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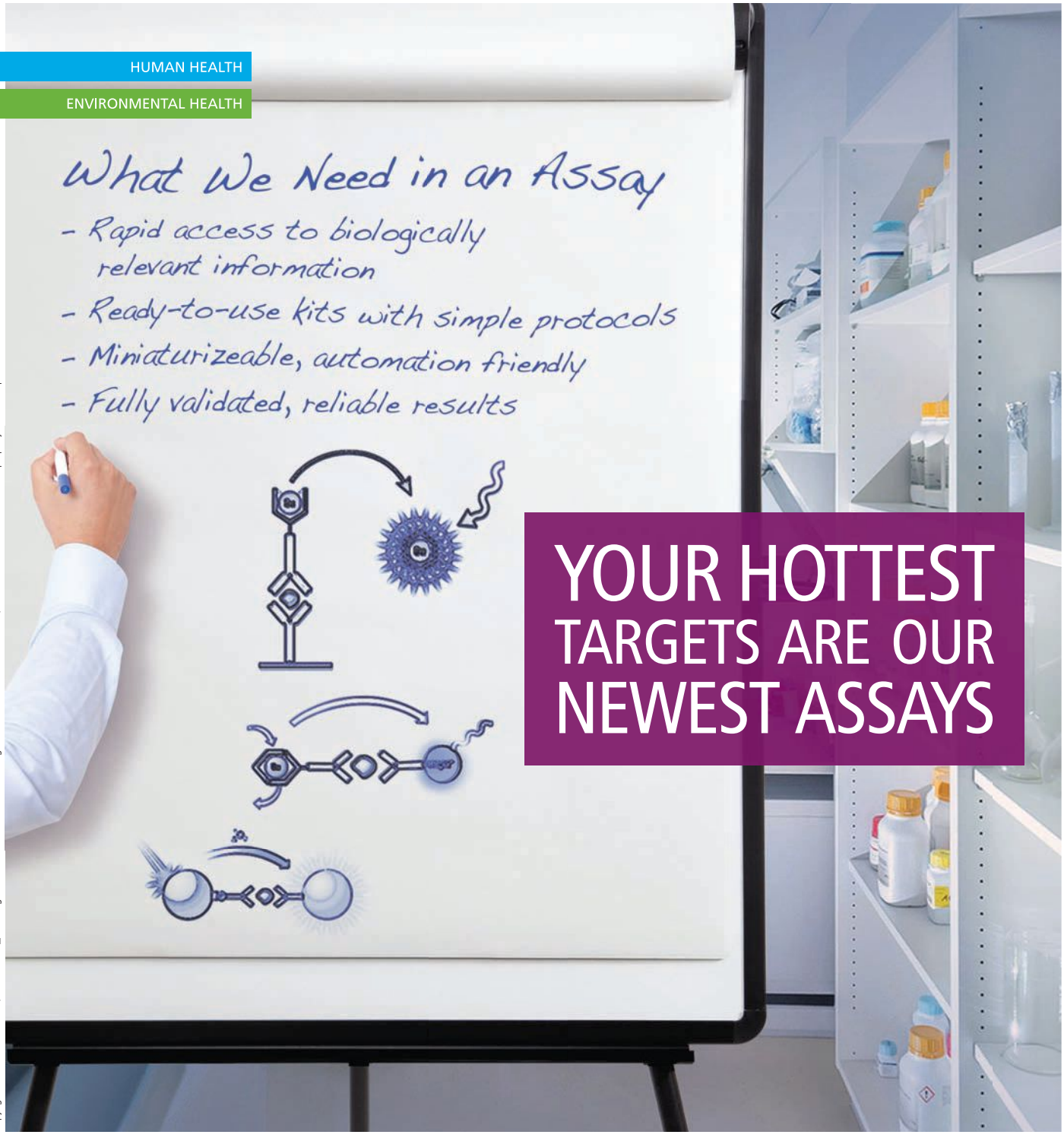

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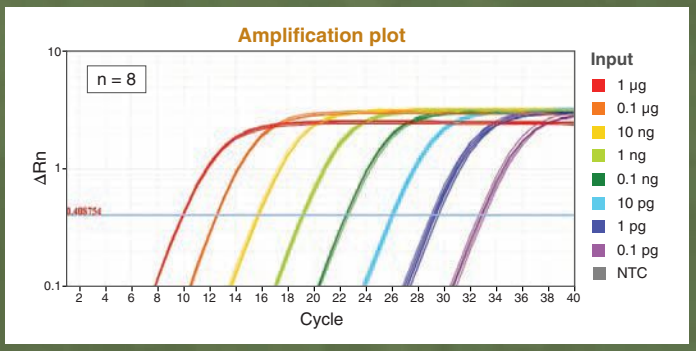

RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 8 replicates at each concentration. Reaction setup and cycling conditions followed recommended protocols, including a 10-minute RT step at 55°C for the thermostable Luna WarmStart® Reverse Transcriptase. NTC = non-template control

Contents

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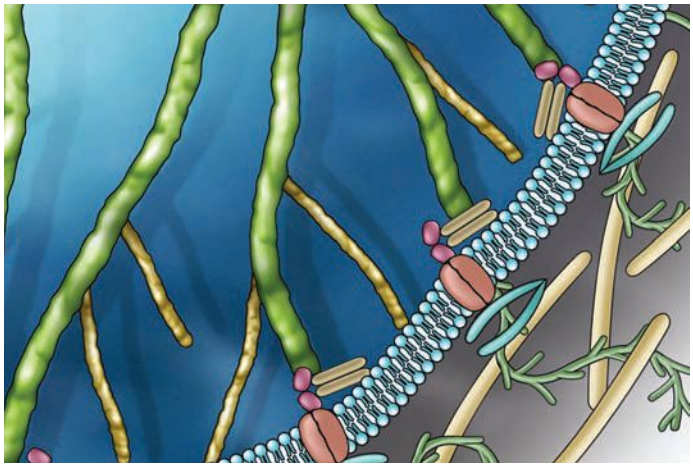

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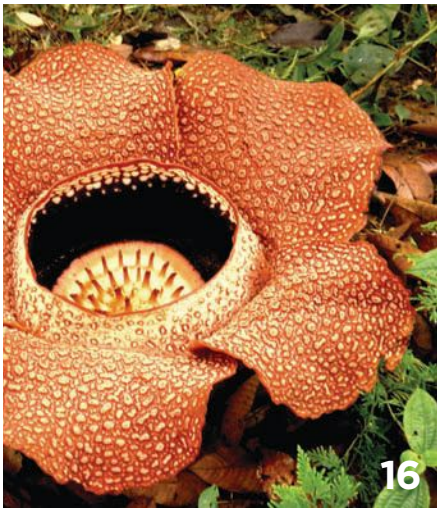

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Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Science Your Plants!
Caltech researcher Elliot Meyerowitz describes how plant genetics influences growth and productivity.

VIDEO
Plant Whisperer
Meet Monica Gagliano, the biologist who studies sound production and reception in plants and whose work with associative learning in plants is making waves in botanical circles.

VIDEO
In Praise of McClintock
Cold Spring Harbor Laboratory's Robert Martienssen discusses the prescience of pioneering geneticist Barbara McClintock, with whom he worked before her death in 1992.

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

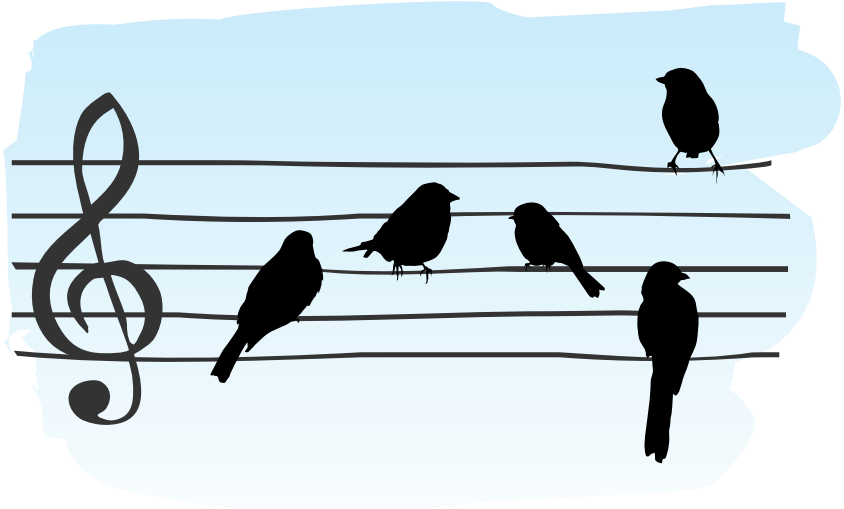
Coming in March

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

Focus on the biology of music

- The evolution of human musicality
- The scientific underpinnings of music therapy
- Song and music in the animal kingdom
- Profile: Erich Jarvis
- Isaac Newton: Color theory as musical notes

AND MUCH MORE



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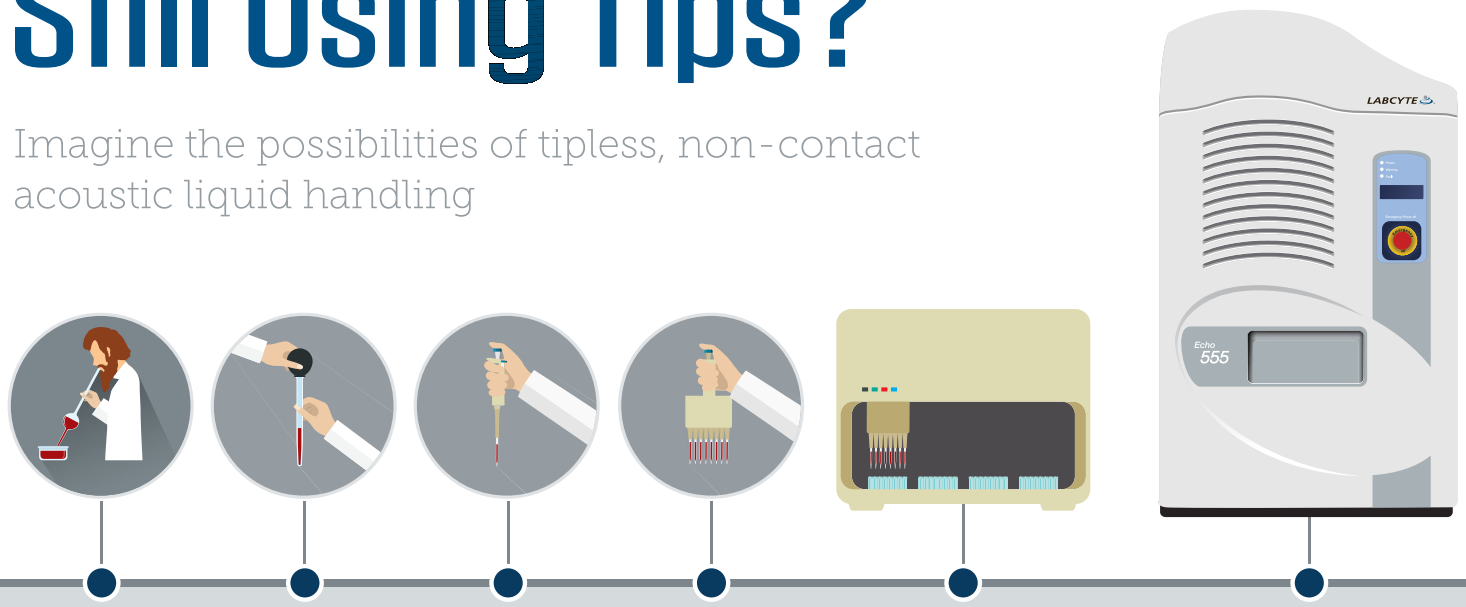

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Contributors

Ning Wang is a professor of biomechanics at the University of Illinois at Urbana-Champaign. Wang started off as a mechanical engineering major in the late '70s at Huazhong University of Science and Technology in China. But after listening to a lecture on the possible role of mechanical properties in soft tissue physiology, a new and unexplored idea at the time, he decided to pursue biomechanics. "I was intrigued by that idea of describing living tissues with mechanics," he says. After earning a master's in biomechanics and an ScD in physiology, Wang worked as a postdoc under Harvard's Donald Ingber, an early proponent of the notion that mechanical tension governs the structure and behavior of cells. In Ingber's lab in 1993, Wang provided the first evidence that integrins mediate mechanical signaling in cells, a discovery now recognized as foundational to mechanobiology. Since then, Wang has continued to characterize cellular mechanosensing and its possible implications for medicine, particularly cancer cell metastasis and stem cell development.

Wang delves deep into the past, present, and future of the field in "May the Force Be with You" on page 44.

"I've always been into natural history, especially some of the more macabre aspects of it," **Bill Schutt** admits. The author of *Cannibalism: A Perfectly Natural History*, and *Dark Banquet: Blood and the Curious Lives of Blood-Feeding Creatures*, Schutt teaches anatomy and zoology at Long Island University–Post and is a research associate in residence at the American Museum of Natural History. Schutt completed a PhD in zoology at Cornell University studying vampire bats, and his interest in the blood-feeders became the inspiration for *Dark Banquet*, his first book, published in 2008. "I like to take subjects that people are grossed out by and turn them around," Schutt says, "so people realize these are natural occurrences" and that "it's not all sensationalism." Schutt now divides his time between writing and teaching. Currently, he is working on *The Himalayan Codex*, a sequel to his 2016 novel *Hell's Gate*, a World War II thriller set in the Brazilian wilderness, the plot of which drew heavily upon Schutt's familiarity with zoology's more lurid side.

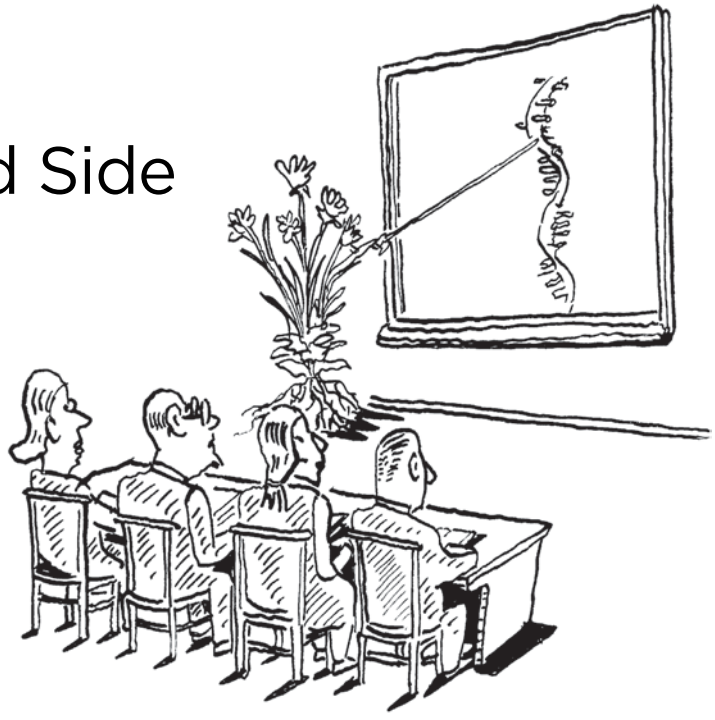
Schutt explains why eating one's own is a regular part of nature on page 63 and in his book *Cannibalism: A Perfectly Natural History*, published this month.

A number of February authors are past contributors to the pages of *The Scientist*. **Sandeep Ravindran** ("What Sensory Receptors Do Outside of Sense Organs," September 2016) writes about novel drug-discovery methods that depend on activating silent gene clusters (page 56). A Notebook by **David R. Smith** describes a parasitic plant that adroitly pilfers mitochondrial genes from its host (page 16); earlier contributions from Smith include four opinions and a January 2013 feature "Steal My Sunshine." **Wolf Frommer** contributes his third opinion piece, "An Ethical Code for Conferences" (page 24), which first appeared online on December 2, 2016.

A Walk on the Wild Side

Plants have so much to teach us.

BY MARY BETH ABERLIN



ANDRZEJ KRAUZE

Scientists' understanding of Kingdom Plantae still has some big gaps, such as exactly when our distant eukaryotic cousins evolved, whether their forebears originated on land or in water, and the shape of their phylogenetic tree. But about one thing there can be no doubt: plants do the damndest things.

A few cases in point: In 1999, the Massachusetts Museum of Contemporary Art (MASS MoCA) opened its doors. Much about the new complex was jaw-dropping, but none more so than the six flame maple trees set in the center of the approach to its entrance. The trees, about 10 feet tall, were growing upside down. The bio-sculpture installation, *Tree Logic,* was conceived and executed by Natalie Jeremijenko, an artist who is no stranger to science. Over the years, the inverted trees took on novel shapes with their trunks and branches curving up toward the Sun, as light trumped gravity.

And then there have been seeds sown in a radically different kind of environment: the International Space Station (ISS). Plants grown some 250 miles above our planet's surface (from wheat to lettuce to zinnias) experience a gravitational tug about 89 percent as strong as that felt by the MASS MoCa trees. A number of the botanical studies on the space station were performed using *Arabidopsis,* a lowly weed dubbed "the lab rat of plant biology," which made its first journey into space in 1982. Versions of the plant went on to glow in the dark on the ISS, and their growth patterns showed that light again trumped gravity, even though both forces worked together to alter the behavior of the plants' roots.

In this special issue highlighting plant biology, you will find *Arabidopsis* mentioned over and over again. Here on Earth, the plant's genome and behavior have been sliced and diced six ways to Sunday. In fact, 2014 saw the 25th International Conference on *Arabidopsis* Research. Elliot Meyerowitz, profiled on page 52, was an early advocate of using the plant as a model for developmental biology, carrying its DNA in his pocket at scientific meetings to share with potential collaborators.

All the probing confirms that, even though the plant and animal kingdoms share a lot of biology, plants really can surprise us. In "Plants' Epigenetic Secrets" (page 28), Senior Editor Jef Akst outlines the distinctly different way plants silence their relatively huge, transposon-riddled genomes, which, unlike animal genomes, have nearly identical methylation patterns in all their tissues. Don't miss the lovely photo of jumping-gene discoverer Barbara McClintock in a greenhouse at Cold Spring Harbor Laboratory with plant biologist Rob Martienssen, who continues to study the phenomenon.

Plants also interact with other species in unusual ways. Senior Editor Kerry Grens reports on the transfer of small RNAs in "Cross-Kingdom Swap Meet" (page 36), presenting evidence from the literature that both plants and parasitic fungi use RNA interference (RNAi) as a mechanism of defense and virulence, respectively. A better understanding of these exchanges may result in new antifungals for crop species.

Peruse the Notebook section (page 16) for more really remarkable things plants do. "Parasite Par Excellence" describes a plant that forsakes photosynthesis, stealing both nutrients and mitochondrial DNA from its host. Botanical "neurobiology" is the subject of "Pavlovian Plants," which covers research demonstrating that plants might be able to perform associative learning just like those famous dogs.

Without a doubt, plants are fascinating Earthlings that continue to surprise, confound, and enlighten researchers. ■

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

Speaking of Science

As you must certainly know, stealing is wrong. . . . Physicians and patients depend on the integrity of the [peer review] process. Such cases of theft, scientific fraud, and plagiarism cannot be tolerated because they are harmful and unethical.

—Tufts Medical Center's **Michael Dansinger,** in a letter addressed to a peer reviewer who rejected his *Annals of Internal Medicine* manuscript and then published it elsewhere with a group of Italian researchers (December 13)

A more subtle, but in many ways more insidious, kind of theft, likely happens even more often. Many researchers can tell stories of being beaten to publication by competitors whom they are fairly sure reviewed their work and delayed it long enough to make sure their own study was published first.

Journalists **Adam Marcus** and **Ivan Oransky,** in a *STAT News* piece that described Dansinger's struggle with the *Annals of Internal Medicine* peer reviewer (December 12)

This paper is really one of the first to prove gun violence functions like a disease and deserves public health and medical resources.

—**Charles Branas,** a University of Pennsylvania epidemiologist, discussing a recent *JAMA Internal Medicine* paper that modeled gunshot violence spreading through social networks in Chicago (January 4)

NIH has benefited greatly, over many years, by being kept outside of partisan jockeying and political interference. The administrations and the Congresses have generally kept hands-off when it came to inserting earmarks or special preferences about where the funding should go. . . . But if this long-standing dynamic were to shift, and decisions started to be made more on the basis of political expediency or special interest, rather than on the basis of scientific opportunity, that would be a major concern.

—National Institutes of Health Director **Francis Collins** talking to *STAT News* about the future of the agency under the Trump Administration (December 16)

Unless we all become partisans in renewed local and global battles for social and economic equity in the spirit of distributive justice, we shall indeed betray the future of our children and grandchildren. . . . To make real progress, we must, therefore, stop seeing the world through our medically tainted glasses. Discoveries on the multifactorial causation of disease have, for a long time, called attention to the association between health problems of great importance to man and social, economic, and other environmental factors.

—Former World Health Organization Director-General **Halfdan Mahler,** who died in mid-December at age 93, espousing his view that health is a fundamental human right, in a speech at the 61st World Health Assembly (2008)

This is the masochism of science. The willingness to suffer the pain of hundreds or thousands of solid, sincere attempts ending in failure just to find a moment of bliss in discovery.

—**Jordan Yaron,** Arizona State University cell biology postdoc, on the rarity of success in biological science (January 4)

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TS Webinars

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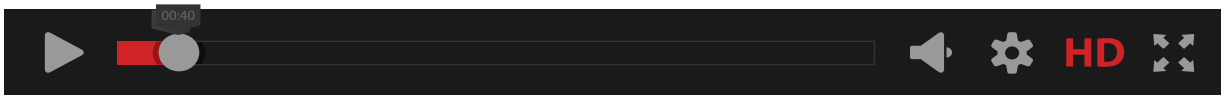
Exosomes encapsulate and transport a wide variety of molecules generated by their cell-of-origin, a process now thought to be a form of cellular signaling. Exosome signaling is common across cell types and species, but it is of particular interest in diseases with an inflammatory component. While exosome isolation and analysis is useful to understanding the mechanisms behind these multifaceted diseases, exosomes may also be exploited for their therapeutic potential. A panel of experts reviews the current knowledge on exosomes in inflammation and explores the potential for exosome-based therapeutics.



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

PETRA HIRSOVA, PhD
Assistant Professor of Medicine
Division of Gastroenterology and Hepatology
Mayo Clinic

JAE-WOO LEE, MD
Associate Professor of Anesthesia
UCSF School of Medicine

TOPICS COVERED:

- The exosomal cargoes released during inflammation, and their potential as therapeutic targets
- How inflammatory diseases are uniquely suited to exosome analysis

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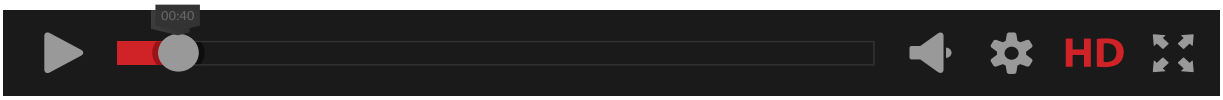
ONDEMAND | Awakening Oligodendrocyte Precursors: Recent Advances in Remyelination

Defects in myelination impair normal action potential propagation and dampen axonal conductivity. Remediating the conductivity between neurons in the pathological setting has proven difficult, as oligodendrocyte precursor cell (OPC) reprogramming has not, to date, been feasible on a large scale. To explore whether or not remyelination is an achievable therapeutic target in diseases such as multiple sclerosis and schizophrenia, *The Scientist* brings together a panel of experts to discuss the current body of evidence.



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STEVE GOLDMAN, MD, PhD
URMC Distinguished Professor of Neurology and Neuroscience
Co-Director, Center for Translational Neuromedicine
University of Rochester Medical Center

YASIR AHMED SYED, PhD
Postdoctoral Researcher
Department of Clinical Neurosciences
Cambridge Stem Cell Institute,
University of Cambridge

TOPICS COVERED:

- Promotion of remyelination by resident OPCs
- Addressing and overcoming demyelination and hypomyelination

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ONDEMAND | Combating Zika Virus with Synthetic Biology and Genome Editing

The wide adoption of synthetic biology and genome-editing technologies has enabled a heretofore unprecedented response to the appearance of Zika virus in the Americas. In concert, these technologies have led to several advances in the study, diagnosis, and prevention of the mosquito-borne virus. To explore the union of urgency and collaboration that has typified the rapid response, *The Scientist* brings together a panel of experts to share their research into understanding and combatting Zika virus, and to explore the lessons learned.

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KEITH PARDEE, PhD
Assistant Professor
Leslie Dan Faculty of Pharmacy
University of Toronto

ZACHARY N. ADELMAN, PhD
Associate Professor
Department of Entomology
Texas A&M University

TOPICS COVERED:

- Disease detection with paper-based diagnostic tests
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Metabolic pathways represent the beautifully orchestrated interplay between cellular systems, working in concert to achieve a shared goal, but – despite their power when finely tuned – they can rapidly fall out of sync, giving rise to disease. To better understand the genesis of these diseases and, ultimately, how to reverse or prevent them, researchers are interrogating the integral cell-signaling pathways and assessing their defects. *The Scientist* brings together a panel of experts to discuss how they are untangling these multifaceted pathways.

