of behaviorism in the early 20th century, there were brave exceptions, such as Edward Tolman, Wolfgang Köhler, Nadia Kohts, and Robert Yerkes, who all proposed that animals are capable of insightful problem solving. These pioneering researchers saw animals think, not just learn. They worked mostly under the radar, however, and were forced to publish in second-tier journals. Nonetheless, they erected the signposts indicating the direction of things to come.

The best way to appreciate animal intelligence is to take the natural behavior of each species into account. The idea of universality—that all animals follow essentially the same rules of learning—is anathema to most biologists. It is impossible to extrapolate from rat behavior to that of the entire animal kingdom. Each species has its own senses, its own natural history, and its own ecological problems to solve. We cannot expect an echolocating bat to have the same cognition as a visual creature like ourselves, or an elephant to approach problems the same way an octopus would. Every species has its own *Umwelt* (German for "the surrounding world"), a concept developed early last century by biologist Jakob von Uexküll. Each organism perceives the environment in its own way, he said. His prime example was the eyeless tick, which climbs onto a grass stem to wait for the smell of butyric acid wafting from mammalian skin. We can only understand an organism if we try to enter its *Umwelt*.

In my book I give many examples of animals solving problems in their own habitats, or problems that we present them with in controlled laboratory settings, always taking into account what their *Umwelt* is like.

The relatively new science of animal cognition is in bloom now that researchers have become more sensitive to the different abilities that each species brings to the table. Instead of trying to find a cookie-cutter cognition, we recognize the immense variation in cognition, and look for ways to demonstrate the diverse highlights. New findings consistently point to sophisticated animal cognition, often with compelling videos to back them up. We hear that rats may regret their decisions, that crows manufacture tools, that octopuses recognize human faces, or that special neurons allow monkeys to learn from each other's mistakes. We speak openly about culture in animals, or their abilities to feel empathy or friendship. Nothing is off limits anymore, not even the rationality that was once considered humanity's trademark.

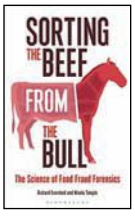


W.W. Norton, May 2016

We live in exciting times indeed. I felt it was time to review how we got here, look back at the historical resistance, and look forward to the appreciation of the animal mind that is providing the study of cognition with a truly evolutionary perspective. ■

Frans de Waal is the C. H. Candler Professor of Psychology at Emory University and Director of the Living Links Center at the Yerkes National Primate Research Center. Read an excerpt from Are We Smart Enough to Know How Smart Animals Are? *at the-scientist.com.*

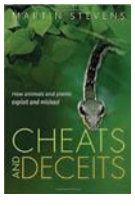
Sorting the Beef from the Bull: The Science of Food Fraud Forensics
Richard Evershed and Nicola Temple
Bloomsbury Sigma, April 2016



We modern humans place an inordinate amount of trust in the people who produce, package, ship, and market our food. And all too often that trust is betrayed. Living ever farther from the sources of nature's bounty leaves space for deception, fraud, and malfeasance to creep into the supply chain. In *Sorting the Beef from the Bull*, biogeochemist Richard Evershed of the University of Bristol in the U.K. and biologist and science writer Nicola Temple tell some of the most egregious tales of food fraud that science has helped expose.

From the unimaginative—advertising horse meat as ground beef, or fobbing off inferior wine as a superlative vintage—to the rather inventive, such as manufacturing phony eggs from a combination of chemicals and algae-derived powders, Evershed and Temple explore cases of food tomfoolery and the science that was used to nab the perpetrators. They also detail the political fallout and provide useful advice to consumers. "Our food system is such that many things lie outside our control, but there are always choices," they write in the book's closing lines. "We are, after all, the consumers, and only we decide what to put in our mouths. Choose well."

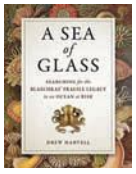
Cheats and Deceits: How Animals and Plants Exploit and Mislead
Martin Stevens
Oxford University Press, May 2016



While humans may be masters of deception and intrigue, other species have millennia on us. Several times over, evolution has favored genes that confer upon their carriers the ability to appear as something they are not. Caterpillars that look like snakes, cuckoos that lay their eggs in brown cowbird nests, and orchids that lure pollinators by smelling like female insects constitute just a taste of the trickery on display in *Cheats and Deceits*, a book by University of Exeter ecologist Martin Stevens.