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

NATHAN COUSSENS, PhD
Senior Research Scientist, Biology
Division of Pre-Clinical Innovation
National Center for Advancing Translational Sciences
National Institutes of Health

BEATRICE HAIMOVICH, PhD
Associate Professor of Surgery
RW Johnson Medical School
Rutgers University

LISA STEHNO-BITTEL, PhD
President
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TOPICS COVERED:

- Studying signal dysregulation as a key to unlocking metabolic disease
- The potential role of leukocyte signaling in insulin resistance

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Notebook

FEBRUARY 2017

HECTOR SATO

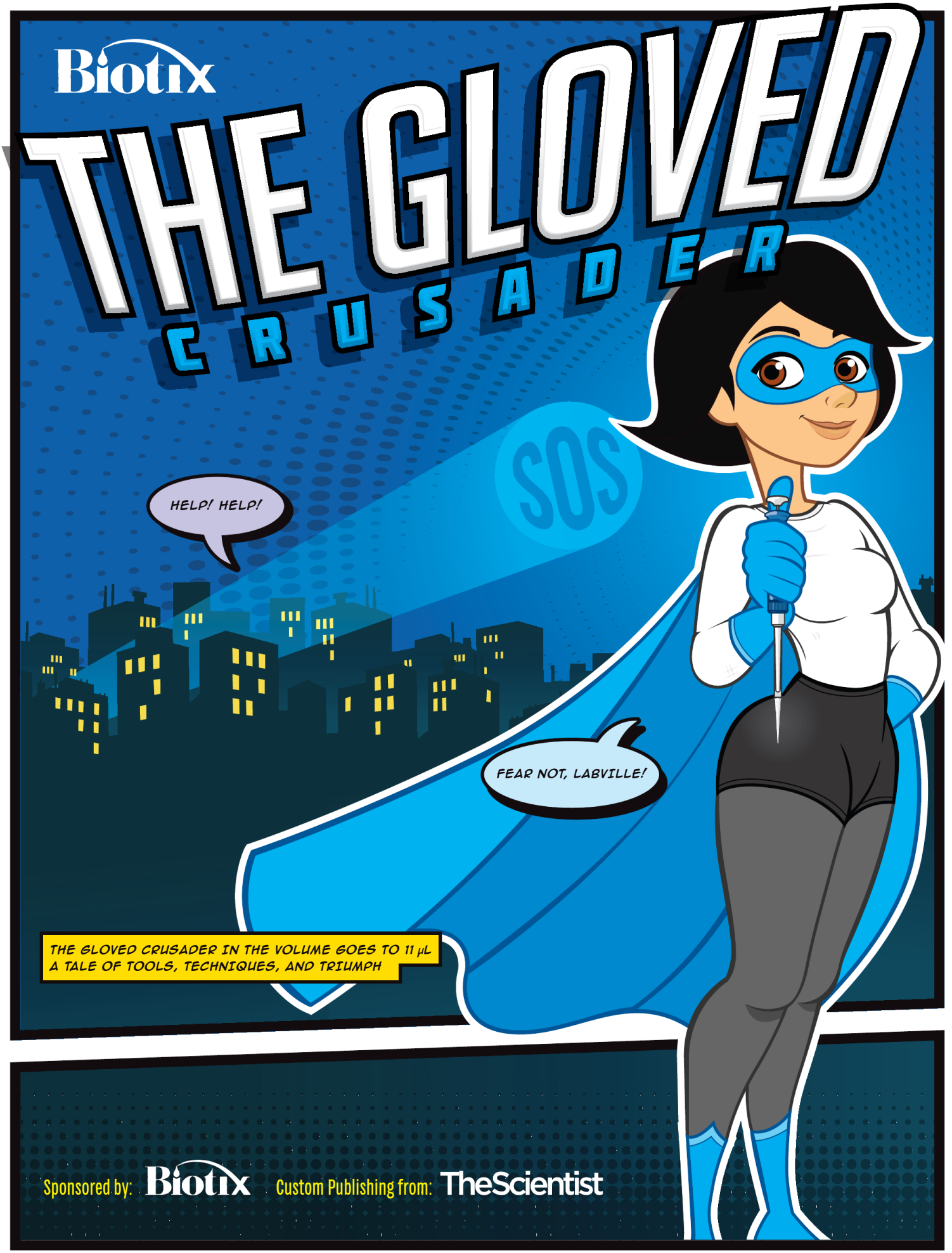
PRETTY LITTLE THIEF: The parasitic plant *Lophophytum mirabile* grows in the Amazon forest, sucking its nutrients (and some mitochondrial DNA) from the roots of other plants.

Parasite Par Excellence

The mitochondrial genomes of land plants are big, bizarre, and often downright bewildering. Take, for instance, the massive, multichromosomal mitochondrial DNA of the sand catchfly (*Silene conica*), which, at 11.3 million bases, is larger than many bacterial genomes; or that of spikemoss (*Selaginella moellendorffii*), with its thousands of RNA editing sites and complete lack of genes for tRNAs. The peculiarities of plant mitochondrial genetics are multifarious enough to make it hard for any one to stand out—that is, until recently, when María Virginia Sanchez-Puerta of Argentina's National University of Cuyo and colleagues sequenced the *Lophophytum mirabile* mitochondrial genome (*New Phytol*, doi:10.1111/nph.14361, 2016).

Lophophytum is a holoparasitic plant, meaning that it has forsaken photosynthesis and is completely reliant on its various hosts for survival. In canopy-darkened South American jungles, the inconspicuous root parasites send spikes of inflorescence upward through the soil while their roots tap into the nutrient supply of plants such as the wilco tree (*Anadenanthera colubrina*). But as it turns out, *Lophophytum* has been stealing much more than just nutrients from its unsuspecting hosts: it has also been snatching their mitochondrial genes, and tossing out many of its own in the process.

Careful analysis of the *Lophophytum* mitochondrial genome sequence, which is about 820,000 base pairs long, revealed 56 genes (44 protein- or RNA-encoding genes, some in multiple copies), which is unremarkable. But what is remarkable about these genes is that at least 37 of them, including 35 protein-coding genes, were acquired via horizontal gene transfer from the plants that *Lophophytum* parasitizes. In other words, *Lophophytum* has swapped almost all its native mitochondrial genes for their foreign equivalents, which is a bit like replacing all the appli-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FEAR NOT, LABVILLE!

THE GLOVED CRUSADER HAS ARRIVED TO PRESERVE YOUR ASSAYS' ACCURACY AND PROTECT YOU FROM BEING VULNERABLE TO VARIABILITY.

YOU'VE ALREADY "MET" THE GLOVED CRUSADER, BUT... THERE ARE TWO KINDS OF SCIENTISTS.

1

2

SCIENCETOWN, ARE YOU READY TO...

UM, ACTUALLY APPROXIMATRIX, IT'S LABVILLE.

CLOSE ENOUGH!

THE MICROPIPETTE TRAIN PULLS INTO CALIBRATION STATION

HANDLE WITH CARE

CLICK!

ALL OF MY MICROPIPETTES HAVE BEEN RECENTLY CALIBRATED TO ENSURE OPTIMAL ACCURACY WHEN MEASURING VOLUMES.

I TREAT THEM CAREFULLY SO THEY STAY CALIBRATED FOR AS LONG AS POSSIBLE.

3

THAT GLOVED WEIRDO ISN'T SO GREAT! I'VE GOT A WHOLE SACKFUL OF PIPETTES.

CRA

SEE?

4

MEANWHILE... IN THE CHAMBER OF ALIQUOTS, THE TUBES ARE LABELED 10 µL, BUT THEY ALL CONTAIN DIFFERENT VOLUMES!

YO, YO, YO! MIX MASTER MIX, TOSS ME ONE OF THOSE ALIQUOTS.

AS I ALWAYS SAY, "AN ALIQUOT IS AN ALIQUOT IS AN ALIQUOT...

UNHAND THOSE UNRELIABLE ALIQUOTS, MIX MASTER MIX! THEY COULD RUIN SOMEONE'S ASSAY.

WHO LET YOU INTO THE CHAMBER OF ALIQUOTS WITH THAT UNCALIBRATED PIPETTE AND LAX TECHNIQUE?

7

THE CARRYOV

A WISE LA
SHARED WITH
ABOUT PIPE
EVER SINCE
I'VE MADE
LOW-RETE
MICROL

8

SHOOTOUT AT THE OK SHARPS CONTAINER

TIP ZEN IS ALL ABOUT BALANCE: PROPERLY FITTING TIPS DON'T NEED TO BE JAMMED AND CRAMMED TO STAY ATTACHED, AND EJECTING THEM DOESN'T REQUIRE A FEAT OF SUPER-STRENGTH. WHEN EJECTING YOUR TIPS, AIM FOR THE NEAREST APPROPRIATE RECEPTACLE AND GENTLY DEPRESS THE EJECTION BUTTON.

FULL

SHARPS

10


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AB MANAGER ONCE
ME A CAUTIONARY TALE
TTE-TIP CARRYOVER.
E THAT FATEFUL DAY,
SURE TO CHOOSE
NTION TIPS WHEN
LITERS MATTER.
ASPIRATE
DISPENSE
ASPIRATE
DISPENSE
ASPIRATE. DISPENSE.
ASPIRATE. DISPENSE. WHO CARES IF
THAT ANNOYING DROPLET HANGS
THERE FOREVER?
NOT ME!
SMASH!
YEEOWCH!
I MAY BE A SUPERVILLAIN,
BUT MY THUMB CAN'T TAKE
THIS ABUSE! I'M REPORTING
THIS TO EH+S, AND THEN I'LL
TAKE A NICE LONG VACATION.


5
THE RIGHT PIPETTE TIP IS LIKE A GLOVE. IT CONFORMS TO YOUR PIPETTE, CREATING AN AIR SEAL FOR PRECISE SAMPLE HANDLING…
AND I KNOW A THING OR TWO ABOUT GLOVES!
6
THIS TIP WON'T STAY PUT ON MY PIPETTE!
YOU WANT TO SEE A FIT? I'LL SHOW YOU A FIT!
BANG!
@#%&
THAT'S THE LEAKIEST SEAL I'VE SEEN SINCE I WORKED AT THE AQUARIUM!
THE END
AND SO, AS THE GLOVED CRUSADER LEFT LABVILLE, CONFIDENT THAT ITS CITIZENS WERE FULLY INFORMED ABOUT THE RIGHTS AND RESPONSIBILITIES THAT COME WITH MICROPIPETTE USE, SHE EXCLAIMED HER LATIN CATCHPHRASE…
IN LIBRIS ACCURATIUS
SUBTITLE: ACCURACY IN VOLUMES
12
13
APPROXIMATRIX? ARE YOU THERE?...
SPROING
...UGH, NEVER MIND.

ADVANCING TIP TECHNOLOGY

BECAUSE YOU START EVERYDAY WITH QUESTIONS, WE START EVERYDAY WITH QUESTIONS. HOW CAN WE PROVIDE TOOLS TO IMPROVE YOUR DATA QUALITY? HOW CAN WE IMPROVE YOUR ACCURACY AND PERFORMANCE? HOW CAN WE USE LESS RESOURCES WHILE PROVIDING MORE FEATURES? HOW CAN WE MOVE SCIENCE FORWARD? INNOVATION IS AT THE HEART OF BIOTIX LIQUID HANDLING PRODUCTS. OUR MISSION IS TO SUPPLY YOU WITH THE HIGHEST QUALITY, MOST INNOVATIVE LIQUID HANDLING PRODUCTS AVAILABLE. WE MANUFACTURE PIPETTE TIPS, TUBES AND RESERVOIRS FOR MANUAL AND AUTOMATED PIPETTING UNDER BEST-CLASS MANUFACTURING AND QUALITY STANDARDS.

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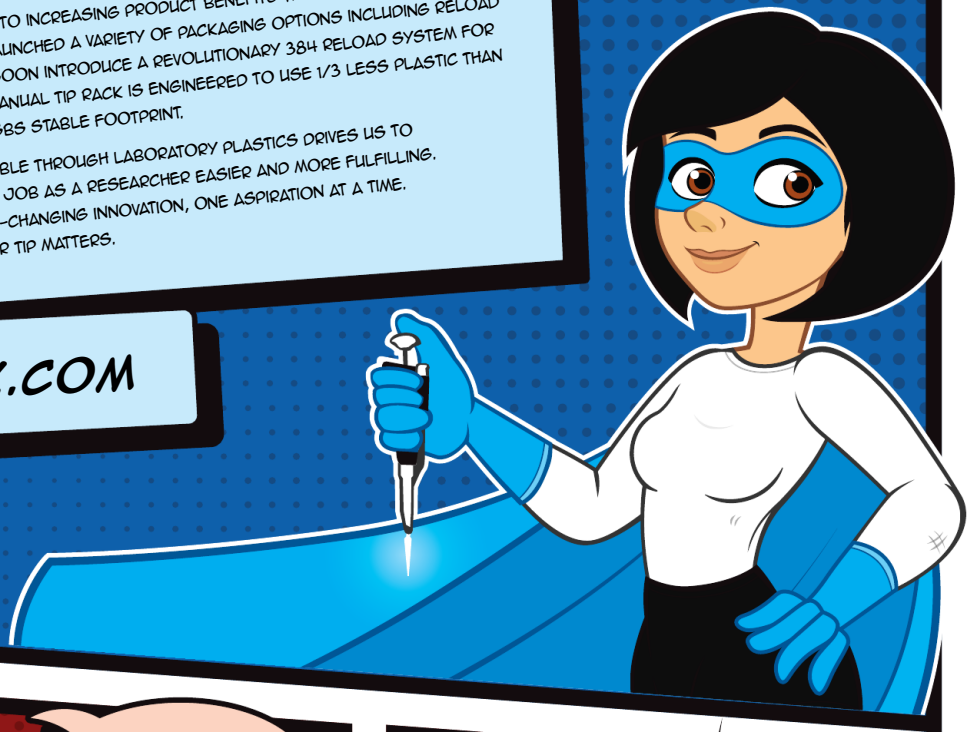


ances in your kitchen with those from a neighbor's kitchen.

Horizontal gene transfer is not uncommon in parasitic plants like *Lophophytum*, which form vascular connections with their hosts, making it easy for them to pilfer water and nutrients. But this open flow from host to parasite also opens the door for the movement of DNA and even entire mitochondria. For instance, researchers recently showed that the holoparasitic plant *Sapria himalayana* acquired many nuclear and mitochondrial genes from its host *Tetrastigma*, a genus in the grape family. In *Sapria*, the foreign gene copies exist alongside native versions, whereas in *Lophophytum*, most of the native homologues of the horizontally acquired genes have been lost completely.

In addition to being a sponge for foreign DNA, the *Lophophytum* mitochondrial genome has an unusual architecture in that it is fragmented into 54 circular chromosomes ranging in size from about 7,000 to 58,000 base pairs, which is in stark contrast to the simpler architecture of most animal mitochondrial DNA. What's more, only 29 of *Lophophytum*'s mitochondrial chromosomes contain intact, known genes, raising the question: what are the functions, if any, of the other 25 chromosomes? Sanchez-Puerta and her coauthors hypothesized that these geneless fragments of DNA might play a regulatory role, or might just be empty baggage.

Finally, a small fraction (0.6 percent) of the *Lophophytum* mitochondrial genome is made up of chloroplast-derived DNA. But, again, these chloroplast sequences appear to have been acquired from the host rather than from the *Lophophytum* chloroplast. In fact, not a single native chloroplast gene was found in the more than 6.5 billion base pairs of *Lophophytum* sequencing data, which was derived from total cellular DNA, suggesting that this parasite might have lost its own chloroplast genome outright. The complete forfeiting of plastid DNA is an extremely rare event, but it is believed to have occurred in the holoparasitic plant *Rafflesia lagascae*—which bears so-called "corpse flowers," so named for their fly-attracting putridity—as well as in the nonphotosynthetic green alga *Polytomella*.

The past year has been a fruitful one for research on parasitic plants. In October, Claude dePamphilis's lab at Penn State University uncovered numerous horizontally acquired genes in the nuclear genomes of parasitic members of the Orobanchaceae (broomrapes), once again illustrating a facility for gene transfer in parasitic plants. DePamphilis and his team have provided strong evidence that horizontal gene transfer plays a crucial role in the adaptation of parasitic plants, possibly helping them to feed on the juicy sugars of their hosts. Sanchez-Puerta and colleagues were open to the idea that the pilfered genes in *Lophophytum* might be benefiting the host-parasite relationship, but they also stressed that, rather than arising via natural selection, the foreign genes could have been acquired and fixed through random, nonadaptive processes and may not necessarily be providing any benefits. More genomic research on parasitic plants is sure to follow. Whatever future studies reveal, these nonphotosynthetic flowers certainly deserve their day in the sun.

—David Smith *is an assistant professor of biology at the University of Western Ontario.*

WIKIMEDIA COMMONS/ROD WADDINGTON

CORPSE FLOWER: Another parasitic plant, *Rafflesia arnoldii*, belongs to a genus that has been caught stealing genetic material from host plants.

Pavlovian Plants

In 2007, plant biologists passionately argued the meaning of the word "neurobiology." The year before, an article published in *Trends in Plant Science* had announced the debut of a new scientific field: plant neurobiology. The authors suggested that electrical potentials and hormone transport in plants bore similarities to animal neuronal signaling, an idea that raised the hackles of many a botanist. Thirty-six plant scientists signed a letter briskly dismissing the new field, calling the comparison between plant signaling—intricate though it is—and animal signaling intellectually reckless. "Plant neurobiology," they wrote, was no more than a "catch-phrase."

Upon close examination, the "neurobiology" debate did not center on very much scientific disagreement. Researchers in both camps agreed on the general facts: plants did not have neurons, nor

did they have brains, but they did possess complicated, poorly understood means of responding to the environment that deserved rigorous study. The community was conflicted over how to talk about these abilities and whether the semantic umbrella of words such as "feel," "choose," and "intelligence" should extend to plants.

The rhetoric surrounding the argument has since cooled, but the debate was never entirely resolved. And in 2016, Monica Gagliano of the University of Western Australia and colleagues provided fresh fuel for the conceptual fire. The researchers conducted an experiment that they say shows plants performing associative learning (*Sci Rep*, 6:38427). This type of learning is the same process by which a dog can learn to associate the sound of a bell, as in Pavlov's famous study, with a treat, and then salivate with anticipation every time a bell rings. The group's experiment, modeled on Pavlov's, was designed to subject pea seedlings to analogous stimuli and find out what the plants could learn.

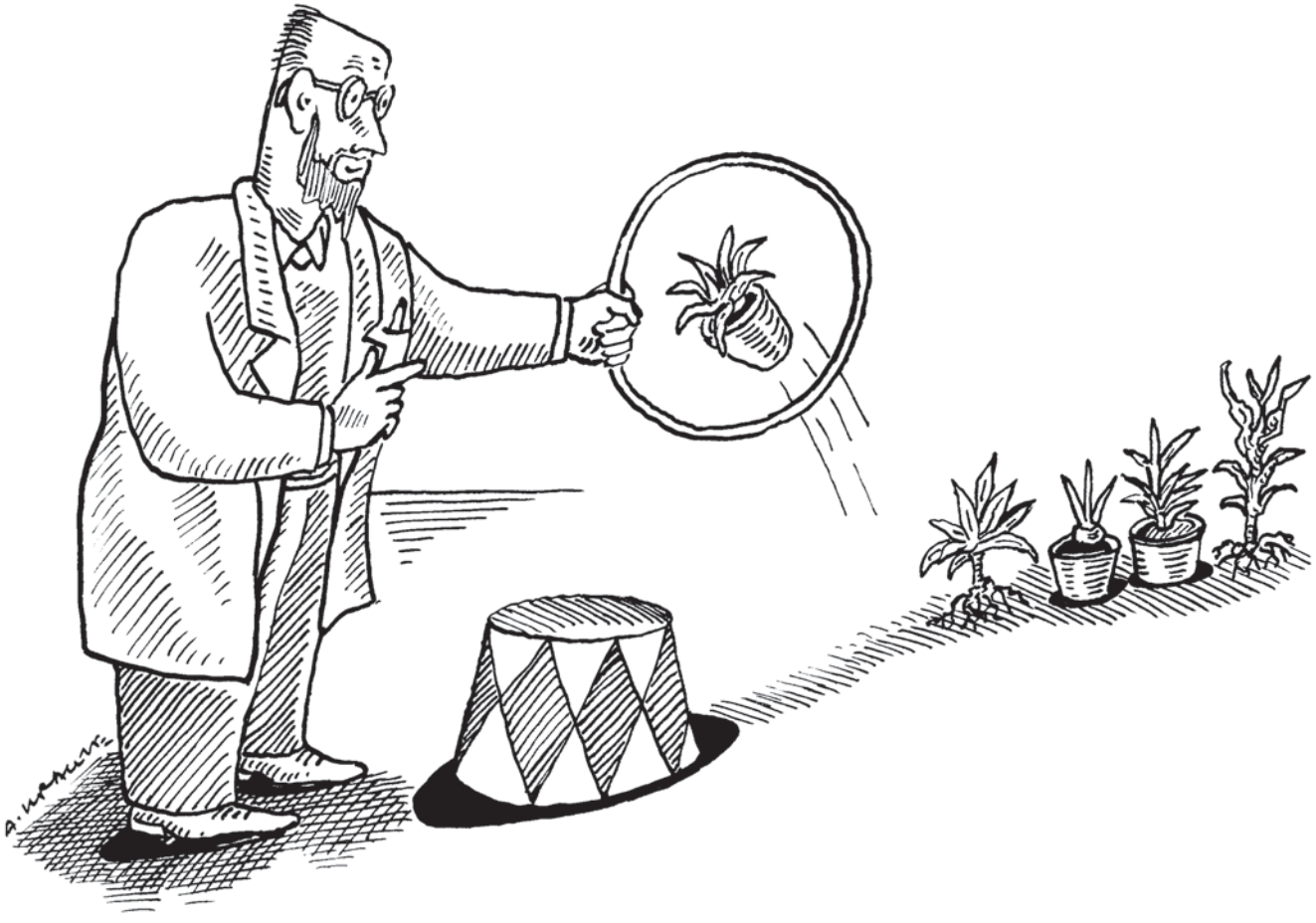
The seedlings were grown in Y-shape tubes for about a week, receiving eight hours of light a day. Then, they were enrolled in a three-day training course.

Unless we really explore the field experimentally, then we are just plant philosophers—and there are already plenty of good philosophers around.

—Monica Gagliano, University of Western Australia

The grow lights were turned off, and three times each day, a small fan blew a light breeze down one arm of the Y-tube for an hour and a half. Beginning after the first hour of that period, the plants were given a one-hour dose of blue light (overlapping for one-half hour with the fan), their only sustenance during otherwise lightless days. For some, the breeze and the light came down the same arm of the Y. For others, the two stimuli came from opposite directions. In both groups, the stimuli were switched randomly from left to right between sessions, and a pilot experiment showed that the breeze had no influence over growth on its own. For all intents and purposes, the breeze was only meaningful to the plants insofar as it predicted where light would soon appear, the authors reasoned.

On the fourth day, seedlings had approached to within a centimeter or so of the bifurcation in the Y, and they were kept in the dark that day. A control group was left undisturbed, while a



ANDRZEJ KRAUZE

test group got the usual three courses of gentle breeze, but this time without the accompanying light. The fan breeze was applied in a direction that "predicted" light to appear opposite the side where it had last appeared.

The seedlings continued to grow, never bumping against the fork of the Y-tube but bending left or right. In doing so they made a choice, so to speak, to grow in the direction of their own survival. Of the 19 plants in the control group, 100 percent extended in whichever direction the light had last come from, exhibiting the well-known affinity of young plants for blue light. The 26 plants in the test group, however, had a decision to make. They could persist in the most recent direction of the blue light like the control group, or grow in the opposite direction, where the fan predicted light should appear—that is, show that they had "learned" something about the meaning of the breeze. Around 65 percent chose this latter option.

"This is exactly what Pavlov did," Gagliano says. "If this were an animal of any kind," rather than a pea plant, "this would be considered learning." However, the experiment is notably limited in scope. A crucial feature of learning in animals is the flexibility to key in on virtually any stimulus. Rats, for example, can just as easily be trained to respond to a light breeze as to the sound of a bell or to vibrations in the ground, because the underlying neural mechanism is not stimulus-specific but all-purpose. Plants are known to respond to many aspects of their environment, from air temperature to soil moisture, but the extent to which a plant can build associations between stimuli other than blue light and a breeze remains to be seen.

The study by Gagliano and her colleagues was also short on controls, says Lincoln Taiz, professor emeritus at the University of California, Santa Cruz, who signed the 2007 letter criticizing the concept of plant neurobiology. Gagliano and her colleagues showed that the fan's light breeze neither attracted nor repelled plants, but did not test whether the breeze interfered with the innate attraction toward light. Taiz points out that the slight rotating motion of a growing shoot's tip, called circumnutation, influences growth patterns such as phototropism and could have been disrupted during the test.

Gagliano acknowledges the need for further work to explore how broadly the associative process can be generalized and to search for a mechanism that might account for it. "This is the beginning," she says. "We needed to first show that learning by association was even pos-

sible." Gagliano is no stranger to controversy, having weathered heavy criticism for her previously published evidence for habituation—a different type of learning—in plants, along with other findings.

As with earlier papers that sought to attribute animal-like behavior to plants, critics are likely to take issue with the authors' use of words typically reserved for animals, including "learning" and "memory," in much the same way researchers debated the moniker "plant neurobiology." Those discussions are valuable and illuminating, says Gagliano, but should not obscure the underlying scientific exchange. "Unless we really explore [the field] experimentally, then we are just plant philosophers—and there are already plenty of good philosophers around."

—Ben Andrew Henry

GRACEFUL DEATH TRAP: Pitcher plants may yield enzymes that can help people with celiac disease digest gluten.

Pouring a Pitcher of Celiac Relief

The pitcher plant *Nepenthes x ventrata* is gorgeous and popular with horticulturists, but it's deadly for the insects that fall into the trap for which it is named. Yet the enzymes in the digestive fluid that fills the carnivorous plants' vase-shape modified leaves might one day provide a service to the animal kingdom, by enabling human celiac disease patients to properly digest the grain protein gluten.

University of Calgary protein chemist David Schriemer didn't set out to identify a celiac disease treatment, even though his 15-year-old niece suffers from the disorder. Instead, he was searching for an alternative to the stomach enzyme pepsin that would be more effective in the low-pH cleavage steps of his proteomics experiments. He turned to plants, looking for enzymes far away on the phylogenetic tree from pepsin. When he tested pitcher plant secretions—which are very effective at digestion and approximately as acidic as human gastric juices—he found that they did something that pepsin could not: snip bonds linking the amino acid proline to other amino acids. (Proline has a ring-shape structure that introduces tight curves into peptide chains.) Such enzymatic activity is relatively rare, Schriemer says. He also realized there might be another application for such an enzyme: prolines make up 15 percent of gluten.

First, Schriemer had to isolate the pitcher plant enzyme responsible for the unique chemical activity, a feat that required more plants than he had in the lab. So in late 2013, he turned to the now-defunct Urban Bog, a carnivorous plant company just southeast of Vancouver, British Columbia. Urban Bog's owners set up a greenhouse filled with 100 *N. ventrata*, each bearing 10 to 20 pitchers, and a *Drosophila* researcher colleague of Schriemer's provided leftover flies for the pitchers' weekly feedings. Over the span of six months, the plants yielded six liters of secretions—enough for Schriemer's group to characterize the transcriptome and proteome of the digestive fluid (*J Proteome Res*, 15:3108-17, 2016).

Next, they reverse-engineered the proline cleaving activity, beginning with recombinant versions of two enzymes they knew were in the mix—the aspartic proteases nepenthesin I and II. Together, these enzymes chopped up proteins effectively, but didn't touch the bonds after prolines in the sequence of amino acids. By comparing their transcriptomic and proteomic data, however, the team discovered a new enzyme that did: neprosin, a prolyl endoprotease. Further tests revealed that a mixture of the nepenthesins and neprosin effectively digested a protein slurry containing gluten in both a test tube and a mouse model of celiac disease (*Sci Rep*, 6:30980, 2016).

Schriemer isn't the first to consider a prolyl endoprotease as a potential therapy for gluten intolerance. The idea dates to 2002, when scientists at Stanford University identified a proline-rich peptide resulting from gluten breakdown that triggered the inflammation characteristic of the celiac immune response (*Science*, 297:2275-79). Although their venture to commercialize bacterial enzymes that break down the peptide has proved unsuccessful, another prolyl endoprotease, from the fungus *Aspergillus niger*, a black mold that grows on fruit, is currently available in the United States as the supplement Tolerase G (or GlutNGo).

Ten years ago, the Dutch company DSM approached immunologist Frits Koning of Leiden University Medical Center about testing AN-PEP, the *Aspergillus* enzyme. Over the following eight years, Koning and his colleagues (who have no financial stake in the product) demonstrated that it digested the inflammatory peptide in test tubes, artificial stomachs, and healthy human subjects (*Aliment Pharmacol Ther*, 42:273-85, 2015). However, it's not yet a wholly effective treatment for celiac disease, partly because its activity depends heavily on a meal's contents. Acidic carbonated beverages boost its activity—but in DSM's native Netherlands, people frequently wash down food with milk, which dampens the enzyme's activity, Koning says. And because AN-PEP isn't gluten-specific, it targets other proteins in the meal, too, reducing its gluten-chopping efficacy. The enzyme is ideal for breaking down trace amounts of contaminating gluten—but not for tackling an entire slice of bread. "It's very hard to guarantee that such an enzyme will perform under all conditions," Koning says.

Whatever we thought we knew about something we discovered in the pitcher plant, it's never quite what it seems.

—David Schriemer, University of Calgary

But Schriemer says the pitcher plant enzymes could get around this problem. His team demonstrated that very low concentrations of nepenthesin and neprosin—as little as 1/12,000 of total protein—were effective at digesting gluten in vitro. Assuming an average protein intake of 50 grams per day, a 5-milligram daily dose of these enzymes would be enough to process any gluten in the diet, Schriemer and his colleagues suggest. Koning is intrigued by that claim, but without a head-to-head comparison with AN-PEP, it's hard to tell whether the pitcher plant–derived proteases are truly more effective, he says.

Schriemer is encouraged enough by early results that he's started a company to commercialize the enzymes. He's been able to make recombinant nepro-sin, and says both potential investors and firms that would produce the recombinant pitcher plant enzymes at scale have expressed interest.

Meanwhile, Schriemer's initial study "is the first in a long series of articles that will describe other applications" of pitcher plant enzymes, says Sissi Miguel of Plant Advanced Technologies, a French biotech searching a variety of plants for new cosmetic, pharmaceutical, and agricultural applications. Earlier this year, she coauthored a study that identified 29 proteins—20 of which had never been described—in the secretions of five species of pitcher plants, not including *N. ventrata* (*Ann Bot*, 117:479-95, 2016). She suggests that examining a large panel of other carnivorous plants, including some of the other roughly 150 *Nepenthes* species such as *N. ampullaria*, which feasts on plant detritus rather than insects, might reveal additional valuable chemicals, such as antimicrobial peptides that help keep the pitchers' fluid free from contamination.

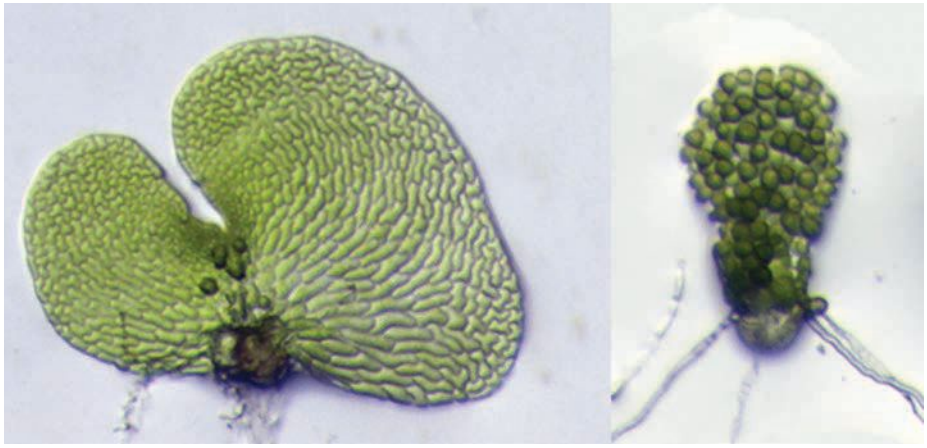
Schriemer agrees that pitcher plants have much more to teach us. "Whatever we thought we knew about something we discovered in the pitcher plant, it's never quite what it seems," he says.

—Jenny Rood

Finicky Ferns

In the mid-1990s, Jody Banks of Purdue University was mutating the genomes of ferns using dimethyl sulfate (DMS) and screening for changes in how the plants determined their sex. Ferns can grow as either males or females (the latter are really hermaphroditic, and in the absence of males can make a few sperm in order to self-fertilize). Normally, *Ceratopteris richardii* ferns grown alone develop into females, but plants grown near genetically identical spores develop into males.

"The female secretes a pheromone telling others to be male," Banks explains. "If the female [pheromone] goes away, the male will switch back to female. It's really cool." This allows plants to outcross when they are near other developing ferns that respond to the pheromone—thought to be a type of gibberellin—but still be able to reproduce by selfing when they are by themselves. "This is a really smart way of making sure, when the population is dense enough, that the female has males surrounding her." There's a catch, however: another hormone, called abscisic acid (ABA), can block the effects of the female pheromone, making ferns develop into females—albeit tiny ones—even when others are growing nearby.



WHAT'S HAPLOIDING, HOT STUFF?: The hermaphroditic (left) and male (right) forms of the haploid gametophytes of *Ceratopteris* ferns

JODY BANKS

Among her mutant ferns, Banks found that some plants didn't follow these normal sex-determining rules. Grown in the presence of the females' pheromone and ABA, some mutants still became normally sized males. Taking away the female-emitted hormone, she could induce the plants to switch to hermaphrodites, which she then selfed to produce offspring that were homozygous for an ABA-insensitive mutation. Knowing that in flowering plants (angiosperms) ABA signaling is critical for proper water conservation, she suspected that these homozygous mutants would not fare well. But to her surprise, the ferns grew just fine. Their leaves did not wilt nor did the plants dry out—phenotypes typical of ABA-insensitive angiosperms.

"We had like 30 independent mutants, and none of them gave us any phenotypes that we expected," Banks recalls. "I was running around the biology department [asking], 'Does anyone know what ABA does in ferns?'" But no one could answer her question.

It would be another 15 years before she got her first clue. In 2011, she read a newly published paper from University of Tasmania researchers who reported that, in contrast to flowering plants, ferns do not use ABA signaling to shut tiny pores on their leaves called stomata when water-stressed (*Science*, 331:582-85). Rather, the stomatal response to ABA appears to have evolved in angiosperm ancestors to help them survive dry environments, the authors concluded. "That was my eureka moment," Banks says. She wrote to coauthor Scott McAdam right away to tell him about her mutants—which she reasoned could provide genetic evidence that McAdam and his advisor Tim Brodribb were right about ABA signaling in ferns.

Upon receiving some of Banks's mutants, McAdam began experimenting with the plants to confirm that they indeed had normal responses to humidity despite being insensitive to ABA. "The ferns had very predictable responses, exactly what we'd expect [of wild-type plants]," he says. Meanwhile, Banks sequenced the ferns' transcriptomes to identify the genes underlying the ABA insensitivity, and identified alterations

to a homolog of *open stomata 1 (OST1)*, a well-known ABA signaling gene in flowering plants. The researchers then used RNA interference to block expression of the gene, which they named *GAIA1*, and saw severely altered sex determination, just as Banks had observed in her original mutants (*PNAS*, 113:12862-67, 2016).

"I was surprised and impressed," says Luca Comai, a plant geneticist at the University of California, Davis, who was not involved in the research. "I did not know that ABA had such an evolutionary history. . . . One wonders, if one looks at all the signaling pathways we have in plants, how many have been rewired?"

Ray Ming, a plant biologist at the University of Illinois at Urbana-Champaign who also did not participate in the study, agrees. The study suggests that "there is remarkable plasticity of genes and gene networks regulating diverse developmental programs during the course of evolution, reflecting adaptation of plants to the changing climate across the geological time scale," he told *The Scientist* in an email.

Although the researchers can't say why ABA signaling was coopted to control stomatal responses to water stress in flowering plants, McAdam suspects there is a link to the pathway's role in ferns' response to low water availability. "Our current thought is that ABA is produced at very high levels when plants are drought stressed; even if [ferns] are not responsive to it in their leaves, they still produce it," he says. This ABA will end up in the fern's spores, causing them to develop into hermaphrodite gametophytes, which can self to form a new adult plant. "It's almost like an emergency mechanism to at least continue [reproducing] if the plant is experiencing extreme conditions."

One wonders, if one looks at all the signaling pathways we have in plants, how many have been rewired?

—Luca Comai, University of California, Davis

Another role ABA signaling plays in flowering plants is ensuring seed dormancy, which may have evolved from ABA control over spore dormancy in ferns, McAdam adds. ABA-insensitive mutants in seed plants have compromised seed dormancy as well. "This hormone has played a major role in drought sensing for over 400 million years . . . so it was a very simple evolutionary step to take that signaling pathway and coopt it."

—Jef Akst

An Ethical Code for Conferences

This fundamental form of scientific communication is threatened by modern recording technology and researchers who refuse to adhere to an age-old ethical code.

BY WOLF B. FROMMER

I recently attended several conferences and saw rampant recording of lectures and posters. Because my talk contained a lot of unpublished work, I asked the audience to refrain from taking pictures. But just five minutes into my talk, I saw multiple cell phones up recording my lecture. I repeated my request, and the people put their phones down. Ten minutes later, however, the very same people did it again. I asked once more, yet one person continued to record my slides.

Scientific conferences are meant to inform the attending audience about the newest results. No one wants to hear only published work; we attend meetings to get the absolute latest information that is coming out of labs. To be able to do that, an honor code exists that conference-goers cannot make use of data presented to advance their own work. While some have broken this code in the past, by and large it has been respected by the scientific community—until now. These days, with the use of new information technologies and social networks, this ethical principle is in serious jeopardy.

We must enforce this old honor code to encourage the sharing of unpublished data and ensure that science can progress effectively.

Modern digital camera technology produces such high-quality images that some people decide to take pictures of slides and posters, or even film entire lectures. This is much easier than scribbling notes, and the resulting files are simpler to show to friends or colleagues. Moreover, some tweeters have started to post pictures of speakers together with their unpublished data. This means that these data are published in the widest possible sense before they are published by the authors themselves. All of these activities can have a detrimental influence on scientific progress, as researchers will begin to refrain from showing their newest data at meetings. Eventually, scientists may choose not to go to conferences at all because they can expect to see talks only on research that is already published or in press.

I thus firmly believe that photographing posters, recording parts of talks, and posting other people's data should be officially banned, and that people who break these ethical standards should be expelled. We must enforce this old honor code to encourage the sharing of unpublished data and ensure that science can progress effectively. Ideally, the scientific community would adopt a generally acceptable and enforceable ethical code for all conferences, make it part of every program, and announce these regulations at the beginning of every meeting, following the examples of Cold Spring Harbor Laboratory symposia and Gordon Research Conferences.

Above all, this code should include the obvious rule that the knowledge you gain from unpublished work ought not be used to compete with the authors. Without adherence to such rules, scientific conferences—and the research they inspire—are at risk of being lost forever. ■

Wolf B. Frommer is a staff member of the department of plant biology at the Carnegie Institution for Science in Stanford, California. A version of this story was published at the-scientist.com on December 2, 2016.

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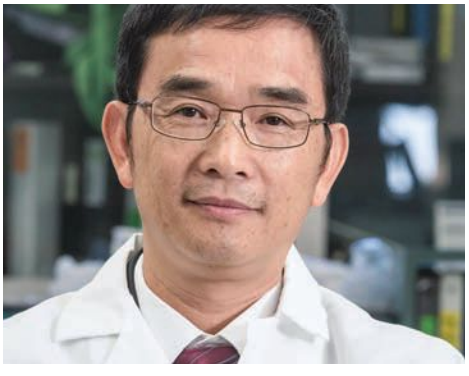

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Deep Pocket Exploration

A modification to traditional docking software enables the examination of a ligand's passage into its receptor.

BY RUTH WILLIAMS

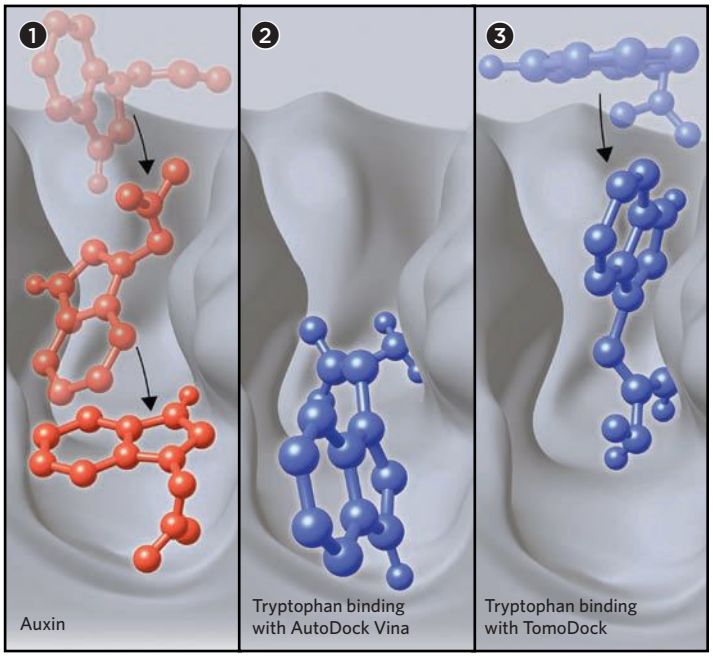
Auxins are a family of small-molecule hormones that control growth and development processes in plants. They are also components of widely used herbicides. In a drive to extend the agricultural and horticultural applications of these hormones, scientists are attempting to design new synthetic auxins. But to do so, they must understand the nitty-gritty of how an auxin molecule binds to it receptor, says Richard Napier of the University of Warwick in the U.K.

Napier's team uses docking software to simulate auxin binding. But there's a problem: the software also allows molecules to dock that, Napier says, are known not to bind in reality—such as auxin's close relative tryptophan. "Getting false positives out of docking [analyses] is absolutely part of the deal," he says. "Docking is not a perfect science."

To reduce such permissiveness, Napier and his colleagues have written additional computational code for a popular docking program, AutoDock Vina. For receptors with deep binding-site pockets (like that of the auxin receptor), the new code mimics the molecule's natural passage by searching for the docking site in a sequence of 0.1-nanometer steps.

Drawing an analogy with a cave, Ning Zheng of the University of Washington says, "The conventional [docking] method just looks at whether a child or adult can be accommodated by the cave interior, but if that cave is separated from the outside by, let's say, a narrow cleft, then . . . maybe it turns out the adult is too big to pass [through]."

Restriction of a molecule's access may not be due to size, but to interactions with residues on the "cave" walls as it enters—which is the case for tryptophan, Napier's team has now discovered. Without simulating a molecule's passage, as per the new method, called TomoDock, such interactions can be missed, Napier says. (*Open Biology*, 6:160139, 2016) ■



MOLECULAR SPELUNKING: AutoDock Vina evaluates a receptor's entire binding pocket at once to find a docking site (lowest-energy binding) for a ligand of interest. The deepest part of the pocket (the actual binding site for auxin shown at the bottom of 1) naturally has a low energy requirement, and this is where, in the case of tryptophan 2, the software suggests a docking site. But by forcing the software to move in incremental steps—starting at the mouth of the pocket and moving inward—TomoDock finds an interaction of tryptophan with residues farther up the pocket 3 that prevents deeper entry. In the case of auxin, however, TomoDock finds the same binding site as that found using AutoDock Vina (step-wise progress of TomoDock shown in 1).

AT A GLANCE

DOCKING PROGRAM	BINDING SITE EVALUATION	APPLICABILITY	ACCESS
AutoDock Vina	A 3-D cuboidal area encompassing the entire binding pocket of a receptor	All ligand-receptor interactions	Free to download from vina.scripps.edu
TomoDock (with AutoDock Vina)	A 3-D cuboidal area big enough to encompass the entire binding pocket, but which is initially positioned at the mouth of the binding pocket and then moved stepwise into the pocket in small increments	Specifically for receptors with deep binding pockets or transport proteins in which molecules of interest pass though a channel	Free to download from www2.warwick.ac.uk/fac/cross_fac/complexity/people/staff/delgenio/tomocode

Extragenomic variation has played key roles in the evolution of plants.

BY JEF AKST

Plants'

GENERATIONS OF EPIGENETICS RESEARCH: Famed transposon discoverer Barbara McClintock and a young Rob Martienssen inspect a maize plant at the Cold Spring Harbor Laboratory greenhouse in 1989.

Epigenetic Secrets

As a postdoc at the University of California, Berkeley, in the mid-1980s, Rob Martienssen discovered a mutation in maize plants that caused their leaves to have a very pale green color. Cells carrying this mutation don't develop chloroplasts, and as a result, the plants couldn't perform photosynthesis. Seedlings would survive for only a week or two on stored nutrients before perishing. "This mutant had a very strong lethal phenotype," Martienssen says. "But from time to time it reverted," he adds. "And it reverted in a very striking way."

Some of his mutant corn plants began to grow striped leaves, with bands of healthy dark-green tissue interspersed with the pale-green cells, and those plants survived a little longer. "From one leaf to the next, you would see more dark-green stripes," Martienssen describes. "The plant eventually grew out of the defect altogether and flowered."

Martienssen and his colleagues knew that the mutant came from a genetic background in which *Mu* transposons—genetic elements that moved around in the genome, sometimes turning traits on and off as they did so—were known to be active. Researchers also knew that these transposons were active when they carried little to no methylation, but silent when marked. Martienssen identified the gene and transposon responsible for the pale-green phenotype and then used Southern blotting to show that the transposon was relatively unmethylated in the light-green parts of the leaf, compared with the highly methylated dark-green swatches.[1] "DNA methylation was suppressing the [mutant] phenotype," he says.

The striped-leaf pattern reminded Martienssen of the multicolored corn kernels in which famed geneticist Barbara McClintock had first identified transposons. "She had noted in the 1950s that transposable elements that landed near genes could change the expression of those genes from one generation to the next in a reversible way," Martienssen says. "She called the phenomenon cycling, which really sums it up." DNA methylation was a popular candidate for the control of transposon activity, and Martienssen's work clearly supported the idea.

In 1989, when Martienssen arrived at Cold Spring Harbor Laboratory to start his own lab, McClintock was still hard at work there. She would take Martienssen out into the field and talk about the control of transposable elements in plant genomes. Both researchers were very interested in how transposons cycled on and off over subsequent generations. "She really liked the DNA methylation explanation," Martienssen recalls.

As the evidence mounted that methylation controlled transposon activity, plant biologists were quickly becoming convinced. But the findings were still correlational. At that time, researchers didn't yet have the means to manipulate methylation in a controlled way to experimentally demonstrate that the epigenetic phenomenon

CSHL ARCHIVES

was suppressing transposon activity. So Martienssen began mutating *Arabidopsis thaliana* plants, searching for one with a defect in its epigenetic machinery.

In 1993, one year after McClintock's death, Martienssen and his colleagues found what they were looking for: the first mutant eukaryote that was defective in DNA methylation. The plants, which carried a mutation in what the researchers named the *decrease in DNA methylation 1* (*DDM1*) gene, had significantly reduced methylation at repeated sequences in the genome, sites associated with transposable elements.[2] A few years later, he and others demonstrated that transposon activation was increased in *DDM1* mutant plants. "Transposons go completely crazy in terms of expression and transposon position," explains Martienssen, who says that it was gratifying to finally have experimental evidence to support McClintock's ideas.

In the two and a half decades since Martienssen's brief overlap with the Nobel-winning botanist at Cold Spring Harbor, the study of plant epigenetics has exploded. In plants, DNA methylation includes not just the CG methylation that's common in mammals and some other animals, but also CHG (where H is any base except G) and CHH methylation. And the mechanisms for maintaining these different methyl marks are diverse and intermingling. It seems that plants—whose genomes are typically large, often containing more than two sets of chromosomes, and riddled with transposons (see "Genomes Gone Wild," *The Scientist*, January 2014)—are extra careful to keep much of their DNA quiet.

"It's very important to keep these transposable elements as silenced and methylated as possible," Martienssen says. "Because of their crazy genomes, [plants] have every possible mechanism to silence and keep things constant."

An emerging understanding of how plants methylate their genomes could also inform our concept of animal epigenetics. For example, it was in *Arabidopsis* that Vincent Colot of the Institut de Biologie de l'École Normale Supérieure (IBENS) of France's National Center for Scientific Research and colleagues identified a methylation mechanism that involves RNA interference machinery better known for its ability to suppress gene expression by degrading transcripts.[3] RNA-directed DNA methylation has since been found to regulate DNA methylation at some specific loci in the mouse genome, as well as to play a role in histone methylation in *Drosophila* and *C. elegans* (and likely in mice as well, Martienssen says).

But there is a key difference in how methylation is inherited between the two kingdoms. While animal cells undergo two rounds of reprogramming during reproduction to wipe clear most of the methyl marks that decorate their DNA and histones, plants leave their epigenomes largely intact from one generation to the next. In plants, this results in epialleles—stably inherited alleles encoded by methylation, rather than by gene sequence—that control subtle phenotypes, such as timing of flowering or fruit ripening.

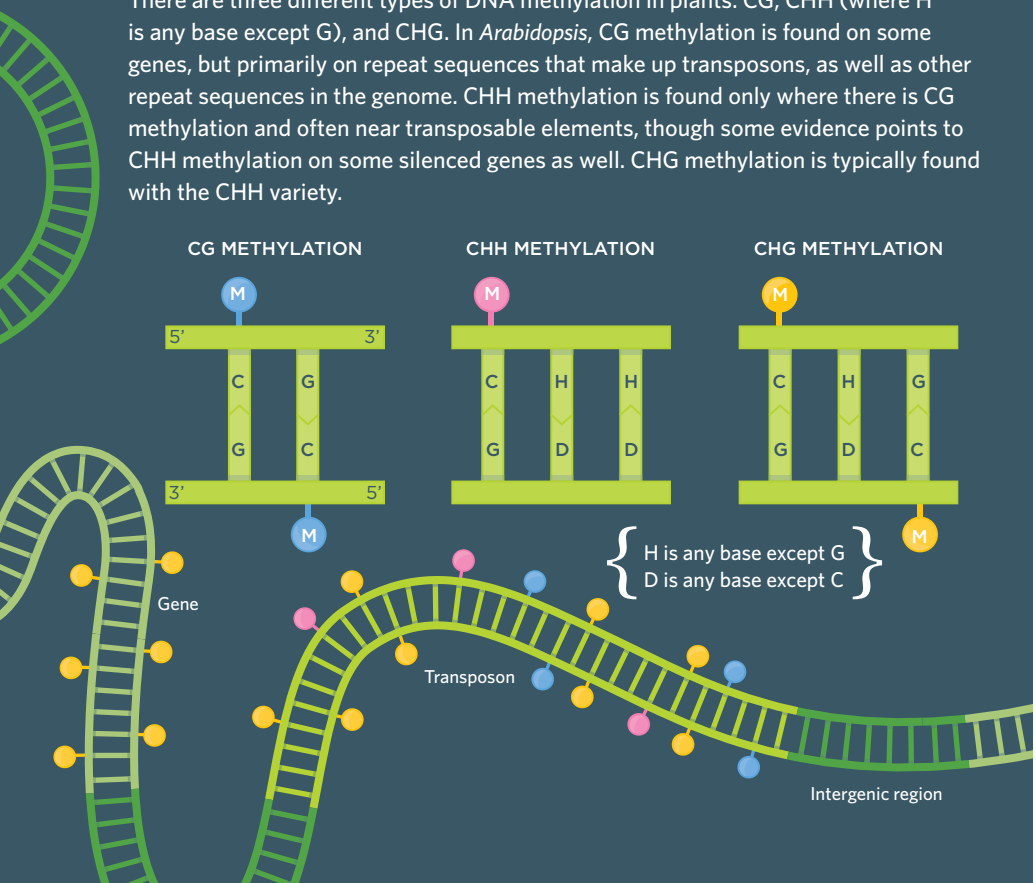
"Most of the differences [between individuals] that we see are caused by genetic variation," says Colot. "But it's not all caused by genetic variation. What would be caused by this epigenetic variation could be as important."

Whether these epialleles can be adaptively altered by the environment remains a matter of debate, and most researchers say there is no convincing evidence for any form of such "Lamarckian" evolution. But there are hints that acquired changes in methylation patterns can impact future generations, and at the very least, errors in transcribing the methylome provide an additional source of new variation, akin to genetic mutation, as mistakes are stably inherited.

"There's a lot more change possible epigenetically, and in some cases only possible epigenetically," says Martienssen. "And selection just acts on that in the same way that selection would act on a genetic change."

PLANT METHYLATION BASICS

There are three different types of DNA methylation in plants: CG, CHH (where H is any base except G), and CHG. In *Arabidopsis*, CG methylation is found on some genes, but primarily on repeat sequences that make up transposons, as well as other repeat sequences in the genome. CHH methylation is found only where there is CG methylation and often near transposable elements, though some evidence points to CHH methylation on some silenced genes as well. CHG methylation is typically found with the CHH variety.

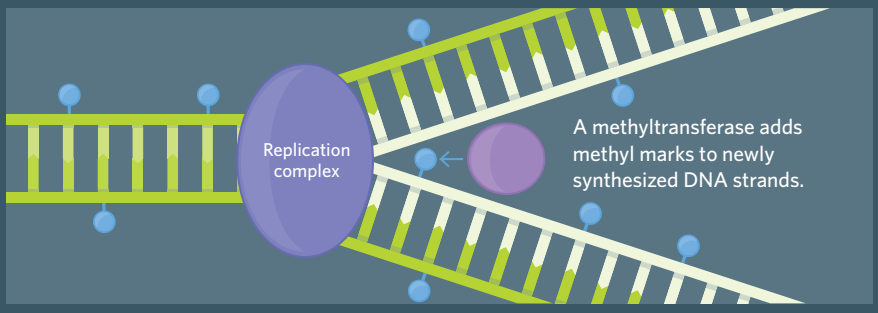


METHYLATION MAINTENANCE

Every time a cell divides, it must replicate its genome and its epigenome. Plants have diverse pathways overseeing the faithful passage of the methylome to daughter cells.