Surveying the sneakier twigs on the tree of life, the book highlights specific instances of deceptive anatomy and behavior in the context of recurring themes or strategies that have arisen independently throughout evolutionary history. Stevens ends the book by considering how the continued study of deception in the natural world will help researchers understand basic biological principles—for example, appreciating how sensory systems work by studying plants or animals that exploit the perceptual quirks of predators, mates, or competitors.

A Sea of Glass: Searching for the Blaschkas' Fragile Legacy in an Ocean at Risk
By Drew Harvell
University of California Press, May 2016

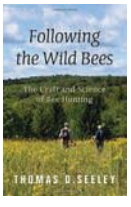


Whether art imitates life or vice versa is open for debate. But that art can inspire science is indisputable, at least in the case of Cornell University ecologist Drew Harvell and her tireless quest of marine discovery. In *A Sea of Glass*, the author relives her journey to the far corners of the planet to seek out the creatures that inspired glassmakers Leopold and Rudolph Blaschka, a father-and-son team that spun graceful, glassy forms to life 150 years ago, fueled by their passion for marine invertebrates.

"I first saw it twenty-seven years ago, broken and dusty, its knowing eye cocked up at me, suckered tentacle stretched across the bottom of its box," Harvell writes of her introduction to the Blaschkas' forgotten art in a storage warehouse outside of Corning, New York. "I was discouraged to see the octopus so damaged, with shattered tentacles and a gaping hole above the eye; it looked to be beyond repair."

But Harvell saw that glass octopus as a metaphor for the fragility of marine life, and it inspired her to "use our glass collection as a time capsule and to see how many of the living representatives we could find in today's oceans." In the richly illustrated book, she recounts finding all of the living representatives of the Blaschkas' menagerie. While the data are still being crunched, Harvell does report that a few species are considered endangered and that biodiversity in the Mediterranean (where the Blaschkas collected most of their muses) has dramatically declined over the past 20 years. (You can see the Blaschkas' ocean-inspired works in person at the Corning Museum of Glass, where they'll be on display from May 14 through January 8, 2017.)

Following the Wild Bees: The Craft and Science of Bee Hunting
By Thomas D. Seeley
Princeton University Press, May 2016



In addition to serving as a primer on the behavior and biology of honeybees, *Following the Wild Bees* just might give readers an intellectually (and physically) stimulating new outdoor activity to take up, just in time for the balmy days of summer. The latest book from Cornell University bee biologist Thomas Seeley is part paean to nature and part practical guide to the lost art of wild-bee hunting.

Seeley adroitly relays the tricks and techniques of tracking wild bees, which involve locating patches of flowers humming with honeybees, capturing and feeding the insects, releasing them, and following them back to their hive, where the hunter can observe the comings and goings of the inhabitants. The author—who also keeps his own hives of domesticated bees—writes most passionately about excursions that end in the pinpointing of a single, wild bee–laden tree among a forest of similar potential habitats. "Like most beekeepers, I love the honey bee colonies that I keep in my hives, for they are easily observed and studied," he writes. "But I am in love with the honey bee colonies that live in the woods." —Bob Grant

New Stand-Alone Imager for Gels and Blots

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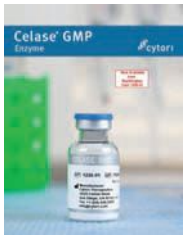

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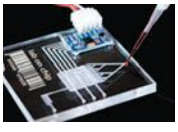

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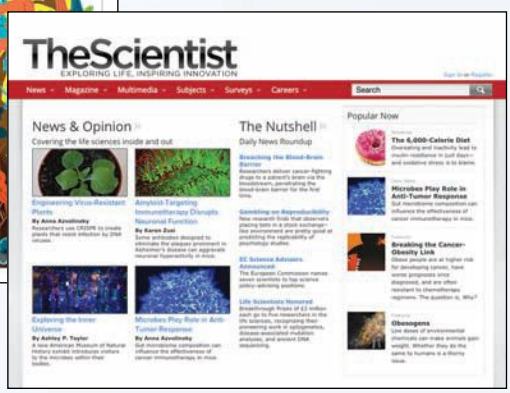

We are pleased to announce that *The Scientist* won a 2015 FOLIO Eddie award for our July 2014 issue and garnered an honorable mention in the Eddies Digital category for our online news coverage. FOLIO awards are presented annually and the Eddies recognize editorial excellence.