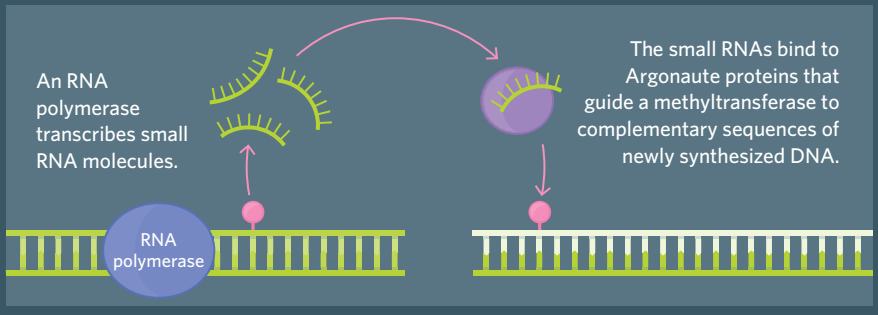
CG METHYLATION

Copying CG methylation patterns to the two daughter strands is relatively straightforward. That's because this type of methylation is symmetrical: the complementary strand is also CG (reading from 5′ to 3′), and that cytosine is also methylated. So when the parent DNA strand splits, the two daughter strands that form will have methylation on the parent-strand side, and that methylation can guide the addition of a methyl group to the newly replicated strands' CG cytosine as DNA is being copied.



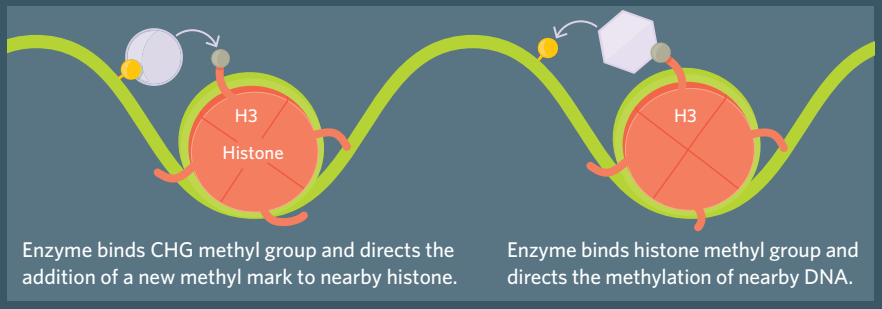
CHH METHYLATION

CHH methylation is inherently asymmetrical, because the H is any base *except* guanine. CHH methylation is passed on to both daughter genomes using a process called RNA-directed DNA methylation, which involves small RNAs that guide RNA interference machinery to methylate complementary regions of DNA.



CHG METHYLATION

Although CHG methylation is symmetrical and thus could in principle use the same methylation maintenance pathway as CG methylation, it also relies on RNA-directed DNA methylation (not pictured below). In addition, this type of methylation is paired with methylation of lysine 9 on histone H3 (H3K9). The histomethyltransferase that methylates H3K9 regions binds to methylated CHG. Conversely, the CHG-methylating enzyme binds to H3K9, then methylates nearby CHG sites, forming a positive feedback loop between the two types of methylation.



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Deciphering the plant epigenetic code

If there's one technology that has revolutionized the study of epigenetics, it's bisulfite sequencing, which converts unmethylated cytosines to uracils before DNA sequencing. In plants, it allows researchers to interrogate not just general methylation patterns—which could be deciphered by Southern blotting and microarrays—but the three specific types of methylation: CG, CHG, and CHH.

In the mid-2000s, several groups published complete methylomes of *Arabidopsis*. Martienssen and colleagues reported maize methylation patterns in 2013,[4] and a handful of other groups followed with more maize methylomes. These surveys have revealed notable variation in methylation patterns among individuals, but unlike animals, which use epigenetics to regulate the patterns of gene expression in different cell types during development and into adulthood, plants have nearly identical patterns of methylation throughout their tissues. "For the most part, if you look at different cell types in plants, the methylation profiles are remarkably similar," says Nathan Springer, a plant geneticist at the University of Minnesota.

This conservation of DNA methylation patterns across the plant's tissues supports the idea that methylation is "part of a strategy to control transposable elements," which are rampant in plants' massive genomes, says Mary Gehring, a plant epigeneticist at the Whitehead Institute. As much as 90 percent of the maize genome, for example, is made up of transposable elements. And indeed, all three types of methylation are found on and around transposon repeat regions. "Transposons very clearly are regulated by methylation, there is no doubt," Martienssen says.

Methylation on genes is much less common, and when it's there, it's most commonly of the CG variety. But epigenetic regulation of genes may begin to crop up as researchers begin to look at other plant species, Martienssen says—in particular, those that, unlike *Arabidopsis* and maize, suffer lethal phenotypes when *DDM1* is mutated. Last year, Bob Schmitz of the University of

Georgia and colleagues greatly expanded the list of plant species with their complete methylomes mapped, surveying 34 species of flowering plants, and documented extensive epigenetic variation. Plants of the brassica family, including *Arabidopsis,* tend to have reduced CG methylation on genes, or none at all; grasses have more gene methylation, but of the CHH variety.[5] "It became apparent, with just a few other plant species, that these rules that we're learning from model systems don't always apply to other species," Schmitz says.

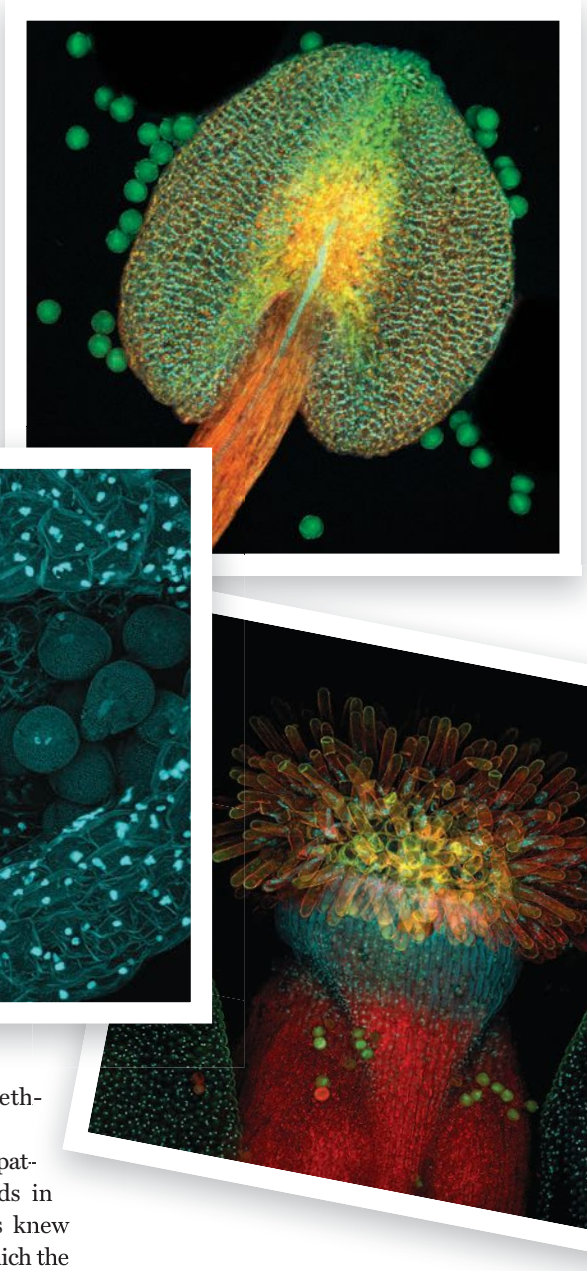
Whole-methylome analysis is a young technique, but more data—and hopefully more answers—are on the horizon, says Martienssen. Both Pacific Biosciences and Oxford Nanopore are "feverishly optimizing" new DNA sequencing technologies that can detect methylation without having to first swap out the methylated cytosines for uracils, he explains. "I would predict that within a year we'll be able to sequence methylated bases just as easily as nonmethylated bases."

Methylation inheritance

After doing a sabbatical in Martienssen's lab at Cold Spring Harbor, Colot returned to France in 2001 to start his own lab at the Plant Genomics Research Unit of the National Institute for Agricultural Research (INRA) with a very specific goal in mind: create epigenetic recombinant inbred lines (epiRILs) of *Arabidopsis*—plants with identical genomes, but different epigenomes. They could be a powerful tool for understanding the function of methylation in different parts of the genome, he reasoned, and allow researchers to track how plant methylomes are passed from one generation to the next.

Colot started with two parent plants with the same genetic background, but one parent was homozygous for a wild-type *DDM1* gene, while the other was homozygous for a mutant version of the gene and thus had dramatically reduced DNA methylation. As had been suggested by previous work, the methylomes of the parents were inherited by the progeny, who each had one set of chromosomes from their wild-type parent with normal methylation patterns and a hypomethylated set from the mutant parent. Colot took one such epigenetically heterozygous female plant, bred it back to a wild-type male, and selected progeny that carried two copies of the wild-type *DDM1* gene. He then selfed those plants, generating 500 separate lines with intact methylation machinery but varied methylation patterns. Even after eight or nine generations, he found that on one-third of the differentially methylated sequences, "the methylation states present in the two parents were segregated according to Mendel's laws," Colot said. "They are truly epigenetic"—extra-genomic variation that is faithfully passed on. The other two-thirds of the sequences he studied had regained wild-type methylation over several generations.[6]

This persistence of methylation patterns across the generations stands in stark contrast to what researchers knew about mammalian epigenetics, in which the CG marks are wiped out in two rounds of reprogramming in the gamete and the early embryo. For plants, this means that once methylation changes arise, they are likely to stick around. These epialleles are now known to control a variety of phenotypes, such as disease resistance, biomass, and flowering time. "There are more heritable epialleles that have been identified in plants than in mammals; presumably that's because of this lack of reprogramming," says Gehring. "That continues to be really exciting."



DOUBLE FERTILIZATION: In the center of an *Arabidopsis* flower (bottom), anthers (zoom shown in top image) harbor the sperm (middle) that will swim through a pollen tube to fertilize the egg and the female central cell.
(See illustration on page 35.)

But can the environment influence the epigenome in an adaptive way? For example, if plants are exposed to a particular pathogen, could a resistance gene shed its methyl mark, improve survival, and spread as a result of Lamarckian-style evolution? Most say the evidence for it is weak. "There's this idea that plants may be able to respond to their environment; an environmental change will affect the methylome, and then that could be inherited," says Gehring. "There's really not much good data that that's true."

Others agree such evolutionary dynamics are unlikely. "There's very little evidence that the environment perturbs DNA methylation in plant genomes," Schmitz says. "A lot of those studies are plagued by not going out far enough. You often see [epigenetic changes in] one generation, but the second or third generation snaps back to the original generation."

> Because of their crazy genomes, plants have every possible mechanism to silence and keep things constant.
>
> —Rob Martienssen
> Cold Spring Harbor Laboratory

One 2016 paper aimed to address that experimental shortcoming. University of Warwick plant biologist Jose Gutierrez-Marcos and his colleagues propagated three lines of *Arabidopsis,* grown in normal or two grades of salty soils for five generations. From each generation, they took seeds and raised offspring and grand-offspring under normal conditions, then compared their methylomes to see whether any epigenetic changes that arose in response to salt stress persisted.[7] "We used a high level of replication in our analysis. We looked at populations of individuals rather than looking at individual plants," Gutierrez-Marcos says. "We were looking for a strong effect, not just effects that could just be spontaneously arising in the experiment."

The researchers did see some changes in DNA methylation that were stably inherited for at least two generations. Although methylation changes began after just one generation in the salty soil, the researchers didn't notice any phenotypic changes until the second generation, when the salt-stressed lines began to show higher germination and survival rates and more-robust foliage.

"Basically they found that not much happened," says Gehring, still a self-proclaimed skeptic of transgenerational effects of this nature. "But they did have a couple of examples—one or two things that seemed like they might be interesting, in terms of [being] induced by stress and inherited."

Over the five generations of the experiment, the subtle adaptive responses did not magnify, suggesting there's a limit to epigenetic adaptation. And in the two generations spun off from each experimental cohort but raised in normal soils, some of the loci that had lost or gained methylation began to revert to the founding plant's methylome. "But there are some particular loci that are very stable, even for four generations," Gutierrez-Marcos noted.

If environment-induced methylome adaptation is happening in nature, it's certainly not common, says Martienssen. "Personally I think it will be found, but obviously it will be rare. It's not going to be a general principle." That said, the environment doesn't have to cause the changes for epigenetic mechanisms to be a driver of evolutionary change, he adds. "We know that epigenetic variation exists. You don't need to have the environment directing the change so long as the changes are there."

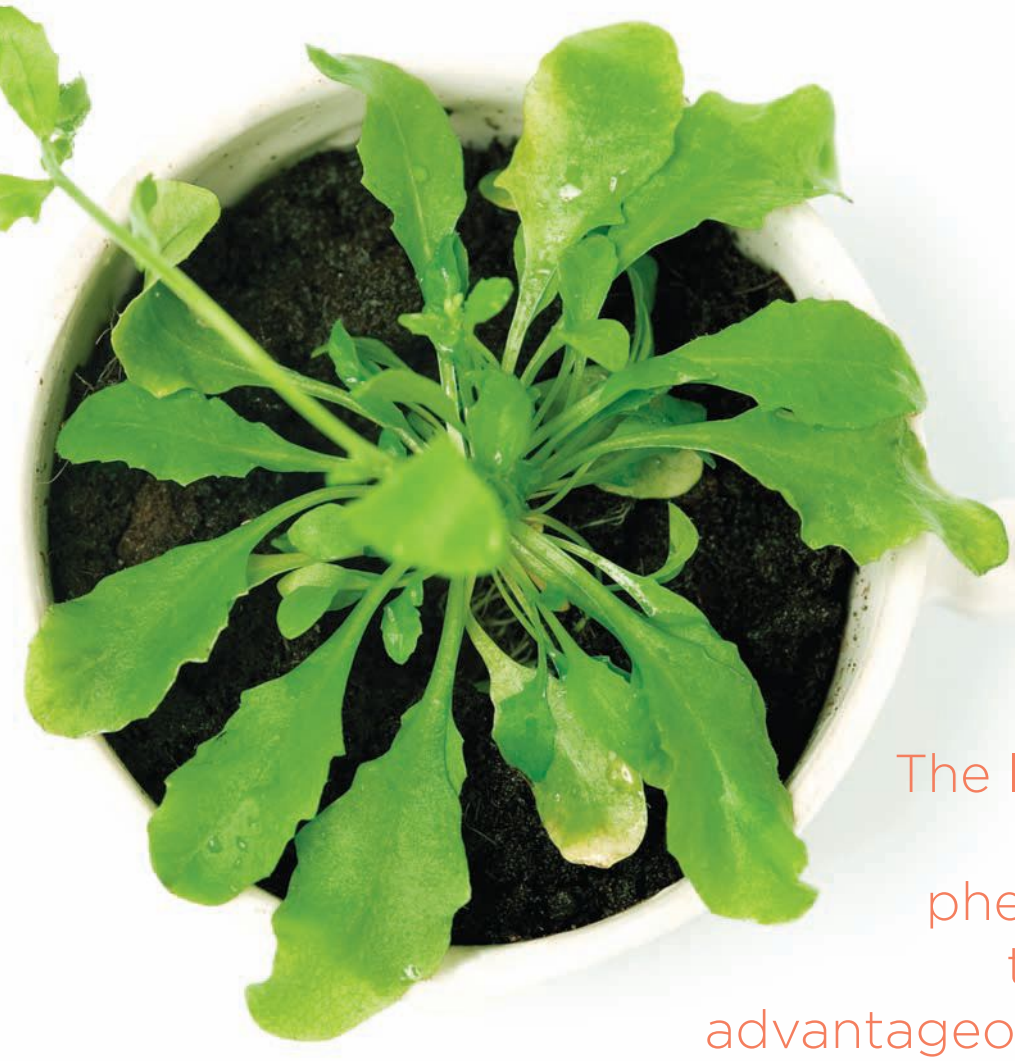
Schmitz says that some of this variation can be chalked up to user error. "Plants work really hard to maintain methylation, and they're pretty good at it, but there are mistakes that occur," he says. And because plants pass down their methylomes in a relatively stable way, "these mistakes can lead to new heritable traits."

Reprogramming exceptions

It's not entirely true, however, that plants do not undergo epigenetic reprogramming. During reproduction, *Arabidopsis* sperm don't erase CG methylation as animals do, but they do wipe out about 90 percent of CHH methylation, which is found along with CG methylation at transposons and sometimes genes as well. In 2009, Martienssen and his colleagues published their analysis of *Arabidopsis* sperm methylomes, comparing the three haploid cells in pollen: two sperm cells and a vegetative cell that helps support the growth of the pollen tube that delivers the sperm to the female gametophyte housing the egg.[8] Although the vegetative cell nucleus loses some CG methylation, that nucleus does not contribute genetic material to the embryo. "By way of contrast—and amazingly—the sperm cells do the opposite: they lose CHH methylation," Martienssen says. (See illustration on page 35.)

After fertilization, RNA-guided methylation pathways restore the embryo's levels of CHH methylation. "It's very interesting that it's so dramatic and that it has to come back again guided by small RNAs," Martienssen says. "It means that the small RNAs found from both parents contribute to the patterns of DNA methylation in the embryo." Sure enough, looking at maize over subsequent generations, he and his colleagues found that the offspring of parents with differing methylomes would acquire methylation at a locus if either parent had it.[4] "And we found literally thousands of places in the genome that underwent a switch that was determined by the small RNAs from either mom or dad," Martienssen says.

The reprogramming of CG methylation in vegetative pollen nuclei is also interesting, as it results in an uptick in transposon activity that might seem detrimental to the plant's health. But Martienssen and his colleagues speculate that there may be benefits to this strategy. For example, release of transposon silencing in



the vegetative nucleus of the sperm could prompt the transcription of small RNAs, which researchers know can travel to the reproductive nuclei of the sperm and serve as guides to reinforce methylation of those transposon sequences. "If you take dead-end cell types that can never go on, why not let them express transposons, [which] get processed into small RNAs and reinforce the silencing of the transposons in the other cells around them?" Springer says of the hypothesis. "You can think of this as almost an immunization."

Although no one has investigated epigenetic changes in plant eggs yet, there is evidence of CG reprogramming in the central cell.[9] Certain nonreproductive plant tissues also undergo a form of epigenetic reprogramming. In 2015, for example, an international team of researchers found that DNA demethylation governs tomato ripening.[10] And last year, plant biologist Pascal Gamas of France's INRA and colleagues found that developing root nodules, which house symbiotic bacteria in legumes, do something similar.[11] In both cases, a specific demethylase that's only expressed in these structures removed the DNA methylation.

"Plants are really good at maintaining DNA methylation, so when you find something like this, it's really neat," says Schmitz. "It's exciting because it's so rare."

Improving agriculture

Many crop species are clonally propagated. In theory, this allows agriculturalists to select those individuals with the most desirable qualities—large, sweet fruits or high oil production—and grow a whole field of them. But while clonal propagation ensures the preservation of desirable genetic sequences, it doesn't take into account the epigenome. The replacement of CHH methyl marks on the DNA in sperm, which is triggered by fertilization, is not able to take place.

In the African oil palm, farmers noticed that between 10 percent and 20 percent of plants weren't producing oil. In 2015, researchers learned that the poorly producing palms suffered the activation of a transposon that had lost its methylation and disrupted a gene critical for oil production.[12] "[CHH methylation] is replaced inefficiently and sometimes in the wrong place," says Martienssen, who was one of many contributors to the study. "So you get a mess."

The loss of methylation may also unveil new phenotypes, including traits that could be advantageous to crop species. And agricultural researchers—who are often forced to cross crop plants with wild cultivars to introduce pathogen-resistance genes, for example, and then backcross the plants to regain the desired traits (see "Putting Up Resistance," *The Scientist*, June 2014)—are anxious to see what's been hiding under all that epigenetic silencing. "If you can change the methylation, you can create heritable phenotypic variation," Schmitz says. Any genes that are typically silenced in plant genomes are "an untapped source of diversity."

Schmidt's goal is to find ways to release that silencing to see if any newly expressed genes are associated with novel, potentially beneficial phenotypes. "There will be a lot of ugly-looking plants that come out of this approach," he says. "But you just need that one that will lead to that trait of interest."

The loss of methylation may unveil new phenotypes, including traits that could be advantageous to crop species.

McClintock would no doubt be impressed by the progress made in understanding DNA methylation in plants, to the point that researchers are starting to tinker with crops' epigenomes in the hopes of making hardier or more nutritious cultivars. And if she were alive today, McClintock might well be getting her hands dirty in a similar pursuit. Even at age 90, Martienssen recalls, "she was surprisingly comfortable with modern molecular biology." And, as it turns out, she was working on the first inquiries in the field. "Obviously they didn't call it epigenetics in those days," Martienssen says, "but we now know that's what it was." ■

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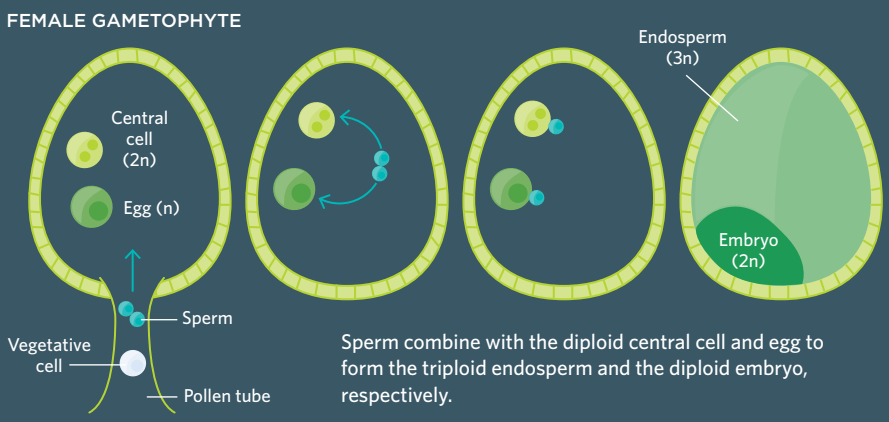
REPRODUCTIVE REPROGRAMMING

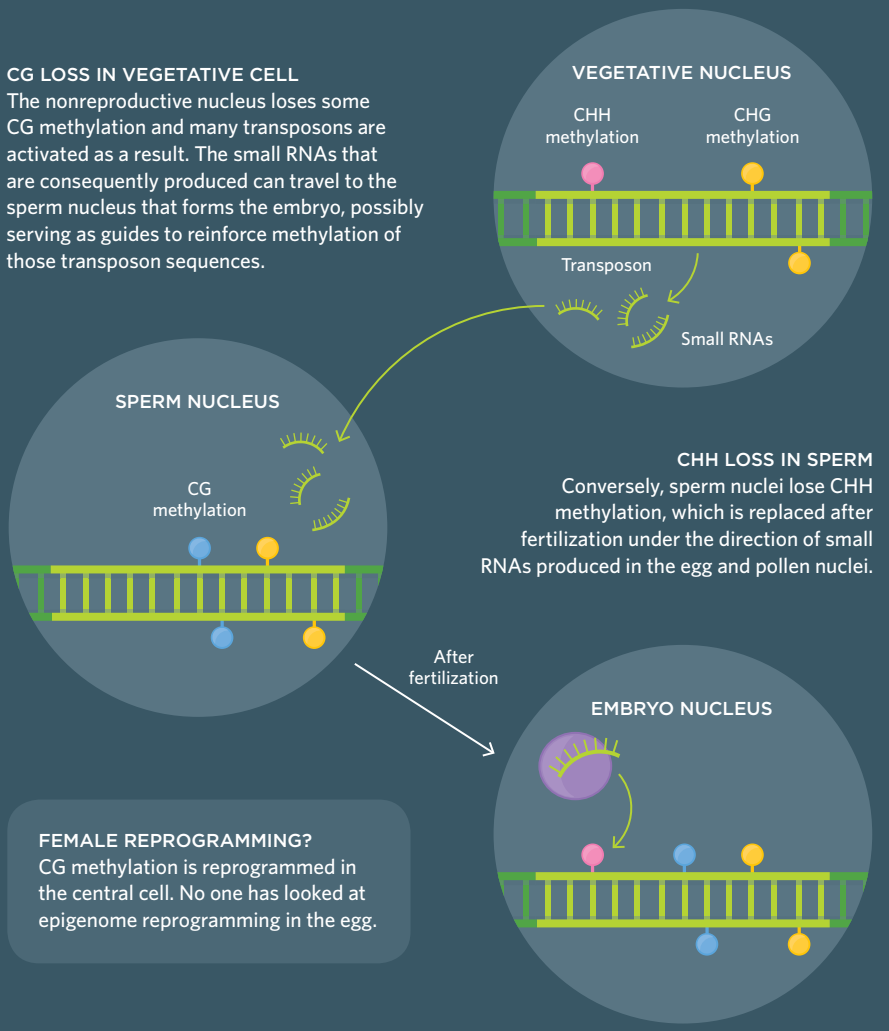
During *Arabidopsis* reproduction, certain types of DNA methylation are reprogrammed in the pollen. *Arabidopsis* pollen grains have three haploid cells: one vegetative cell that helps produce the pollen tube and two sperm. One sperm fertilizes the egg to make the embryo, while the other fuses with the female's central cell to form the supportive tissue known as endosperm.



Sperm combine with the diploid central cell and egg to form the triploid endosperm and the diploid embryo, respectively.

CG LOSS IN VEGETATIVE CELL

The nonreproductive nucleus loses some CG methylation and many transposons are activated as a result. The small RNAs that are consequently produced can travel to the sperm nucleus that forms the embryo, possibly serving as guides to reinforce methylation of those transposon sequences.



CHH LOSS IN SPERM

Conversely, sperm nuclei lose CHH methylation, which is replaced after fertilization under the direction of small RNAs produced in the egg and pollen nuclei.

FEMALE REPROGRAMMING?

CG methylation is reprogrammed in the central cell. No one has looked at epigenome reprogramming in the egg.

CROSS-KINGDOM SWAP MEET

Plants and fungi can use conserved RNA interference machinery to regulate each other's gene expression—and scientists think they can make use of this phenomenon to create a new generation of pesticides.

BY KERRY GRENS

Plants, silent as they are to our ears, are in constant conversation with their environment. As scientists have developed evermore-sensitive tools to eavesdrop on this molecular chatter, they've discovered not only dialogue among the cells of an individual plant and with the plant's immediate surroundings, but between different individuals, sometimes of different species and even different kingdoms. The alphabet of this lingua franca is A, C, G, and U.

Noncoding RNAs are well known for their ability to control gene expression in cells. And as scientists have demonstrated repeatedly, protein production can be affected not just by RNAs made in the same individual, but by RNAs from altogether different organisms. In recent years, researchers have taken advantage of the ability to traffic RNA between distantly related taxa to selectively inhibit the expression of genes in fungi important for their growth, an approach they say might lead to the development of disease-resistant crops. Scientists have also shown in the lab that this cross-kingdom RNA transfer can go both ways: fungi are also sending RNA dispatches to their plant hosts, and the covert operation could be aiding their invasion.

In this conversation between plants and fungi, the organisms rely on a well-worn mechanism of gene-expression regulation that has stood the test of evolutionary time: RNA interference (RNAi). Listening in on the RNA crosstalk between plants and their pathogens could reveal previously unknown facets of basic plant biology, and point the way toward a successful strategy to fend off crop pathogens. Yet, scientists' manipulation of cross-kingdom RNAi using plants predates their full understanding of exactly how it works or how often it happens in nature.

Plant protection

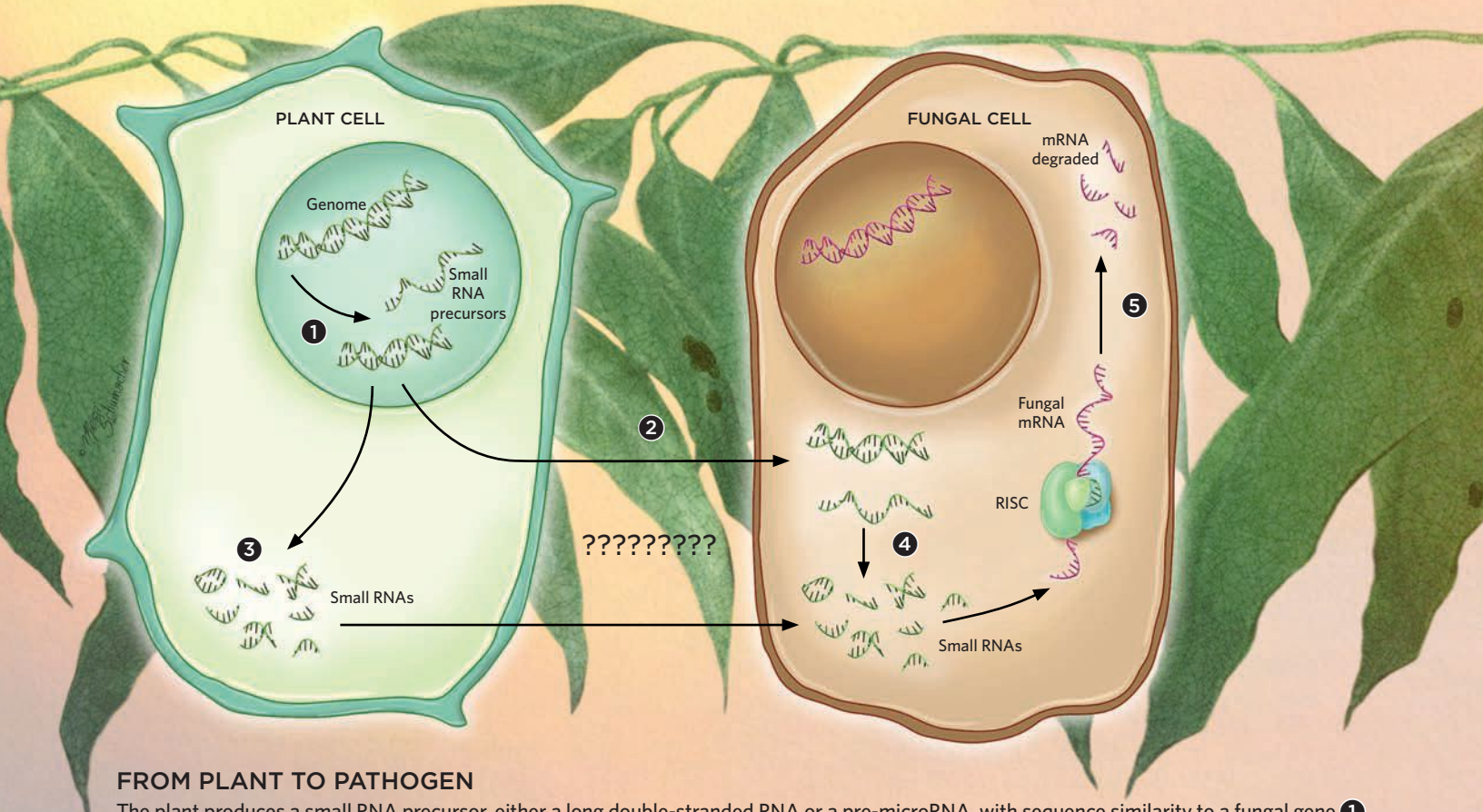
RNAi is a widely conserved mechanism used during development, in routine cellular processes, and in response to foreign invaders—especially viruses—entering a cell. The cell produces small RNAs that are then integrated into an aggregation of proteins called the RNA-induced silencing complex (RISC), which targets messenger RNA molecules (mRNAs) containing the small RNA's complementary sequence. RISC then chops up bound transcripts, thereby tamping down gene expression.

Over the past decade, scientists have demonstrated RNAi's ability to protect numerous plants against nonviral pathogenic foes. In 2007, for instance, Monsanto endowed corn with the ability to fend off western corn rootworm by providing the crop with a gene for an RNA that targeted transcripts of an essential gene in the insect. The transgenic plants suffered less damage, presumably because the insects ingested the interfering RNAs and died.[1] Around the same time, research groups showed that the approach—called host-induced gene silencing (HIGS)—could also ward off parasitic worms, and since then, laboratory experiments with transgenic plants have produced an ever-expanding list of animal pests susceptible to engineered RNAi.

In recent years, genetic engineers have successfully applied HIGS to combat pathogenic fungi. In 2010, a team based at the Leibniz Institute of Plant Genetics and Crop Plant Research in Germany showed

CROSS-KINGDOM RNAi

Evidence from laboratory studies of plants and their fungal pathogens indicates that both parties can fling RNAs back and forth into the other's cells. Plants appear to use these molecules to resist infection, while fungal microbes call upon RNA to enhance their spread. Both types of organisms achieve their desired outcomes through the same molecular process: RNA interference (RNAi), which disrupts gene expression by destroying target messenger RNAs.



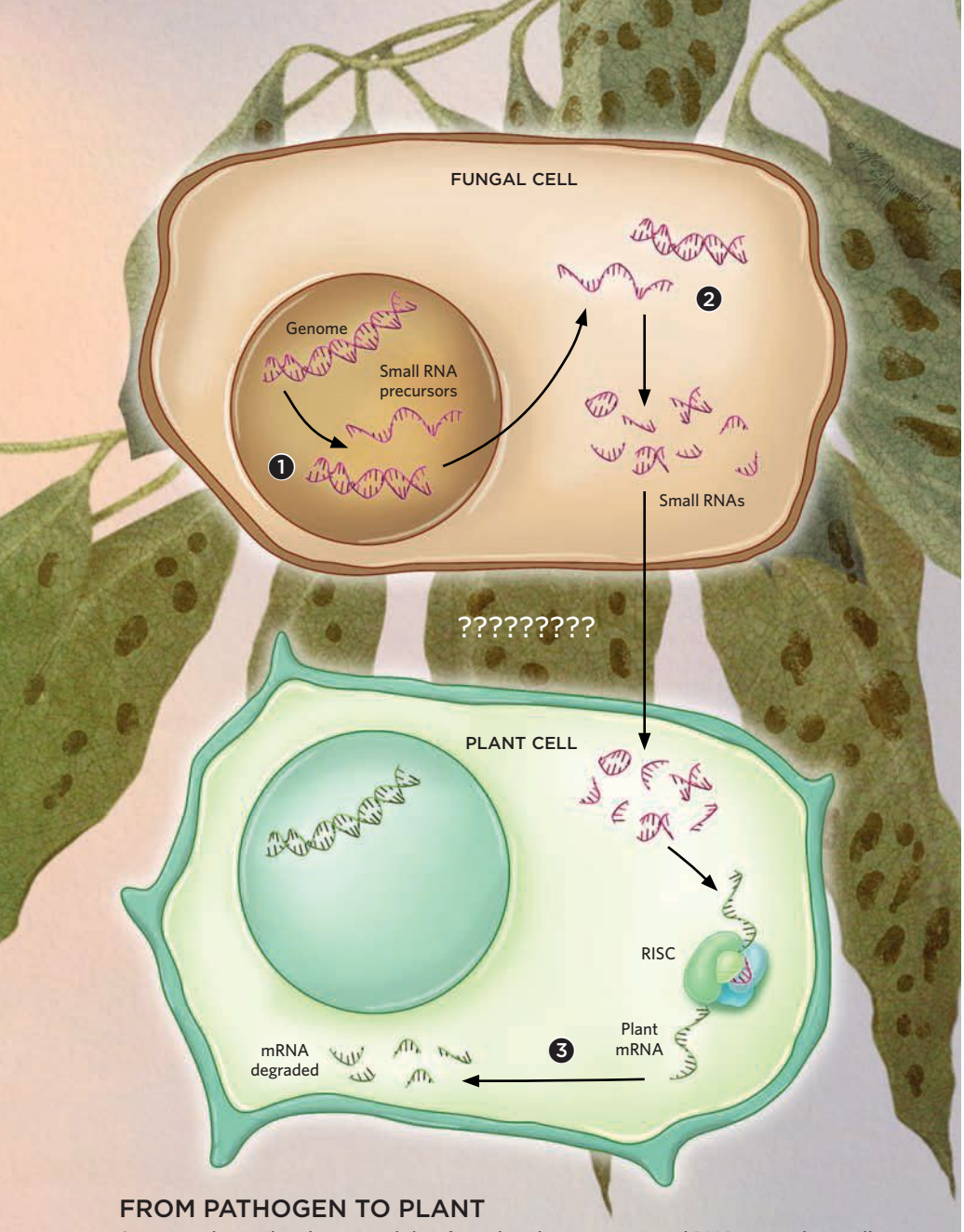
FROM PLANT TO PATHOGEN

The plant produces a small RNA precursor, either a long double-stranded RNA or a pre-microRNA, with sequence similarity to a fungal gene 1. Researchers have engineered the sequence into the genomes of crop plants or model organisms and demonstrated superior fungal resistance, although one recent study showed plants may naturally encode sequences to protect themselves against pathogens.

Evidence points to the idea that the small RNA precursors can pass directly to the fungal cell 2 or undergo processing into small RNAs prior to transfer 3. If the precursor leaves the plant intact, the fungus's processing machinery chops it up 4. In either case, the result is a plant small RNA inside the fungal cell, though the mechanism of transfer remains unknown.

Upon additional processing in the fungal cell, a single strand of the small RNA becomes part of the RNA-induced silencing complex (RISC), which then destroys an mRNA with a matching sequence 5. If the transcript is essential to fungus growth, the pathogen dies and the plant staves off disease.

that the powdery mildew fungus (*Blumeria graminis*) was susceptible to RNAi perpetrated by small RNAs engineered into its host plant's cells. A few years later, Karl-Heinz Kogel's group at Justus Liebig University in Germany demonstrated that HIGS against fungi was possible in whole plants. His team engineered *Arabidopsis* and barley plants to produce small RNAs that inhibited the expression of certain genes in the fungus *Fusarium graminearum*, which causes head blight.[2] The products of these fungal genes are the very proteins upon which conventional fungicides work. "We didn't use a chemical to target the protein; we used small RNAs to target the gene," says Kogel. "And it is highly effective."



FROM PATHOGEN TO PLANT

Scientists have also discovered that fungal pathogens can send RNAs into plant cells to aid their invasion. Similar to the reverse process, the fungus generates small RNA precursors whose sequences complement those of plant mRNAs (1). A fungal protein slices up the small RNA precursors to produce small RNAs (2), which are then passed over to the plant cell via unknown means.

Inside the plant cell, the small RNAs are incorporated into the plant's RISC and direct the complex to degrade the target transcript (3). If the genes affected are involved in plant immunity, the fungal infection expands.

Tweaking that methodology, a number of groups have now shown that HIGS can protect lab-grown plants from several fungal diseases, including potato late blight, downy mildew (which affects lettuce), cereal rust, and wheat leaf rust. All of these experiments rely on naturally occurring RNAi machinery already present in both the plant and the pest, including RISC and proteins that process the precursors into small RNAs. Hailing Jin, who studies plant immunity at the University of California, Riverside, says success with HIGS in the lab suggests the technique could be an effective fungicide in the field "because those RNAs can be specifically designed to target the pathogens you want. Since the design is relatively easy, you have the potential to target multiple pathogens [at once]."

Researchers have already completed a field experiment that demonstrates the effectiveness of engineering plants to send interfering RNAs to fight off fungal pathogens. In 2015, a team in China designed transgenic wheat plants that produced RNAs targeting essential genes in two fungal pathogens, *Fusarium* head blight (FHB) and *Fusarium* seedling blight. Mutant plants grown in the field in Wuhan ("among the most severe wheat FHB epidemic regions in China," the authors noted in their study) were resistant to the disease.[3]

> I wouldn't be surprised if there is small-RNA traffic between fungi and plants outside of laboratory experiments. They do everything else in their war against each other.
>
> —John Pitkin, Monsanto

"This is an area we're definitely watching," says John Pitkin, the Global Disease Management Lead at Monsanto. "It clearly looks like there's movement of RNA between plants and fungi through the expression of transgenes. Whether those reach the level of commercial efficacy is still in question." (See "Using RNAi to Protect Crops" on page 40.)

Despite progress on the biotechnology front, scientists haven't been able to say definitively that the phenomena they can encourage in the lab occur in nature. While the actions of RNAi against viral pathogens within the plant cell have been appreciated for years, it's unclear whether plants in the wild send RNA mercenaries into fungi and other invading eukaryotic pathogens.

A few months ago, scientists reported perhaps the first evidence that small-RNA transfer between plants and fungi does

indeed occur without the intervention of genetic engineers. Hui-Shan Guo at the State Key Laboratory of Plant Genomics at the Chinese Academy of Sciences in Beijing discovered that cotton plants ramp up the production of certain small RNAs after infection by a fungal pest, *Verticillium dahliae*. Not only do these plant RNAs tamp down the expression of two essential genes in the pathogen, but mutating the fungus's genes to be resistant to the RNAi made the pest more virulent.[4] "Our works are the first direct experimental evidence of the mobility of . . . RNA molecules from plants to fungal cells and inducing target gene silencing in fungal cells," Guo wrote in an email to *The Scientist*.

Although Guo's study stands alone as evidence that plants use RNAi to punch back at pests in the field, plant biologists are nevertheless convinced it's a widespread defense strategy. "Assuming there's a mechanism for transferring RNAs from host to pathogen, it makes sense this would be one avenue nature could exploit to kill the pathogen," says Phillip Zamore, who studies RNAi at the University of Massachusetts Medical School. "It's a case of nature got there first."

"I wouldn't be surprised if there is small-RNA traffic between fungi and plants" outside of laboratory experiments, says Pitkin. "They do everything else in their war against each other."

Pest maneuvers

For more than a decade, Jin at UC Riverside has been investigating the ways small RNAs come to plants' defense. Knowing that RNAi machinery is conserved among eukaryotes, Jin decided to look at the other side of the equation: Do plant pests, as part of their attack strategy, produce small RNAs that target specific host genes?

The bane of many a farmer, the fungus *Botrytis cinerea*, also known as gray mold, covers plants in a coat of fuzz that rots leaves, stems, flowers, and fruits. Jin's group infected *Arabidopsis thaliana* and tomato plants with *Botrytis* and profiled the expressed RNAs within the cells of the plants' leaves. "We found a lot of *Botrytis* small RNAs are enriched after infection," she

USING RNAi TO PROTECT CROPS

Field experiments testing the use of genetically engineered RNA interference against eukaryotic pathogens, an approach called host-induced gene silencing (HIGS), are just now embarking on the long road to commercialization. But even if HIGS is successful at warding off disease-causing insects or fungi, cost may be an insurmountable hurdle. John Pitkin, Global Disease Management Lead at Monsanto, says the commercialization of a transgenic crop costs on the order of $130 million to $140 million. "Finding one single disease that reaches that bar for a HIGS approach is a pretty daunting task," he says. In other words, can the financial burden of a pathogen justify the mammoth expense of getting a transgenic, RNAi-protected crop on the market?

Then there's the public's discomfort with genetically modified crops, particularly in Europe, notes Karl-Heinz Kogel, a plant biologist who uses HIGS at Justus Liebig University in Germany. To get around genetic manipulation, he and others have tested the possibility of an RNA spray. Rather than introducing small RNAs via transgenes in the plant, it may be possible to just apply the interfering molecules directly to the crop, an approach called spray-induced gene silencing (SIGS). Several months ago, Kogel's team reported on its experiments spraying barley plants with a long noncoding double-stranded RNA—a precursor to the small RNAs used in RNAi—targeting the same genes critical for *Fusarium graminearum* survival that he attacked using HIGS in 2013. It worked: the plants suffered far less disease (*PLOS Pathog*, 12: e1005901, 2016).

Interestingly, the researchers found that the RNA was taken up by the plant and transferred into the fungus—results that add to another finding that RNAs could directly enter the pathogen as well. "Not only does the fungus take it up from the surface and is then killed, but the plant takes it up, transports it through the plant body, and then the fungus takes it up again," says Kogel. He adds that direct RNA intake by the fungus is much less efficient, and thus the uptake and transfer by plants may be necessary to elicit the protective effect of the spray.

says. "One obvious but very exciting hypothesis would be that those small RNAs would have the potential to target host genes."

To find out, she enlisted a bioinformatician to predict the *Botrytis* RNAs' target genes in the hosts. She set the bar high, including only those small RNAs that matched up with both *Arabidopsis* and tomato genes. "Even then, we got 70-something *Botrytis* small RNAs that can have very good *Arabidopsis* and tomato targets," Jin says.

To see if these had any functional significance, she and her colleagues selected three of the more abundant fungal RNAs and expressed them in *Arabidopsis*. Not only were the plant's target genes subsequently suppressed, the plants were more susceptible to infection, supporting the idea that the fungus is tamping down its host's immunity by transferring small RNAs.[5] "This demonstrated the first example of cross-kingdom RNAi used as a virulence mechanism in fungal pathogens," says Jin.

Kogel calls Jin's discovery "sudden and unexpected. . . . The small RNAs in the fungus are very, very similar to the mRNA structure of genes in the plant, and they inhibit the gene function by degrading these plant RNAs." Just how the fungus gets its RNAs inside the plant leaves remains a mystery, notes Kogel. "This is a black box."

Jin recently put a clever twist on the cross-kingdom RNAi story—using the gene-suppressing mechanism against itself. Her team engineered *Arabidopsis* and tomato plants to produce RNAs to destroy *Botrytis*'s RNAi machinery—specifically, to silence a key RNA-processing enzyme called Dicer-like protein 1. Because the fungal enzyme must trim the RNAs with Dicer-like protein 1 before their transfer to plants, it was like having the plant host lob a bomb at the fungus's bomb factory. And it worked. The fungus didn't grow as well on the transgenic plants.[6]

Trafficking mechanisms

Cross-taxa RNAi isn't limited to plants and the organisms they interact with. It occurs among a variety of organisms, including humans. In 2012, for instance, researchers reported that microRNAs from human blood cells could transfer into the malaria parasite *Plasmodium falciparum*, target a particular transcript, and tamp down gene expression.[7]

Nematode parasites can also pass RNAs to their mammalian hosts. A few years ago, Amy Buck, who studies RNA at the University of Edinburgh, and her colleagues discovered that *Heligmosomoides polygyrus*, a helminth parasite that lives in the guts of mice, secreted exosomes containing, among other cargo, microRNAs that suppressed the expression of certain mouse genes.[8] The RNA-carrying exosomes secreted by the worms also included the protein Argonaute, the member of RISC that chops mRNA, suggesting these cellular blebs come fully loaded to interfere with the recipient's gene expression.

FORTIFYING CROPS: Researchers are using RNAi to ward off animal and fungal pests, including corn rootworm and *Fusarium* head blight that attacks wheat.

Buck's results implicate extracellular vesicles as the vehicles for the transfer of RNAi between organisms. "The vesicles are functional and get into [host] cells," says Buck. "My sense is that vesicles moving cargo between cells or even between organisms is ubiquitous and ancient, and we just haven't appreciated it." Jin suspects that something similar is occurring between plants and fungi.

Buck says one of the questions about the transfer of RNAs within exosomes is whether the concentration of RNA molecules is sufficient to actually have an effect on the host. Just because one organism can produce small RNAs and ferry them over to another organism for interference doesn't mean that they are indeed having a meaningful influence on the recipient. "You need an enormous number" to have a function, says Zamore—one interfering RNA for every target mRNA. "You can't beat the laws of thermodynamics."

Other mechanisms of RNA uptake may be at play. A few months ago, Jin's group published evidence that *Botrytis* can bring in from the environment both small RNAs and longer double-stranded RNAs from which they are derived.[6] And Kogel, too, has found that cultured fungi could absorb externally applied RNAs.

PARASITIC PLANTS

Dodder, also known as strangleweed (genus *Cuscuta*), grows in many parts of the globe, sapping nutrients from other plants. It wraps its leafless, yellow-orange stem around its victim and burrows in, forming direct contact with the host's vasculature. Dodder's root then dies back, and the parasite sends off new shoots to find other plants to sink their haustoria into. "It looks like a mat of tangled coils and webs of tissue," says Jim Westwood, who studies strangleweed at Virginia Tech. "It looks like somebody has thrown a bunch of straw out into a patch."

All manner of material pass from the host to the dodder, including, Westwood has found, RNA. A decade ago he captured and sequenced messenger RNAs passing from tomato and pumpkin plants into the parasite (*C. pentagona* Engelm.), and found them to represent a handful of genes from each host genome, totaling about 20 in number (*Plant Physiol,* 143:1037-43, 2008). Shortly after, another group led by Neelima Sinha of the University of California, Davis, also found a few mobile transcripts transferred from tomato to dodder (*New Phytol,* 179:1133-41, 2008), but the full extent of what was going on was far from appreciated.

A few years later, as next-generation sequencing was becoming more affordable, Westwood's team grew dodder on tomato and *Arabidopsis* plants. Westwood anticipated that there would be many more mobile mRNAs that hadn't been identified by the earlier work, and perhaps he could nab a couple hundred more in this go round. But when the results came back, "it was not a few hundred. It was thousands."

Most surprisingly, Westwood says, mRNAs were passing bidirectionally—also moving from dodder to host (*Science,* 345:808-11, 2014). "It suggested RNA movement is much more common than we had thought," he says. But the effect of these incoming messages on the plants or their relationship "is a big unknown," he adds. "We don't have any evidence for an effect of that in the host-parasite interaction."

However, like the mechanism of RNA transfer between plant and fungus, how this transfer happens is still unclear.

Despite lingering questions about the function of cross-kingdom RNAi, its use in the lab is becoming a powerful tool for experimentation. The mere ability to control the gene expression of pest organisms with transgenic plants has opened up research opportunities previously closed to scientists. Hans Thordal-Christensen, who studies plant immunity at the University of Copenhagen, says the fungus he works with isn't amenable to genetic transformation. But with HIGS, he can manipulate fungal gene expression, especially of the effector genes that the pathogen secretes upon infection. "It's opened up for us being able to study these things that otherwise we really couldn't." ■

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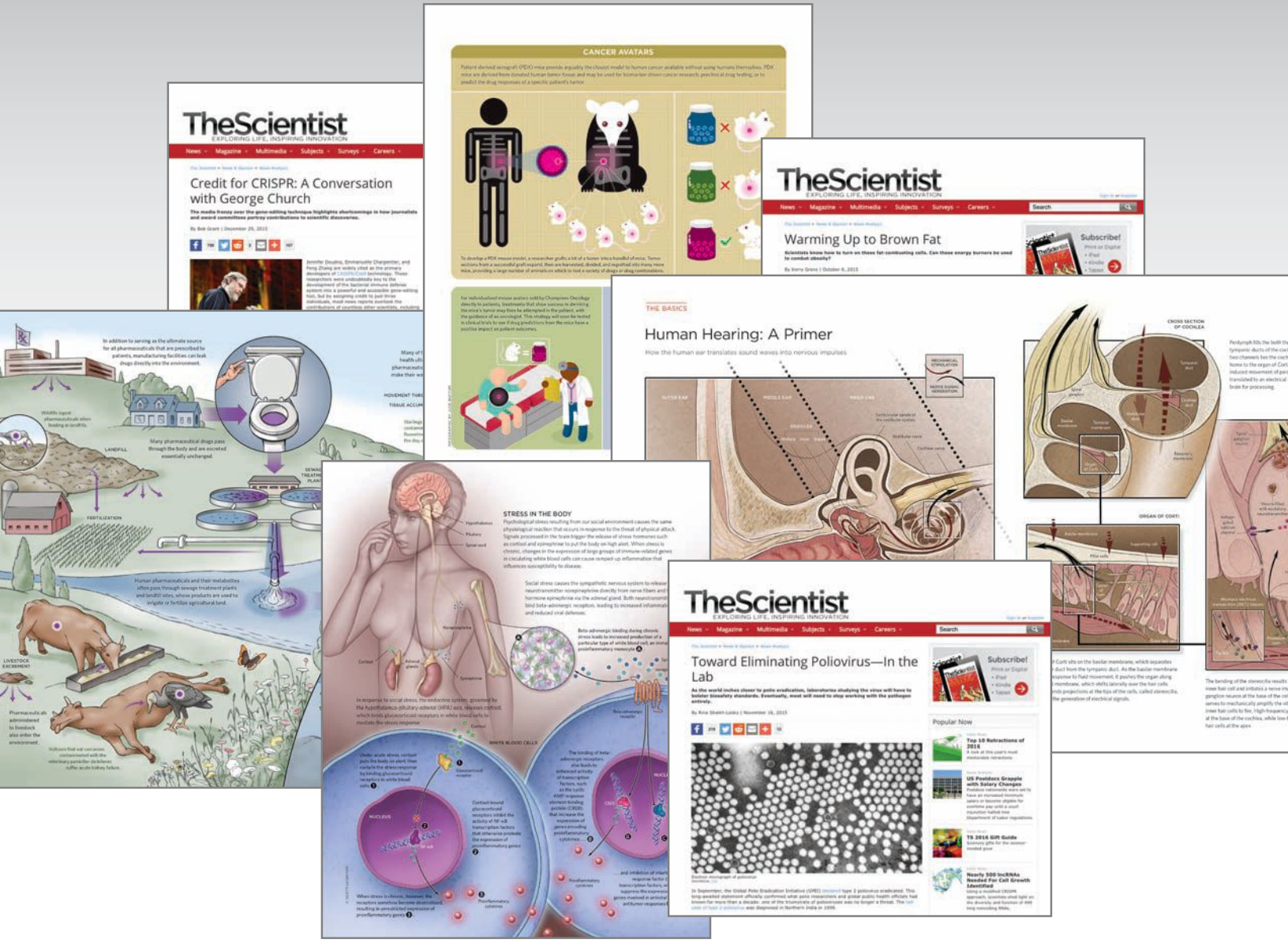

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CELL SCAFFOLDING: This composite super-resolution microscopy image shows actin (on a scale from blue to magenta/red, for earlier to later time points of imaging) in a living pig kidney cell.