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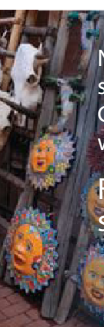

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Picturing Inheritance, 1916

BY AMANDA B. KEENER

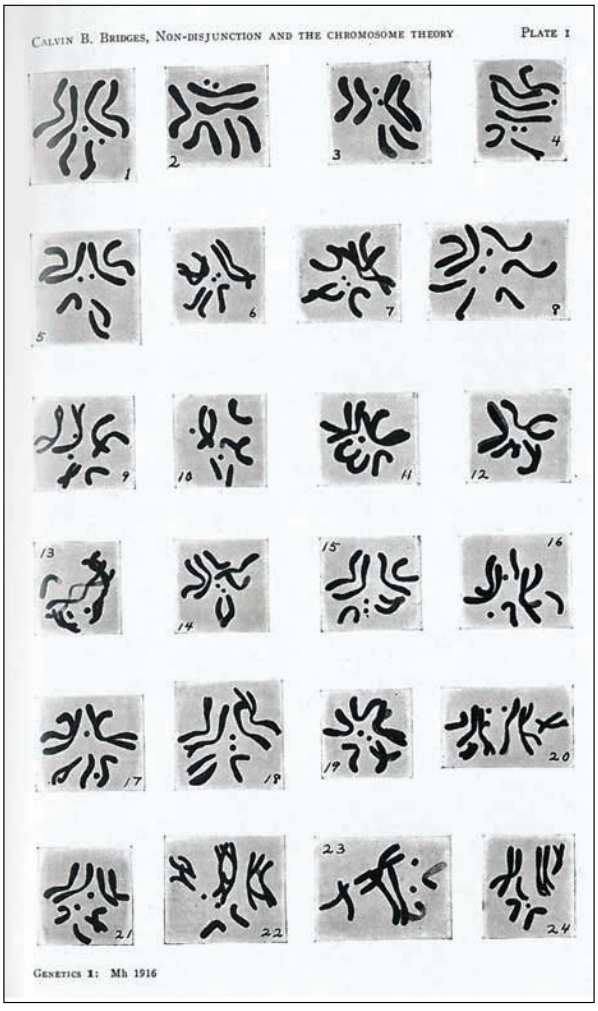
In an era of whole-genome sequencing, epigenetic profiling, and precision gene editing, it's easy to take for granted the basics of genetic inheritance. But 100 years ago, it was not at all clear how heritable information was passed from one generation to the next. Although cytologists who studied cell division hypothesized that chromosomes were involved in heredity, they lacked experimental data to back up the idea. It took an observant student named Calvin Bridges and some rule-breaking fruit flies (*Drosophila melanogaster*) to confirm the hunch.

In 1910, Bridges began working in the famous Fly Room at Columbia University as a member of Thomas Hunt Morgan's lab. Morgan's group had already described sex-linked inheritance: male fruit flies, which receive just one X chromosome from their mothers, express any recessive genes on that chromosome, such as an unusual eye color. Females, on the other hand, receive an X chromosome from each parent, so recessive X-linked traits emerge only among homozygotes—at least, that's what happens most of the time. Bridges encountered rare errors in this pattern. About 1 in every 1,600 heterozygotic females expressed an unusual eye color or some other recessive X-linked trait borne by her mother, suggesting she had not received an X chromosome from her father. And an equal number of males expressed X-linked traits only their fathers displayed.

Bridges took note of these odd flies and proposed that during meiosis, some chromosomes failed to separate, causing one haploid gamete to receive two sex chromosomes and one to receive none. He called this distribution of chromosomes nondisjunction.

The rarity of this event made it difficult for Bridges to test his theory. So he bred his unusual females with normal males to produce atypical females at a much higher rate. He dissected the offspring's ovaries, and with just the aid of a light microscope, examined oocytes undergoing meiosis. As predicted, he found that the females always had two X chromosomes and one Y. He concluded that during fertilization after nondisjunction, if an XX egg receives an X chromosome from the father, the egg is inviable, while a Y chromosome results in XXY females. For eggs with no X chromosome, only those that receive the father's X chromosome survive, and Bridges found that those develop into sterile males. His results, published in two articles in the inaugural issue of *Genetics* in 1916 (1:1-52 and 107-63), also confirmed that in *Drosophila*, sex is determined by the number of X chromosomes; one X chromosome makes a male and two make a female.