MAY THE FORCE BE WITH YOU

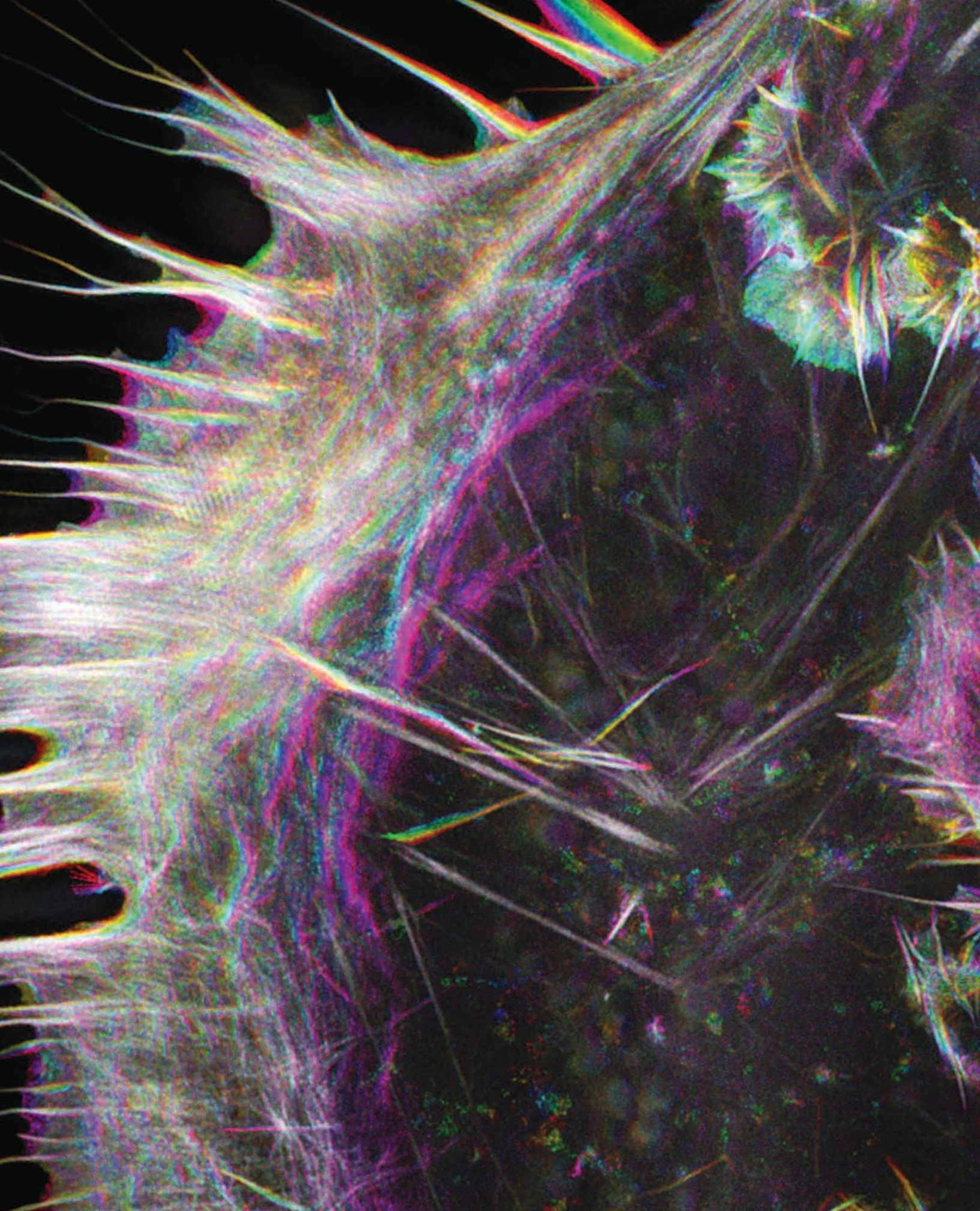
The dissection of how cells sense and propagate physical forces is leading to exciting new tools and discoveries in mechanobiology and mechanomedicine.

BY NING WANG

It is well known that some human diseases are related to changes in mechanical properties of tissues. In patients suffering from arteriosclerosis, the arteries lose some of their elasticity and become thicker and stiffer. In liver or lung fibrosis, excessive fibrous connective tissue has a similar hardening effect on those organs. And patients with aneurysms have balloon-like bulges in their blood vessels that, if left untreated, can expand under pressure until they burst.

Of course, mechanical properties and forces aren't just important in disease, but in health as well. Almost all living cells and tissues exert and experience physical forces that influence biological function. The magnitudes of those forces vary among different cell and tissue types, as do cells' sensitivities to changes in magnitudes, frequencies, and durations of the forces. Touch, hearing, proprioception, and certain other senses are well-known examples of specialized force sensors. But force detection and sensing are not limited to these special cases; rather, they are shared by all living cells in all tissues and organs. The underlying mechanisms of force generation and detection are not well understood, however, leaving many open questions about force dynamics; the distance over which a force exerts its impact; and how cells convert mechanical signals into biochemical signals and changes in gene expression.

In recent years, biologists have begun to uncover the molecular players that mediate force sensation and propagation at the cellular level, and they're collecting clues as to how mechanical stimuli influence biological function. Such work could pave the way for a deeper understanding of how physical forces influence biological functions in embryonic development, normal physiology, and complex diseases. Translating this research into the clinic may help create new ways of treating certain diseases using mechanics- and engineering-based tools.



Generating and sensing physical forces

In the early 1980s, Donald Ingber of Harvard University and Mina Bissell of Lawrence Berkeley National Laboratory independently proposed that the extracellular matrix (ECM) that surrounds and supports cells could affect cell/tissue organization and function as well as gene expression. But at the time, experimental evidence was scarce, and the mechanism was not clear.

Over the next several years, researchers began to report that cells experience mechanical stimuli via cell surface receptors. In 1986, MIT's Richard Hynes and colleagues cloned one such receptor, which they called integrin, that turned out to be the primary transmembrane molecule that mediates cell adhesion to the ECM. Most researchers at the time presumed that integrins (there are now 24 known subtypes) primarily functioned in chemical signaling. But in the early 1990s, while working as a postdoc in Ingber's lab, I provided the first experimental evidence that integrins, and associated intracellular protein complexes known as focal adhesions, mediate mechanical force transmission to the cytoskeleton.[1]

Using Arg-Gly-Asp peptide–coated magnetic beads that clustered integrins and induced the formation of focal adhesions beneath the beads on the inner surface of the cell membrane, I applied measured amounts of stress to the surfaces of living cells and found that cell stiffness increased with the magnitude of the forces. By disrupting cytoskeletal filaments such as filamentous actin (F-actin), I could abolish the transmission of force into the cell. This study changed the scientific community's view of integrins, which are now recognized as key molecular force sensors.

Applied forces concentrate at actin stress fibers and propagate over longer distances in the cytoplasm.

Subsequently, using a laser tweezer, Mike Sheetz's lab at Columbia University independently confirmed that focal adhesions transmit external forces into the cell.[2] In addition, two other groups—those of Yu-Li Wang, now at Carnegie Mellon University, and Benny Geiger at the Weizmann Institute of Science—found that focal adhesions also transmit forces generated inside the cell by powerful molecular motors such as myosin II, which binds to F-actin in the cytoskeleton, out into the ECM. This research showed that focal adhesion–mediated transmission of mechanical signals is bidirectional.[3,4]

Another class of mechanosensors used by the cell are stretch-activated ion channels on the plasma membrane. Over the past decade, Martin Chalfie of Columbia University and other labs have worked on several candidate channels that, in response to stretch, open to allow ions to flow into the cytoplasm. The result is mechanoelectrical transduction—analogous to a neuronal action potential—that can activate enzymes or proteins in the cytoplasm to affect intracellular activities, or even influence gene expression. The detailed mechanisms of this are still unclear, however.

More recently, many labs have searched for intracellular mechanosensors downstream of integrins at focal adhesions. For example, Sheetz and his colleagues have found evidence of a mechanosensing role for the focal adhesion protein talin,[5] while Martin Schwartz's group at Yale has demonstrated a similar role for the focal adhesion protein vinculin.[6] But a fundamental question remains: How does a living cell integrate forces sensed by different mechanosensors and respond in a coherent manner?

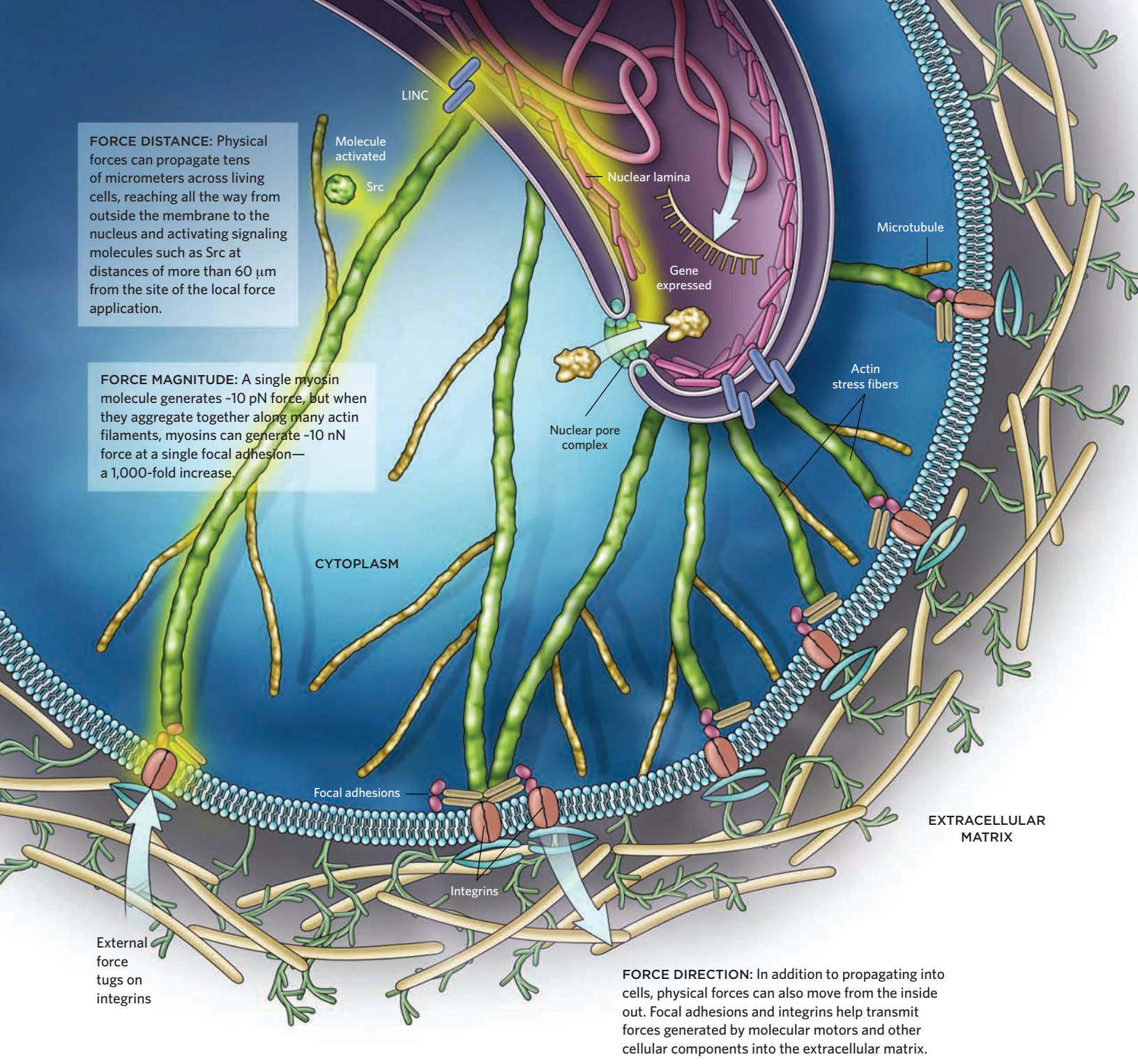
Ingber first proposed the model of cellular tensegrity (tensional integrity) in the early 1980s, emphasizing the importance of tension among cytoskeletal structures in the cell's ability to configure a holistic sense of the forces at play. If there is tension in the cytoskeletal filaments, then a local change in one part of the filament is quickly transmitted to all connected parts.

Experimental evidence now supports this model. In the early 2000s, collaborating with the Ingber lab, my group used chemicals to either contract or relax the cytoskeleton of cultured human smooth muscle cells, which systematically varied the cells' inherent tension, or "prestress," without changing their shape. We found that the cell stiffness changed accordingly. In other words, cell stiffness is determined by the cytoskeleton's tension.[7] Moreover, after disrupting the cell's microtubules with specific drugs, we found that the force transmitted out of the cell increased.[8] This suggests that microtubules, which are relatively stiff components of the cytoskeleton, are essentially balancing some of the cell's endogenous prestress, and when they are disturbed, that tension is transferred to the ECM.

Mechanotransduction at a distance

For many years, the prevailing view in the field of mechanotransduction was that forces transmit only a short distance in living cells, and thus a local force can only exert significant effects at the periphery of the cell. From a materials science point of view, this limited reach would be reasonable if the material was homogeneous and isotropic—in other words, there is no difference in its stiffness or other mechanical properties when the force direction is changed. In this case, a local stress would rapidly decay as the distance increases. However, the cytoplasm of a living cell is neither homogeneous nor isotropic; it is heterogeneous and anisotropic, meaning that the material's mechanical properties do depend on the direction of force. Importantly, there are stiff, prestressed actin bundles (also called stress fibers) in the cell. Applied forces concentrate at these actin bundles and propagate over longer distances in the cytoplasm.

Since the early 2000s, my group has demonstrated that forces do propagate across relatively vast cellular distances—on the order of tens of micrometers—in living cells, and that this long-distance signal is dependent on the inherent tension in the cytoskeleton.[9,10] Just as a violin string can only ring with the correct resonance and sound the right note if it has proper tension, when the prestressed actin bundles are disrupted, force propagation becomes short-range (acting over only a few μms). The higher the tension, the farther the force will be propagated.



FORCE DISTANCE: Physical forces can propagate tens of micrometers across living cells, reaching all the way from outside the membrane to the nucleus and activating signaling molecules such as Src at distances of more than 60 μm from the site of the local force application.

FORCE MAGNITUDE: A single myosin molecule generates ~10 pN force, but when they aggregate together along many actin filaments, myosins can generate ~10 nN force at a single focal adhesion—a 1,000-fold increase.

FORCE DIRECTION: In addition to propagating into cells, physical forces can also move from the inside out. Focal adhesions and integrins help transmit forces generated by molecular motors and other cellular components into the extracellular matrix.

FOLLOWING THE FORCE

Physical forces generated outside a cell can be transmitted to the cytoskeleton via cell-surface receptors known as integrins and associated protein complexes called focal adhesions. Once believed to act over only short distances, such forces are now recognized to propagate tens of microns across cells via cytoskeletal filaments such as actin stress fibers and microtubules.

Such forces can even travel all the way to the nucleus, where they may influence gene expression. On the outside of the organelle, the nuclear envelope is tethered to the actin cytoskeleton via the LINC (linker of nucleoskeleton and cytoskeleton) complex. Just inside the envelope, the nuclear lamina comprises a layer of intermediate filament proteins called lamins that are critical for force-induced changes in gene expression. Forces can also affect the translocation of certain molecules through the nuclear pore complex.

Most recently, we have found that specific signaling molecules—in particular, the tyrosine kinase Src and the GTPase Rac1—can be activated at distances of more than 60 μm away from the site of the local force application via integrins at the cell membrane.[11,12] Importantly, this activation is fast, taking less than 300 ms from force application to the activation of Src and Rac1, making mechanotransduction much faster than the 10 to 20 seconds it takes a soluble growth factor–induced signal to travel over the same distance.[11]

Mechanotransduction in the nucleus

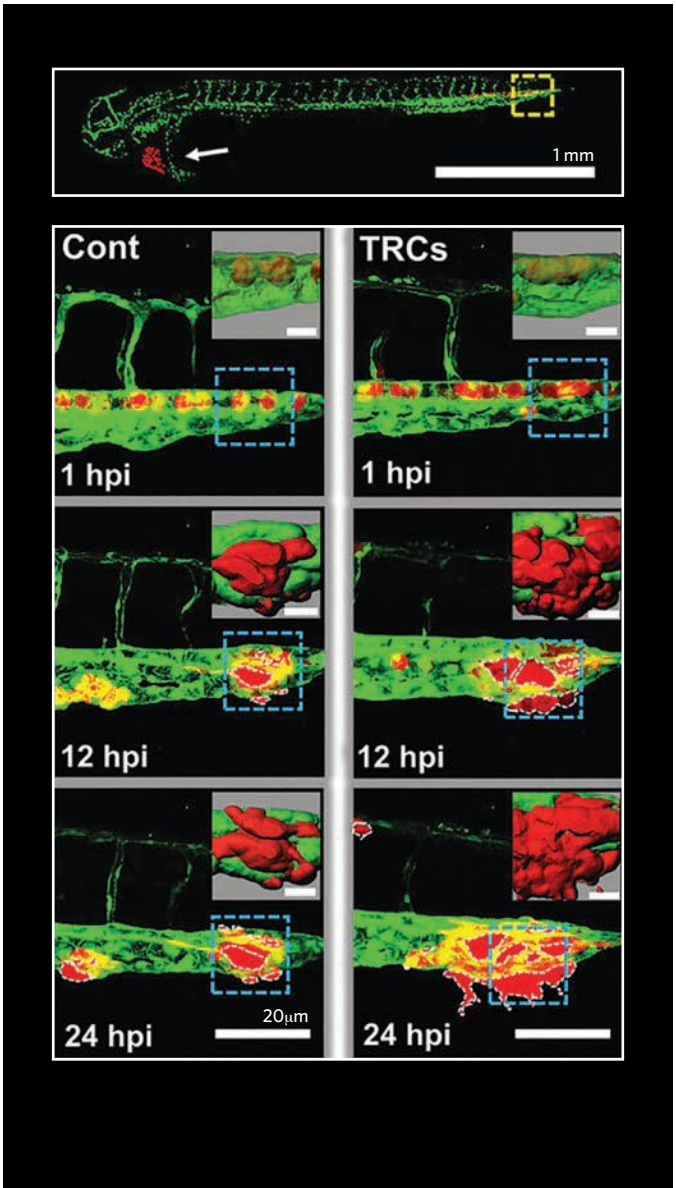
In contrast to the emerging picture of force propagation in the cytoplasm, we know very little about nuclear mechanotransduction. The nuclear envelope is physically tethered to the actin cytoskeleton via the LINC (linker of nucleoskeleton and cytoskeleton) complex. In the late 1990s, Ingber and colleagues published the first evidence that force-carrying connections reach from the plasma membrane to the nucleus, perhaps playing a role in the regulation of gene expression. Using a micropipette coated with fibronectin to attach to the cell surface, the researchers pulled on the cell and found that the nuclear envelope distorted.[13] Later, my group revealed a force-induced protein-protein dissociation inside the nucleus.[14] This change was dependent on both a properly stressed cytoskeleton and an intact nuclear lamina, a layer of intermediate filament proteins called lamins that line the inside of the bilamellar nuclear envelope. Subsequent research has demonstrated that lamins are mechanosensors critical for extracellular matrix stiffness–directed differentiation,[15] and for regulation of transcription factors.[16]

To more directly investigate whether a physiologically relevant force can directly deform chromatin structure in a living cell to regulate specific gene expression, my group recently teamed up with Andy Belmont's lab at the University of Illinois at Urbana-Champaign. Belmont's team used bacterial artificial chromosomes to insert multiple green fluorescent proteins and the gene for dihydrofolate reductase (DHFR), an essential enzyme for the synthesis of thymine, into the same chromatin domain in Chinese hamster ovary (CHO) cells. My lab applied a local force to those modified cells via integrins. Sure enough, we measured an uptick in DHFR transcription in response to the applied force. Conversely, disrupting cytoskeletal tension, or the force transmission pathways from the cell surface to lamins and to the nuclear structural proteins that connect to the chromatin, abolished force-induced DHFR expression.[17]

This work provides the first evidence that externally applied forces can stretch chromatin and promote gene expression. As expected with physical force–mediated processes, the response was rapid; we were able to quantify DHFR transcription upregulation within 15 seconds after force application. Interestingly, force-triggered transcription is sensitive to the angle and direction of force relative to the actin bundles: the higher the stress angle, the greater the transcription. Because endogenous forces are constantly generated inside a living cell, these findings suggest that gene expression might be incessantly regulated by physical forces via this direct structural pathway and the indirect pathways of matrix rigidity–dependent nuclear translocation of certain factors, such as yes-associated protein (YAP) and TWIST1. More research is needed to understand the relative contributions of each of these mechanisms in determining overall gene expression levels in any given cell.

From mechanobiology to mechanomedicine

Mechanobiology is becoming increasingly relevant to stem cell biology. For many years, researchers have cultured cells on top of rigid plastic or glass coverslips. However, it is well known that various types of living cells in soft tissues attach to matrices of varying stiffness. Tuning the substrate stiffness in a controlled manner, Yu-Li Wang and colleagues demonstrated that the size



TUMOR POTENTIAL: When melanoma cells were injected into embryonic zebrafish pericardium (red cells indicated by white arrow), the membrane that surrounds the heart, the cells travel to the tail in just one hour post injection (hpi). Differentiated melanoma cells cultured on stiff substrates (Cont) were less efficient than undifferentiated tumor-repopulating cells (TRCs) cultured on a soft matrix at establishing new tumors.

J. CHEN, "EFFICIENT EXTRAVASATION OF TUMOR-REPOPULATING CELLS DEPENDS ON CELL DEFORMABILITY," *SCI REP*, 6:19304, 2016.

and dynamics of focal adhesion complexes as well as the migration of living cells are dramatically altered by substrates of different rigidity.[3] Later, Adam Engler of the University of California, San Diego, and Dennis Discher of the University of Pennsylvania reported that mesenchymal stem cell differentiation can be directed by extracellular matrix stiffness.[18] And my lab has demonstrated that applying local force can spur the differentiation of a single embryonic stem cell.[19] Physical forces also appear critical in the patterning and organization of germ layers during early mammalian embryonic development.

Mechanomedicine is poised to emerge as an exciting branch of medicine that uses mechanics- and engineering-based principles and technologies for precision diagnostics and effective therapeutics.

Researchers are also considering mechanical forces in cancer research. For example, despite decades of study, it is still unclear why only a few cancer cells out of thousands are able to metastasize. The answer may lie in the tumor's physical environment. Scientists have shown that, in primary tumors, high mechanical tension and matrix stiffening are important in cancer progression, and high fluid/solid pressure in the primary tumor often accompanies tumor growth. (See "The Forces of Cancer," *The Scientist*, April 2016.) However, secondary metastatic sites of tumors appear to be softer—suggesting that they have lower forces—than the surrounding normal tissues.

Using a 3-D soft matrix made of fibrin gels, my group has managed to isolate and grow cells that are highly tumorigenic and malignant, called tumor-repopulating cells (TRCs), from several murine or human cancer cell lines.[20] Interestingly, melanoma TRCs cultured in soft 3-D matrices are less differentiated—and thus more tumorigenic—than melanoma cells grown in stiff matrices or on rigid plastic, suggesting that low matrix stiffness drives TRC growth.[21] These soft-cultured melanoma TRCs also move out of the blood vessels in zebrafish to secondary sites more efficiently than more-differentiated melanoma cells cultured on stiff substrates.[22] These findings suggest a common thread in metastatic colonization of malignant tumors: a few tumorigenic cells are able to survive, metastasize, and grow at the secondary sites of soft matrices because these cells are undifferentiated.

And the role of physical forces in biology is by no means limited to stem cells and cancer biology. Across the life sciences, researchers are continuing to draw on insights into mechanobiology to better understand and treat a wide variety of conditions. Human organs-on-a-chip for novel drug screening, shear force–activated cleaning of thrombosis, mechanically tuned hydrogels for bone formation, and tumor cell membrane–derived therapeutic microparticles for reversing cancer drug resistance are just a few recent examples of clinical applications of mechanobiology-based technologies. Mechanobiology-based medicine (mechanomedicine) is poised to emerge as an exciting branch of medicine that uses mechanics- and engineering-based principles and technologies for precision diagnostics and effective therapeutics of diseases that are beyond the reach of existing toolboxes. ■

Ning Wang is the Leonard C. and Mary Lou Hoeft Professor in the Department of Mechanical Science and Engineering at the University of Illinois at Urbana-Champaign and adjunct professor at Huazhong University of Science and Technology.

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The Literature

EDITOR'S CHOICE IN CELL & MOLECULAR BIOLOGY

The Meteorologist Molecule

THE PAPER

M. Legris et al., "Phytochrome B integrates light and temperature signals in *Arabidopsis*," *Science*, 354:897-900, 2016.

Researchers discovered in the 1930s that they could cause lettuce seeds to germinate just by shining red light on them. It turns out, as scientists later revealed, that the proteins responsible for this phenomenon are photoreceptors called phytochromes, now appreciated for their roles in regulating many aspects of plant development beyond germination, from stem growth to the sprouting of leaves to bud flowering. A pair of papers published last October in *Science*, however, describes an entirely new role for one of the pigments: sensing temperature.

The experiments were conducted on phytochrome B (phyB), which, like other phytochromes, is activated by red light but deactivated by light on the far-red end of the visible light spectrum. When turned on, phyB shuts off a class of transcription factors needed for stem growth in seedlings. If the seedling is in the shade of another plant—a potentially fatal situation—far-red light filters through the leaves and triggers a growth spurt.

Jorge Casal, a researcher at the Agricultural Plant Physiology and Ecology Research Institute in Argentina and a coauthor on one of the papers (cited above), initially hypothesized that phyB would be unaffected by changes in temperature. "We know that light signaling is very precise," he says, which led him to suspect plants have evolved means to maintain that precision on warm days and cold days alike. But when he and his colleagues started searching for such imperturbability in phyB, they found the exact opposite.

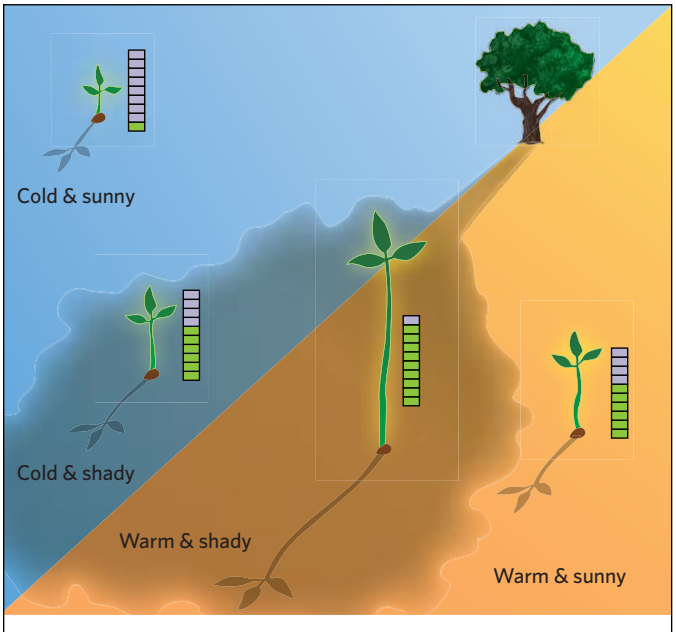
Spectroscopic analysis of purified phyB revealed that warmer temperatures drastically accelerated the reversion of active phyB to its inactive form. The researchers grew *Arabidopsis* seedlings under varying light and temperature conditions. Turning up the heat deactivated phyB and spurred the seedling's growth, tweaking the phyB pathway in much the same way as placing a plant in the shade.

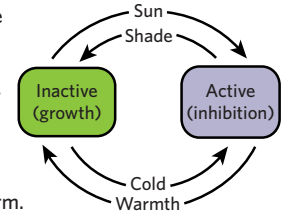
The implication is that phyB serves not just as a light-detector but as a thermosensor, too. The discovery was reported simultaneously in another study by a team of European researchers, who identified the genetic regulation behind the phenomenon (*Science*, 354:886-89, 2016).

Previous work in *Arabidopsis* established that temperature influences various stages of plant development, but the mechanisms by which plants sense temperature has until now been obscure. The adaptive explanation as to why the phyB pathway responds to both temperature and light is not clear, Casal says. However, the effect of warmer weather on increasing growth is particularly strong in dim light, so it's possible the system encourages shaded plants to capitalize on warm days in order to grow tall enough to escape the shade.

Christian Fankhauser, a University of Lausanne biologist who studies light signaling in plants, predicts that phyB is only one of many temperature-sensing pathways in plants, all part of the finely tuned apparatus that times every step of plant development. "A little seedling is a fragile thing," Fankhauser says, so when it makes a break for open air, "you better do it at the right time."

—Ben Andrew Henry





LIGHT SWITCH: The active form of the photoreceptor phytochrome B (phyB) suppresses stem growth in seedlings. In lab experiments, low light inactivates phyB, triggering growth. Warm temperatures turn out to also inactivate phyB, evidence that this pathway monitors temperature as well as light to coordinate growth. This may explain why plants grow the most in warmer, shady conditions compared to cooler shade, and why plants in full light—a growth-slowing condition—grow more if it's warm.

COST CUTTING: Scientists are working to improve photosynthesis in tobacco plants like these.

CELL & MOLECULAR BIOLOGY

Photosynthetic Boost

THE PAPER

J. Kromdijk et al., "Improving photosynthesis and crop productivity by accelerating recovery from photoprotection," *Science*, 354:857-61, 2016.

FOOD SHORTAGE

The United Nations' Food and Agriculture Organization predicts that by 2050 the world will need to produce 70 percent more food than it does currently. Along with improving food storage and transport, increasing crop yields is seen as a primary solution, says Stephen Long of the University of Illinois at Urbana-Champaign.

IDENTIFYING INEFFICIENCY

One option is to boost crops' photosynthesis, so Long's team focused on a rather inefficient part of the process called nonphotochemical quenching (NPQ), which protects plants from intense light. NPQ dissipates excessive light energy as heat to prevent the destruction of light-responsive structures critical to photosynthesis. But when light intensity drops, as when a cloud covers the sun or one leaf shades another, NPQ is slow to switch off. Energy thus continues to be diverted even when not in excess. Computer modeling suggests this lag may reduce a plant's potential yield by "anything between 8 and 40 percent," says Long.

SPEED IS THE SOLUTION

By overexpressing three NPQ-controlling genes in tobacco plants, Long and colleagues increased the speed with which NPQ switches on and off. Importantly, this increase boosted dry weight yield by 14 to 20 percent per plant in field conditions. Tobacco is a technically amenable crop, says Long, explaining that the ultimate aim is to modify food crops.

FIELD OF DREAMS

With such a significant improvement from tackling just one aspect of photosynthesis, says Christine Foyer, a plant scientist at the University of Leeds, "the 70 to 100 percent increase in yield that we desperately need by 2050 is looking much more like a reality."

—Ruth Williams

STOWAWAYS: Fungal pseudoflowers, in the form of a chalky white coating on leaves, lure in pollinators to spread pathogenic spores to neighboring plants.

ECOLOGY

Sweet Fraud

THE PAPER

S.H. McArt et al., "Floral scent mimicry and vector-pathogen associations in a pseudoflower-inducing plant pathogen system," *PLOS ONE*, 11:e0165761, 2016.

UNWANTED COMPANY

Shriveled, sickly white berries are an unwelcome sight on any blueberry farm, and are symptoms of a crop-wasting infection called mummy berry disease. Researchers recently profiled the modus operandi of the fungus responsible, *Monilinia vaccinii-corymbosi (Mvc)*, uncovering a previously unappreciated tactic for spreading its spores.

FAKING IT

The fungus starts off as an airborne spore riding on the breeze, waiting to land on a new leaf of a blueberry plant. There, the spore multiplies and exudes a sticky, sugary, fragrant, spore-laden film, which is called a pseudoflower for its ability to attract insects. Visiting pollinators carry the spores to real flowers, giving the fungus the opportunity to invade nascent fruits that eventually shrivel, fall to the ground, and crack open to release more spores to the wind.

TOOLS OF THE CON

The adaptive role of the pseudoflower has not been clear, says Cornell University ecologist Scott McArt. Other fungal pseudoflowers fall into two categories: some mimic their host flower, while others form their own, unique kind of flower. To draw the distinction, McArt and his colleagues analyzed the volatile compounds responsible for the *Mvc* pseudoflower's scent and discovered a close match to those released by actual blueberry flowers. "The degree of the mimicry is pretty extraordinary," says University of Oregon ecologist Bitty Roy.

AIDING AND ABETTING

A genetic analysis found *Mvc* fungal DNA on 56 percent of bees and wasps and 31 percent of flies captured, implicating them as spore vectors. But behavioral data on the preference for infected versus uninfected plants was equivocal, leaving the insects' exact roles to be quantified in future field studies.

—Ben Andrew Henry

PROFILE

From the Ground Up

Instrumental in launching *Arabidopsis thaliana* as a model system, Elliot Meyerowitz has since driven the use of computational modeling to study developmental biology.

BY ANNA AZVOLINSKY

In 1980, Elliot Meyerowitz was a newly minted assistant professor of biology at the California Institute of Technology (Caltech) studying *Drosophila melanogaster* development. He was asked to teach a graduate genetics seminar, and after leading the session on plant development and discussing the subject with his then graduate student Robert Pruitt, he decided, with Pruitt, to dabble in plant genetics using *Arabidopsis thaliana*. Meyerowitz had become interested in plant genetics in graduate school and thought that there were opportunities to apply molecular cloning—a new technique that he had learned as a postdoc at Stanford University—to plants.

"The literature indicated that the *Arabidopsis* genome was relatively small, which at the time, was a prerequisite for molecular cloning. And the attempts to do mutagenesis in plants showed that chemical mutagenesis appeared very effective and that the genes segregated in a Mendelian fashion, which is not true in many plants because of polyploidy," Meyerowitz says. "*Arabidopsis* self-fertilizes, so you can get homozygous mutants quickly and have more than 10,000 seeds from these small plants. You could grow a million plants in a boiler room instead of needing 50 acres to grow corn!"

"I think if animal developmental biologists were more open-minded about plant research, biology overall would benefit."

Supplied with *Arabidopsis* seeds by Pruitt's uncle, a plant breeder at Washington State University, the Meyerowitz lab began to fill with postdocs and graduate students interested in studying plant biology. Meyerowitz soon became an advocate of making *Arabidopsis* a model organism for studying plant genetics. The lab also continued to work on *Drosophila* until the 1990s, when the funding finally shifted completely from fruit flies to the study of *Arabidopsis* flower and plant development, regeneration, and stem cells. With a focus on developmental biology, Meyerowitz has driven advances in imaging and in computational approaches to understanding how plant stem cells form patterns and employ chemical and mechanical signaling to develop into a mature, flowering plant.

Here, Meyerowitz advocates for more awareness of plant research, talks about why he used to carry DNA in his pockets at plant meetings, and offers advice on how to help postdocs succeed.

MEYEROWITZ'S MOTIVATION

Expanding horizons. Meyerowitz was born in Washington, DC, and raised in a Maryland suburb. He was interested in science from as early as he can remember, and in the summer of 1967, his junior year of high school, worked at the National Naval Medical Center in Bethesda, Maryland, analyzing lipids from rats using thin layer chromatography. Meyerowitz's chemistry teacher encouraged him to apply to other universities besides the local University of Maryland.

A fishy start. Meyerowitz entered Columbia University in 1969. The biology department had recently undergone a number of changes, shifting its focus from traditional zoology and botany to the newly emerging molecular biology, thanks in part to professors such as Cyrus Levinthal, a physicist turned molecular and computational biologist. During his sophomore year, Meyerowitz sought Levinthal's advice on finding a part-time paid position in a research lab to help defray his college costs. Because Meyerowitz had shown so much enthusiasm for Levinthal's research during their meeting, Levinthal offered him a spot in his lab. Meyerowitz worked on the Amazon molly (*Poecilia formosa*), a so-called gynogenetic freshwater fish, in which sperm from males stimulates the eggs to develop but does not contribute genetic material to the offspring. "The question I was addressing was: To what degree was the morphology of neurons the same in animals that were genetically identical?" says Meyerowitz. He learned how to do painstaking serial sections of fish embryo brains and how to analyze neuronal patterns using computer programming. "It turns out that the neuron patterns were identical down to about one micron and that the pattern of midline crossover of the optic nerve was neither random nor all the same, but rather a one-third/two-thirds distribution. I used computational modeling for the analysis, so in some sense my approach has never changed since my undergraduate days." Meyerowitz also went with Levinthal's lab to the Woods Hole Marine Biological Laboratory for two summers. There he became fascinated by developmental biology.

The eyes have it. Meyerowitz entered Yale University's biology PhD program in 1973, joining Douglas Kankel's fruit-fly developmental genetics lab as Kankel's first graduate student. There Meyerowitz examined whether information transmitted from the embryonic fruit-fly eye to the brain is important for brain development and whether neuronal brain connections influence eye development. After first analyzing every available fly mutant with badly developed eyes, he focused on three, which had both a neuronal and an eye phenotype. Meyerowitz used genetic mosaics to show that if the mutation was only in the eye, the axons in the fly's brain were also highly disorganized, but that the same mutations present only in brain tissue did not result in malformed eyes, providing evidence

FAST FORWARD

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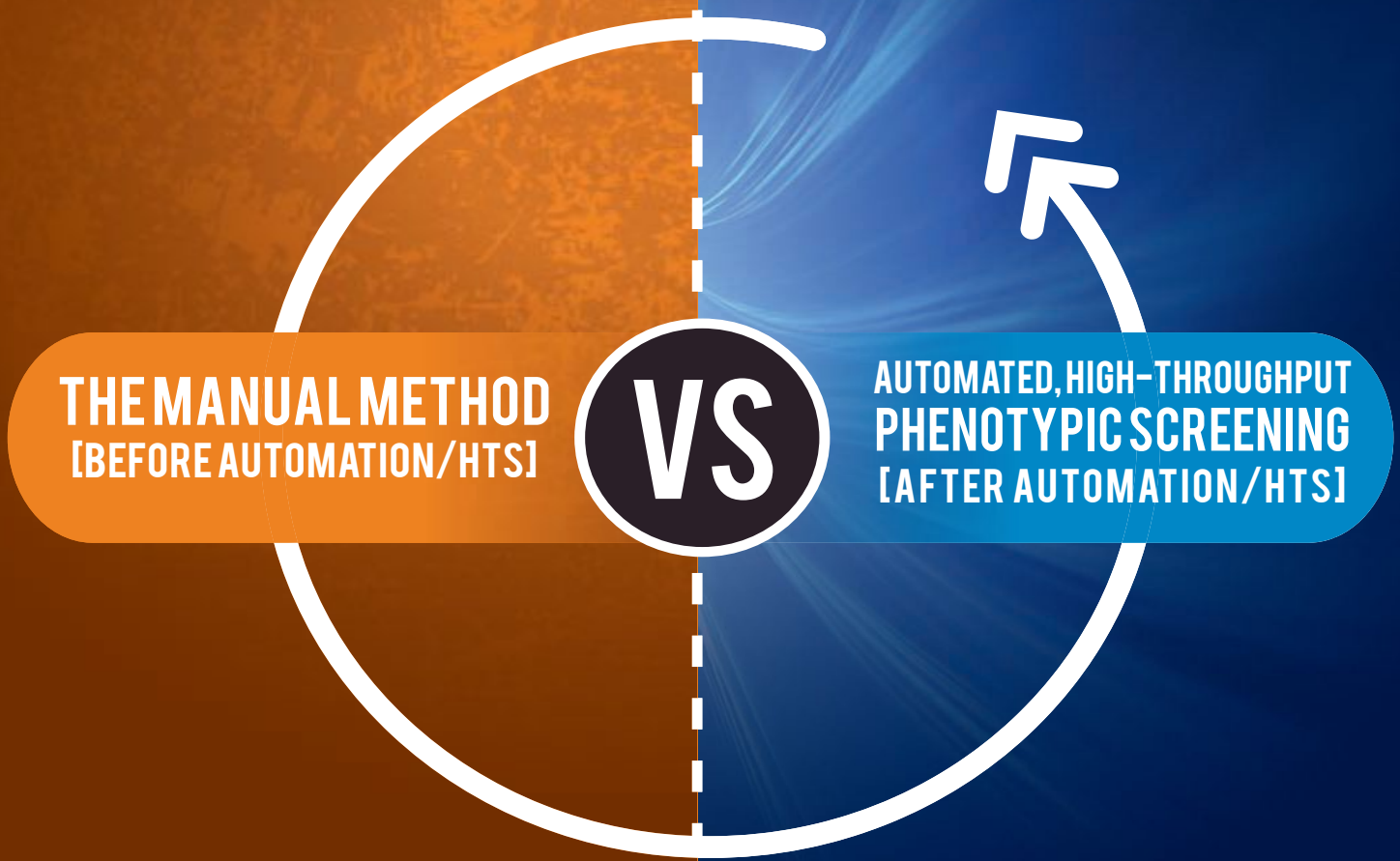

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THE MANUAL METHOD
[BEFORE AUTOMATION/HCS]

VS

AUTOMATED, HIGH-THROUGHPUT PHENOTYPIC SCREENING
[AFTER AUTOMATION/HCS]

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The length of this step depends on the quality of your sequencing data and the complexity of your target.

EXECUTE YOUR CRISPR EDITING STRATEGY

Manual pipetting is time-consuming, and prone to liquid-dispense variability.

CONFIRM YOUR CRISPR EDIT(S)

DESIGN YOUR CRISPR EDITING STRATEGY

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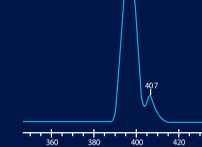
EXECUTE YOUR CRISPR EDITING STRATEGY

Automated liquid handling increases efficiency, reduces manual intervention, and ensures precise reaction volumes, every time.

CONFIRM YOUR CRISPR EDIT(S)

Sending your CRISPR product out for sequencing could add days to your workflow. Alternatively, you could move forward with unconfirmed edits, potentially adding significantly to your downstream screen times.



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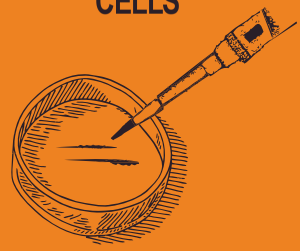

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3D cultures more closely recapitulate in vivo conditions, allowing deeper analysis of cell-cell interactions.

CELL CULTURE

IMAGE YOUR CELLS: ONE WELL AT A TIME

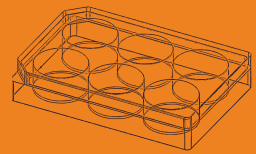
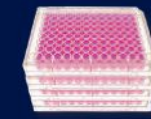


Image collection can be the most labor-intensive step of your CRISPR workflow, particularly if you took a shortcut and failed to confirm your edits.

PHENOTYPIC ANALYSIS

IMAGE YOUR CELLS: PLATES AT A TIME



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INTERPRET YOUR DATA

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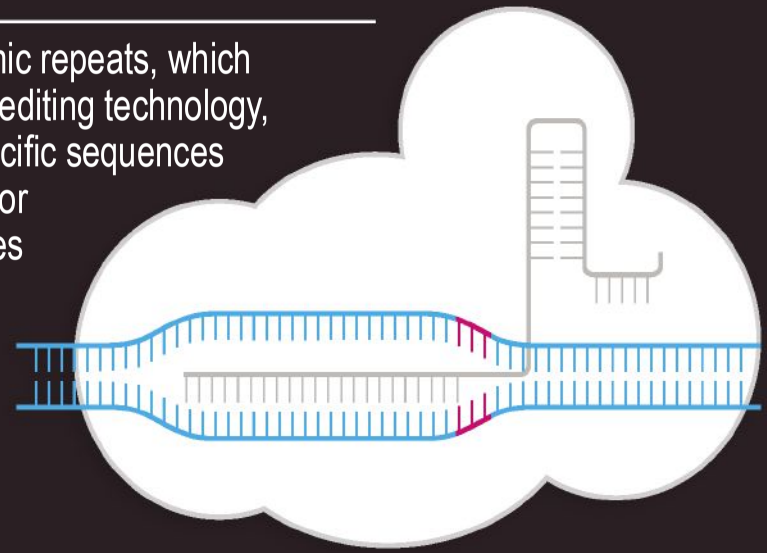
NEW TO CRISPR?

CRISPR stands for clustered, regularly interspaced, short palindromic repeats, which were first discovered as an immune function in archaea. As a gene-editing technology, CRISPR relies on Cas proteins with nuclease activity to identify specific sequences in the genome, cut the cognate sequence, and introduce insertions or deletions. CRISPR has all-but-replaced traditional cloning techniques in laboratories across the globe.



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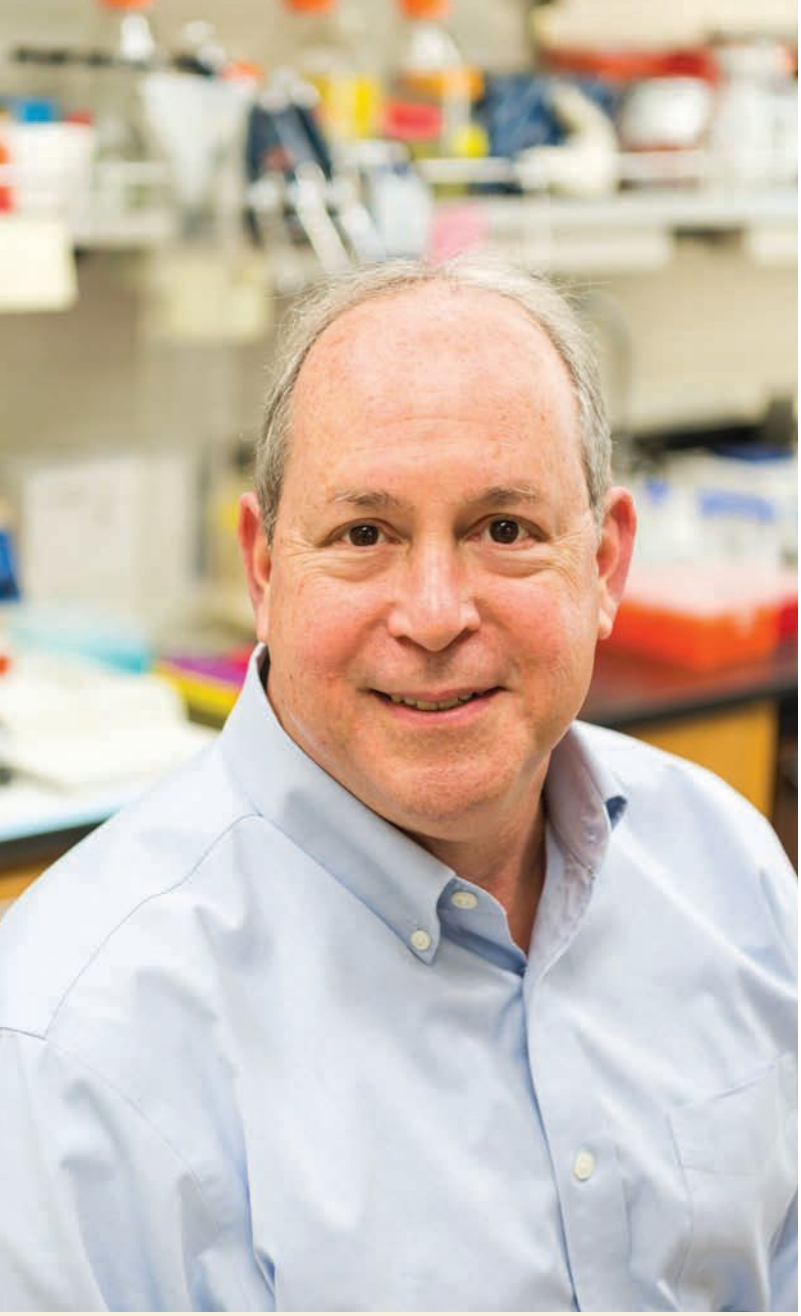

ELLIOT MEYEROWITZ

George W. Beadle Professor of Biology
California Institute of Technology
Investigator, Howard Hughes Medical Institute

Greatest Hits

- Instrumental in establishing *Arabidopsis thaliana* as a widespread model plant system
- First cloning of a plant hormone receptor, for ethylene, with grad student Caren Chang and postdoc Tony Bleecker
- First identification of a peptide signaling pathway in plants, with postdocs including Steven Clark and Jennifer Fletcher
- With John Bowman and David Smyth, proposed the ABC model, the basic model of plant organ specification
- Collaborating with computational biologists Eric Mjolsness and Henrik Jönsson, initiated a novel computational modeling–based approach to understanding plant development

that the organization of the optic lobe in the brain depends on information from the fruit fly's eye.

Cloning pioneer. Meyerowitz decided to try a warmer climate for his postdoc and in 1977 joined David Hogness's lab at Stanford, where molecular cloning was just taking off. "I learned all of the current molecular biology there," says Meyerowitz. The first *Drosophila* cDNA library had just been created and Meyerowitz helped to make some of the first fly genomic libraries. To do that, he first developed new vectors into which larger pieces of DNA could be cloned. Using these lambda phage and cosmid vectors, he explored the gene organization of the *Drosophila* salivary gland polytene chromosomes. Meyerowitz also met his wife, then a graduate student in Arthur Kornberg's lab, "which is probably more important than anything I learned scientifically," he says.

MEYEROWITZ'S MODEL

A new model system. Meyerowitz chose to begin his career as a principal investigator at Caltech because "there was already a rigorous tradition of developmental biology and genetics. And I would have to be on the ball to keep up with everyone there, which was a challenge I wanted to take." Not wanting to lose any time while his lab space was being renovated, Meyerowitz conducted fly crosses in the kitchen of his apartment for six weeks. Although he continued to study fly polytene chromosomes, *Arabidopsis* became a new focus in the lab. By creating a genomic DNA library, his team confirmed that the plant's genome was relatively small (about 10^8 base pairs) with few repetitive elements.

By the mid-1980s, Meyerowitz had formed close relationships with other *Arabidopsis* researchers and, along with Chris Somerville, Maarten Koornneef, David Meinke, and others, used scientific meetings to espouse the adoption of the plant as a research model. "We decided to make sure people shared their materials, and to promote that, I used to bring with me in my pocket the DNA clones that we had mapped along with the restriction polymorphism DNA maps we had created for chromosome walks," says Meyerowitz. The community grew quickly, and with the help of the National Science Foundation, there were soon *Arabidopsis* stock centers and an international committee set up to sequence the plant's genome. Meyerowitz's graduate student Caren Chang first cloned and sequenced an *Arabidopsis* gene, the gene for alcohol dehydrogenase, providing proof that molecular genetic tools could be readily applied in plants. "I think because these community resources we developed began along with the community of researchers, it all really took off," says Meyerowitz.

The ABC model. Graduate student John Bowman and a visiting professor from Monash University in Australia, David Smyth, collected *Arabidopsis* flower mutants and observed homeotic phenotypes in which one flower organ is transformed into another, such as petals into stamens. "We recognized that the mutants could tell us something fundamental about organ specification in plants," says Meyerowitz. The group found that their mutants fell into one of three classes they called A, B, and C, which affected the identity of petals, sepals, and stamens, and, in turn, the whorls of the flower—the concentric rings into which the flower organs organize.

"This led us to propose the ABC model in 1991, still the basic model for floral organ specification," says Meyerowitz. The model predicted the phenotypes of the single, double, and triple mutant flowers the researchers generated. "I don't think it is technologies that are the primary roadblocks in science," says Meyerowitz. "I think it's our own ability to think about how organisms work. An example is the ABC model. There was not a single method or even type of mutation that hadn't existed 50 years earlier."

Research offshoots. The Meyerowitz lab continued to work on flower development, focusing on the shoot apical meristem (SAM), which houses plant stem cells capable of regeneration. In 1993, they identified the role of the *CLAVATA1* gene, which regulates meristem and flower development. In 1999, the lab found that CLAVATA3, a secreted peptide, and CLAVATA1, a transmembrane receptor kinase, control the balance in the SAM between stem cell renewal and differentiation.

As the list of genes involved in flower development grew, Meyerowitz thought he needed a new approach to developmental genetics, not just for the *Arabidopsis* model system but for all organisms. Rather than gene diagrams showing the direction of informational flow, Meyerowitz wanted to use computer analyses to create quantitative hypotheses that captured the strength and feedback of gene interactions in order to generate predictive models of how cells interact with each other. His lab collaborated with computer scientists, physicists, and mathematicians. The modeling took Meyerowitz in many different directions, including analyzing the molecular basis for plant phyllotaxis, or leaf and flower patterns. In 2006, the lab showed that, starting in the SAM, polarized transport of the plant hormone auxin results in *Arabidopsis* flower patterning.