In addition to demonstrating that misbehaving chromosomes were behind the unusually inherited traits, says Barry Ganetzky, a fly geneticist and emeritus professor at the University of Wisconsin–Madison, Bridges was also the first to describe an error in meiosis—the type of error that is the most frequent cause of birth defects in humans (see "A Scrambled Mess" on page 28). Ganetzky adds that he is impressed Bridges even took note of a 1-in-1,600 aberration. He suspects most scientists would disregard the chance event rather than divert resources from a planned project. "I hate to admit that's probably what I would have done." ■



MAKING EXCEPTIONS: In the drawings illustrating one of his 1916 *Genetics* articles, Calvin Bridges compared the karyotypes of wild-type fruit flies (1–4) with those expressing traits contrary to the normal pattern of sex-linked inheritance (5–24). Normal flies have four pairs of chromosomes, including a pair of X chromosomes for females (1–3) and one X and one Y (hook-shape chromosome) for males (4; the extra spot here was thought to be of no significance). Bridges found that females expressing their mothers' recessive X-linked traits always had an XXY karyotype as a result of nondisjunction during meiosis (5–19). Figures 20–24 depict the XXYY karyotype of a female produced by mating an XXY female and an XYY male, the parents themselves offspring of an XXY female.

C.B. BRIDGES, "NON-DISJUNCTION AS PROOF OF THE CHROMOSOME THEORY OF HEREDITY (CONCLUDED)," *GENETICS*, 1:107-63, 1916.

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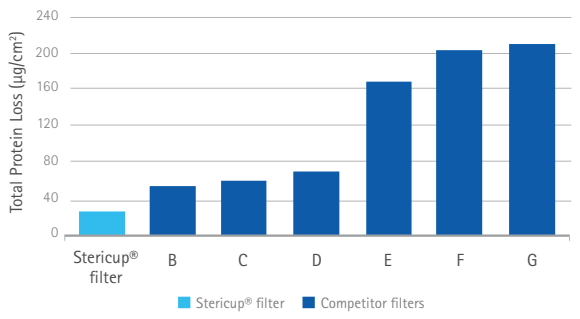

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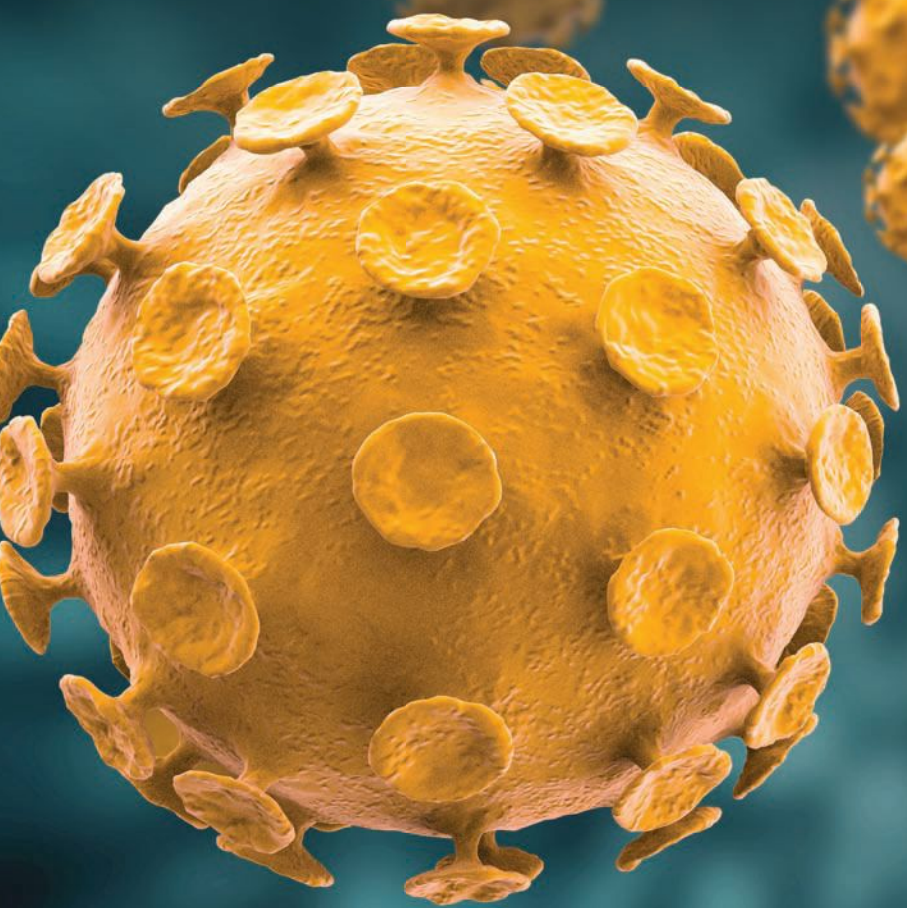

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