Meyerowitz also works on the role of mechanical signaling between cells and tissues. Work from his lab suggests that mechanical signaling is as important for plant patterning and growth as peptide and hormone signaling. "The work on peptide signaling, hormone signaling, phyllotactic patterns, and mechanical signaling is all converging on a computational model that might explain everything about how the SAM works," says Meyerowitz. His lab also developed live-imaging methods using confocal microscopy that are now widely used to observe plant development. The impetus, says Meyerowitz, was to test some of the lab's computational models of gene function and cell-cell interactions. In 2005, the lab observed, in real time, cycles of auxin buildup and depletion in the SAM that facilitate the spiral pattern of leaves around the stem of *Arabidopsis*.

Looking to the future. "The *Arabidopsis* revolution has pretty much answered the fundamental plant biology questions that existed 30 years ago. We know how most of the plant hormones work; we know a lot of the modalities by which plants interact with the environment; we know ever so much more about plant differentiation, stem cells, and development," says Meyerowitz. "Today, we have new ways of thinking about these topics. It's now a question of going from the gene to the whole-organism level and integrating information at every level. We can start to put the whole picture together, using the sorts of computational methods those in the field have worked so hard to develop. Maybe before the end of the century, we'll have computational models that represent everything that's happening so as to understand the full complexity of plants."

MEYEROWITZ'S MEASURE

Plant promotion. "About 15 years ago, I gave a talk on plant development in a session focused on animal development at the American Society for Cell Biology meeting. Before I even got up on stage, about half of the 10,000 people in the audience started to walk out. That's an example of animal scientists not having any interest in plants. And I don't care that they learn from me necessarily, but I think if they were more open-minded about plant research, biology overall would benefit. I think a lot of people don't realize that many things found in animals were first discovered in plants—viruses, the cytoskeleton, transposable elements, microRNAs. Of the three major theories in biology—on cells, genes, and evolution—the cell and gene theories originated from the study of plants."

Seeking balance. "Funding for plant research is another issue. Only about 2 percent of funding in the U.S. goes to fund plant research, which is not well balanced if you consider the importance of these organisms for our lives. The way our funding situation is set up, most of the money goes to health issues. But being able to eat is also an important health issue, as is living in air without too much carbon dioxide in it."

Scientific pursuit. "I try not to compete with my former postdocs. They take what they want with them to their new academic positions, and it's my job to find new ideas for my lab. The goal of having a postdoc is to train that person to go out and be an independent researcher or industry researcher or whatever they wish to be. You have to do whatever you can to help that. If you don't, you are not contributing as much to the academic system as you ought to be. It's the same when you review a paper. The goal is to help the authors, to tell them what they could do to make the paper better, not give the editor an excuse to turn down the paper. If you think about what goals are necessary to advance science and the scientific community, it becomes clear how we need to deal with these things." ■

Andrea Eveland: Passion for Plants

Assistant Member and Principal Investigator
Donald Danforth Plant Science Center. Age: 39

BY KAREN ZUSI

After years of working in plant nurseries and flower shops, newly graduated biologist Andrea Eveland went looking for lab experience. She found it as a research assistant at Torrey Mesa Research Institute, an agricultural research branch of the biotech company Syngenta.

Mentored by the postdocs and other scientists in her lab, Eveland began learning about molecular biology and crop improvement. "I started applying to graduate programs in plant biology, and I came back to corn and agriculture immediately because I saw the future in that," Eveland says.

In 2002, she entered a PhD program at the University of Florida under maize physiologist Karen Koch. Eveland's research focused on the genes and enzymes that control sugars moving into developing maize kernels and how stress affects those pathways. As part of her work, she developed a strategy to analyze the expression of closely related genes, using high-throughput sequencing to profile the 3′ untranslated region of messenger RNAs in maize.[1]

But to continue working with gene networks, Eveland knew she needed a stronger foundation in bioinformatics. "She realized very early on that the ability to analyze very large sets of data, particularly genome data, was going to be tremendously important," says Elizabeth Kellogg, a plant geneticist currently working with Eveland at the Donald Danforth Plant Science Center in Missouri.

After earning her PhD in 2008, Eveland became a postdoc at Cold Spring Harbor Laboratory, co-mentored by maize developmental geneticist David Jackson and computational biologist Doreen Ware. There, she studied the development of maize inflorescences: the plant's male flower (tassel), the source of pollen, and female flower (ear), where kernels form.

Eveland and her colleagues matched physical features of developing inflorescences with high-throughput gene expression data to identify gene networks that control inflorescence growth. "I was helping generate data in the developmental biology lab and then analyzing those data sets in the bioinformatics lab, so it became a really integrated experience," Eveland says. Over the course of the research, she discovered that one of the primary genes controlling leaf architecture also plays a key role in inflorescence development.[2] The team also identified the role of transcription factor FEA4 in regulating the size of the meristem, the site of pluripotent stem cells that give rise to a plant's form and structures.[3]

In 2014, Eveland joined the Donald Danforth Plant Science Center, where she continues to concentrate on the developmental genetics and genomics of plant architecture in cereal crops. "She's very passionate, very focused and excited," says Sarah Hake, a plant developmental geneticist at the University of California, Berkeley, and one of Eveland's collaborators.

One of Eveland's current interests, a carryover from her postdoctoral research, is integrating molecular and phenotypic data to define gene networks involved in multiple aspects of plant development. She hopes that understanding these networks will help breed crops that are more productive under stress.

With Kellogg, Eveland is studying inflorescence development in green millet (*Setaria viridis*), an emerging model system for corn and other food crops. Her team is using CRISPR-Cas9–based genome editing to selectively disable *Setaria* and maize genes to dissect their function. ■

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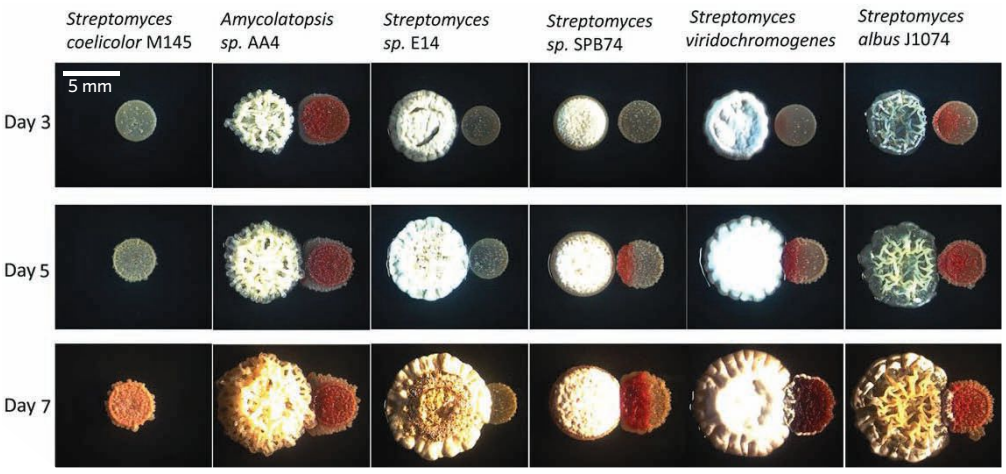
Novel Antibiotics Speak Up

Methods for activating silent gene clusters to discover new drugs

BY SANDEEP RAVINDRAN

Antibiotic resistance is a major threat to global health, and researchers have struggled to identify new antimicrobial compounds. By the late 1990s, the bacterial reservoirs from which almost all clinically useful antibiotics had sprung appeared to run dry. As bacterial genome sequencing became more widespread in the last decade, however, researchers discovered many potential sources of new drugs hidden in these genomes. Now they just need to learn how to mine them.



MIXING IT UP: The bacterium *Streptomyces coelicolor* produces a variety of novel compounds when grown in combination with other bacterial species, and these interspecies interactions result in differences in colony development and pigment production when viewed under a microscope.

Most antibiotics are derived from small molecules produced by bacteria, and the genes that synthesize, regulate, and export these molecules typically occur together in groups called biosynthetic gene clusters, which range in size from just a handful to several dozen genes. Sequencing efforts have revealed that the genomes of antibiotic-producing organisms, such as *Streptomyces coelicolor*, contained a large number of gene clusters that were "silent" or "cryptic" and were not expressed under laboratory conditions. "We were culturing these bacteria for decades, and we had only found a handful of compounds in each of these key, industrially relevant strains," says Mohammad Seyedsayamdost, an assistant professor of chemistry at Princeton University. "It was just remarkable that they had so much more potential that we hadn't tapped into."

Bioinformatic tools can now identify many silent biosynthetic gene clusters in bacterial genomes. The next step is figuring out how to activate them. "That would profoundly change natural product discovery, and also drug discovery," says Seyedsayamdost. Researchers have tried many approaches, such as expressing these gene clusters in other bacterial species, modifying their promoters or regulators, and changing how the bacteria are cultured. "Many of these strategies are not scalable or generally applicable," says Huimin Zhao, a professor of chemical and biomolecular engineering at the University of Illinois at Urbana-Champaign. "There's a lot of luck involved."

But that may be changing, as more reliable and high-throughput methods to activate these clusters come online. Advances in synthetic biology, small-molecule screening, and mass spectrometry have led to promising new ways to induce gene clusters and identify their products. Some hurdles remain—scientists are still trying to develop better ways to pinpoint the clusters that will yield new and useful compounds, as well as methods to more quickly and accurately identify the molecules produced by individual clusters. In addition, the new approaches for turning on gene clusters may help elucidate how this activation is regulated.

The Scientist talked with researchers about some of these new approaches. Here's what we learned.

HARNESSING MICROBIAL INTERACTIONS

INVESTIGATORS: Matt Traxler, Assistant Professor, Department of Plant & Microbial Biology, University of California, Berkeley, and Roberto Kolter, Professor of Microbiology and Immunobiology, Harvard Medical School

PROJECT: Take advantage of the natural interactions between bacteria to activate silent gene clusters.

APPROACH: Past work has shown that bacteria undergo very different physiological changes when grown in the presence of other microbes than when they're grown by themselves. Kolter and Traxler wondered if they could harness the crosstalk between different bacterial species to activate silent gene clusters. They systematically tested this hypothesis by growing different combinations of bacteria together and using mass spectrometry to identify and compare the compounds produced. "It became very

mBIO, DOI:10.1128/MBIO.00459-13, 2013

clear that many very different metabolites get made when things are grown in co-culture," says Kolter. When they paired one species of actinobacterium (*Streptomyces coelicolor*) with five others, the researchers further found that the set of compounds that were produced were different for each of the five co-cultures (*mBio*, doi:10.1128/mBio.00459-13, 2013). "We can see a lot of interactions that lead to antibiotic biosynthesis," says Traxler. "That suggested to us that there is still some deep potential here for drug discovery."

ADVANTAGES: This approach is relatively simple to implement and doesn't require any prior knowledge of the bacterial genomes. In addition, "it's linked directly to the ecology of these organisms, and I see that as a major strength," says Traxler. He suggests that co-cultures replicate some of the complex ecological interactions that bacteria face in their natural habitats, which might force them to activate more novel gene clusters than methods that rely only on single-species bacterial cultures. "There may be a limit to what we're able to find in single culture, and we may only be able to cross that by going to co-culture or even in situ, to find antibiotics we may never find otherwise," he says.

LIMITATIONS: The main drawback of the method is that it can be slow and labor-intensive, especially when trying out many different combinations of bacteria. "High-throughput for us means a hundred combinations, not hundreds of thousands or millions of combinations," says Kolter. In addition, once the researchers identify interesting compounds being produced by bacteria in co-culture, they still need to comb through the bacterial genomes to try to figure out which gene clusters they came from. "We have to work backwards to identify which processes we have woken up," says Kolter.

LOOKING AHEAD: Traxler is trying to use microfluidics to make this approach higher-throughput, and hopes to eventually test tens of thousands of combinations using this setup. He is also planning to go from looking at simple binary interactions to looking at more-complex interactions in natural settings.

HOW TO USE: "It's pretty cheap and easy to do this," says Traxler. Just set up your co-cultures and watch them grow.

LOOKING FOR ACTIVATING SIGNALS

INVESTIGATOR: Mohammad Seyedsayamdost, Assistant Professor, Department of Chemistry, Princeton University

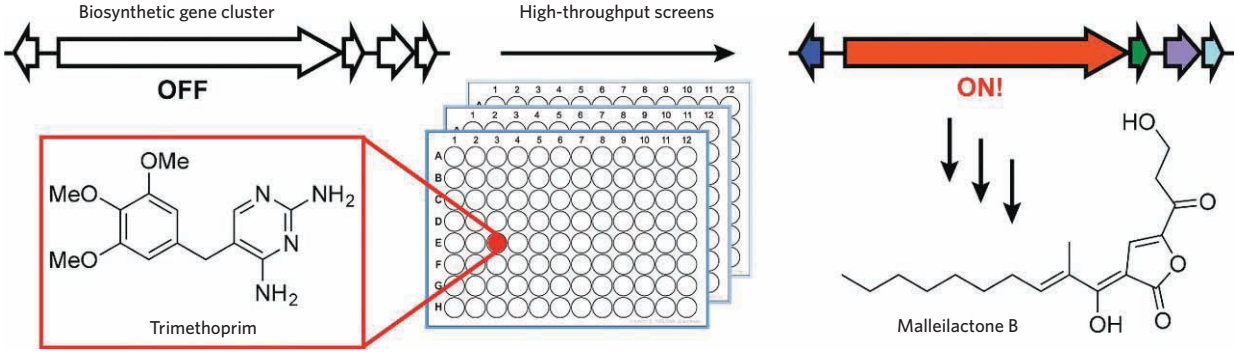
PROJECT: Screen a small-molecule library for compounds that activate silent gene clusters.

APPROACH: Seyedsayamdost and his team postulated that certain natural compounds turn on biosynthetic gene clusters, so they developed a high-throughput screen to search out these elicitor molecules. Seyedsayamdost's group first inserts a genetic reporter, such as lacZ, into a gene cluster of interest. They then expose the bacteria to a large library of natural products and bioactive small molecules, looking for those that induce the expression of the gene cluster. The approach can pinpoint molecules that activate a specific silent gene cluster as well as global elicitors that activate many different clusters (*PNAS*, 111:7266-71, 2014). "Interestingly, it turned out that antibiotics in these compound libraries in many cases turn out to be the elicitors," Seyedsayamdost says. This suggests that old antibiotics could be used to find new antibiotics.

ADVANTAGES: "One of the advantages of our method is that we can find naturally occurring inducers," says Seyedsayamdost. Identifying global elicitors that activate many gene clusters could also help researchers identify common regulatory mechanisms that could be deployed to activate silent clusters. In addition, the approach can be used to screen tens of thousands of small-molecule elicitors at the same time.

LIMITATIONS: The main challenge of the system lies in genetically modifying the bacteria with a suitable reporter, which can be difficult and time-consuming depending on the species.

ELICITING A SIGNAL: Researchers insert a reporter gene inside a silent gene cluster of interest (white arrows), then screen a small-molecule library for signals that can turn on the cluster (colored arrows). When this approach was used in *Burkholderia thailandensis*, the antibiotic trimethoprim was found to activate the silent gene cluster that produces the cytotoxin malleilactone B.



FEMS MICROBIOLOGY REVIEWS, DOI:10.1093/FEMSRE/FUW035, 2016

Seyedsayamdost has successfully applied the technique to the gram-negative soil bacterium *Burkholderia thailandensis*, but getting it to work in less genetically tractable species will require more time and effort, he says.

LOOKING AHEAD: Seyedsayamdost is expanding the technique to different bacterial species. "We're now getting it to work in *Actinomycetes*, which are responsible for many antibiotics," says Seyedsayamdost. In organisms where it's difficult to insert a genetic reporter, he envisions using mass spectrometry as a readout for bacterially produced small molecules. He is also trying to use more than one reporter to simultaneously assay multiple gene clusters, and eventually hopes to use RNA-seq to monitor an elicitor's effects on an organism's entire transcriptome.

HOW TO USE: The approach involves standard microbiology and genetics techniques. The main expense comes from the small-molecule screening. "Each screen may cost something like $500 to $5000 with a 5,000- to 10,000-compound library," says Seyedsayamdost. "For the amount of data you get, it's actually fairly affordable. Once the screen is done, you're off to the races."

PLUG-AND-PLAY GENE EXPRESSION

INVESTIGATOR: Huimin Zhao, Professor, Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign

PROJECT: Use synthetic biology to re-create and express silent gene clusters.

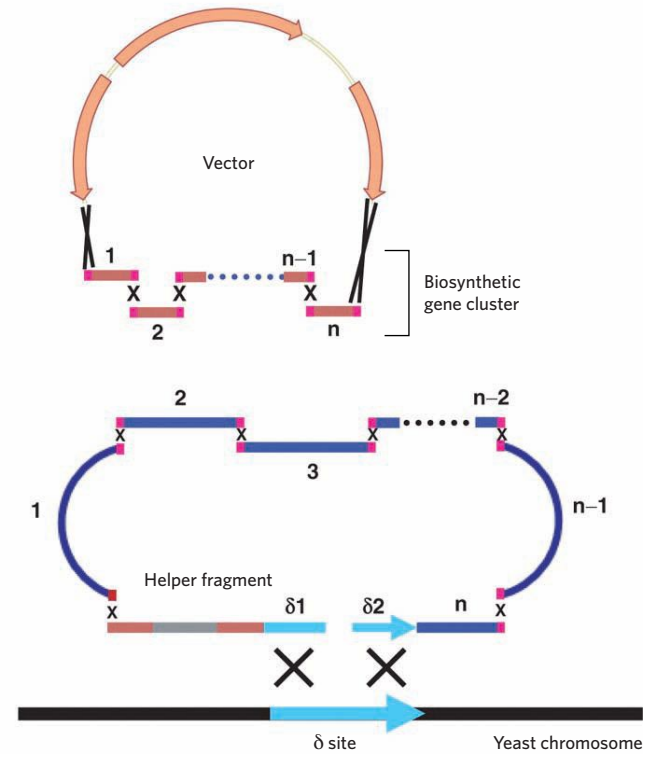
APPROACH: Zhao's team uses bioinformatics to home in on a gene cluster's structural genes—those that don't encode regulatory proteins but are instead directly involved in the synthesis of the compound. The researchers then either amplify these genes using PCR or synthesize them anew. They clone these genes into a previously developed plug-and-play scaffold with standardized promoters for expressing biosynthetic pathway genes (*Nucleic Acids Res*, doi:10.1093/nar/gkn991, 2009; *Mol Biosyst*, 7:1056-59, 2011). Finally, they express the genes in a nonnative, or heterologous, host. "The whole idea is to basically rebuild the pathway from scratch," Zhao says. The researchers used this method to activate a silent biosynthetic gene cluster from *Streptomyces griseus* and identify potential antibiotic compounds (*Nat Commun*, 4:2894, 2013).

ADVANTAGES: By focusing on the structural genes from the biosynthetic cluster, Zhao's approach aims to circumvent the expression of any regulatory genes that might repress the gene cluster. Using well-characterized and standardized promoters and heterologous hosts, the technique also reduces the number of variables that researchers need to troubleshoot in order to activate a particular cluster. The method also makes it easy to delete or alter specific genes in a cluster; by deleting different genes, Zhao was able to dissect the individual steps of a *Streptomyces* biosynthetic pathway. By tweaking the scaffold and changing the heterologous host used, the technique could be extended to other species of bacteria and eukaryotes. "You cannot just work on one organism; you have to work on many different ones," Zhao says.

LIMITATIONS: Gene clusters from some bacteria, such as *Streptomyces*, can be hard to amplify using PCR. Synthesizing the gene cluster is an alternative, but could get expensive if the cluster has a lot of genes. In addition, the host used may lack some of the precursors necessary to create the final product of the cluster.

LOOKING AHEAD: Zhao's eventual goal is to automate the synthetic expression of silent gene clusters. "In order to make an impact, we have to scale up the process," he says. "Given the recent progress, I'm very optimistic."

HOW TO USE: The protocols used in Zhao's technique have been published and all the necessary tools are commercially available. "[Researchers] just need to follow the protocols, they're all very straightforward," he says. He estimates that it should cost a few hundred dollars to synthetically construct and express a particular gene cluster. ■



EASY ASSEMBLY: A silent biosynthetic gene cluster of interest (genes 1 to n) is reconstructed in a vector (orange, top) and integrated into a yeast chromosome through recombination at the δ site (bottom). The assembled biosynthetic cluster can then be isolated from yeast and transferred into a heterologous host, where it is expressed and its products analyzed by mass spectrometry and nuclear magnetic resonance spectroscopy.

ADAPTED FROM *NUCLEIC ACIDS RES*, DOI:10.1093/NAR/GKN991, 2009

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Getting Personal

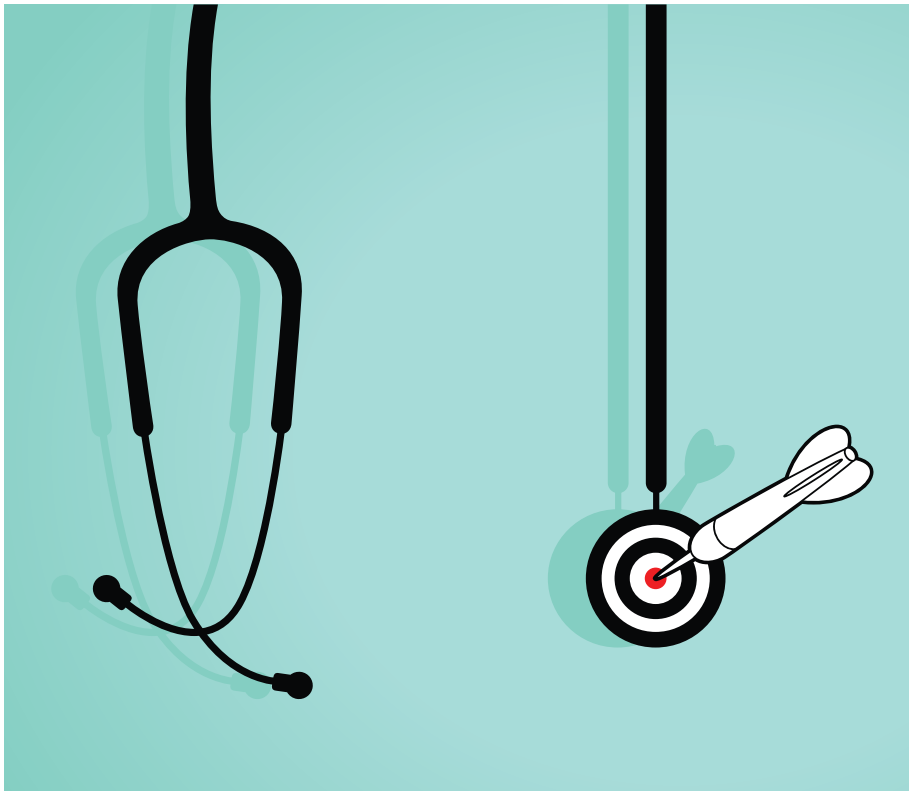
Drug developers and diagnostic firms collaborate to advance precision therapies to market.

BY CATHERINE OFFORD

In 2003, the US Food and Drug Administration (FDA) approved the first targeted therapy for non-small cell lung cancer (NSCLC). AstraZeneca's Iressa (gefitinib) had shrunk some patients' tumors in small clinical trials, and despite some concern about the robustness of the results, regulators OK'd the therapy as an option for patients when chemotherapy and other generalized cancer-attacking treatments had failed. But after only a few months on the market, it was clear that something was amiss: for up to 90 percent of patients, the drug simply didn't work.

While AstraZeneca struggled to explain this disappointing outcome, within a year three academic groups independently came to the same conclusion: gefitinib, it appeared, was only effective in patients harboring certain mutations in *EGFR*, a gene coding for a cell-surface protein receptor involved in cell signaling; patients without these mutations had no noticeable response to the drug. Based on gefitinib's inefficacy in most patients, the FDA withdrew approval in 2005. Ten years later, however, AstraZeneca was able to get gefitinib reapproved for NSCLC—this time alongside a diagnostic test to identify patients with the relevant *EGFR* mutations.

In retrospect, gefitinib's initial failure hardly seems surprising: patients are genetically diverse, and such variation can affect how people respond to particular therapies. But until recently, doctors didn't have much of a choice in treatments, says Ronenn Roubenoff, head of global translational medicine in musculoskeletal diseases at Novartis Institutes for Biomedical Research. "In the old days, we used to just have a few drugs and so we'd give them to everybody," he explains. As a result, outcomes were often poor—prompting some patients to stop taking (and purchasing) their meds altogether—and neither physicians nor drug companies could explain why, he adds. "Everybody's kind of bumbling around in the dark."



Today's emerging field of precision medicine—previously referred to as personalized medicine—takes advantage of this genetic diversity to tailor treatments to those patients most likely to respond. Rather than administering therapies based solely on the disease, new strategies to identify biomarkers, such as *EGFR* mutations in NSCLC patients, help doctors select the best therapies for individual patients.

This evidence-based approach could reduce trial-and-error prescribing and help avoid adverse reactions to drugs—and might even lower the ultimate cost of health care. In 2015, in recognition of this potential, President Obama announced a nationwide research effort, the Precision Medicine Initiative, to support the approach's use in oncology (where precision medicine has had the most success so far) and for diverse other conditions, including neurodegenerative, autoimmune, and cardiovascular diseases.

This enthusiasm is mirrored by the drug development industry. That same year, 28 percent of drugs approved by the FDA were considered precision therapies, and an October 2016 report predicted the global precision medicine market will reach nearly $113 billion by 2025. But

such a transition inevitably brings challenges. For companies built on the traditional, so-called blockbuster model of drug development—which aims to produce drugs that are applicable to many patients, thus generating high returns—precision medicine presents a very different commercial landscape.

By definition, precision drugs target only a subset of a particular patient population, reducing pharma's ability to recoup development costs through sales. Moreover, as gefitinib's setbacks illustrate, drug design is now just part of a therapy's development. Diagnostic tests to identify responsive patients must be created alongside a new targeted therapy, and determining which biomarkers are reliable indicators of a therapeutic response can often involve sifting through unprecedentedly large volumes of data early in preclinical and clinical testing.

Faced with these challenges, leaders in pharma are looking for opportunities to partner with other drug companies, tech firms, regulators, and even patients themselves. For precision medicine to work, "as an industry, we need to collaborate more," says Sy Pretorius, chief scientific officer of life sciences consultancy firm Parexel. "It's rare that one team can do it all by itself."

"There's a recognition that the problem requires a broad swath," agrees Peter Bergethon, vice president for quantitative medicine at Pfizer's neuroscience and pain research unit. "The idea that a single person, single group, or single discipline has the answers to solve a problem like personalized medicine is now appreciated as not being true. It's in the nature of the problem."

Timing it right

To realize the potential of precision medicine, health-care providers need biomarkers predictive of a therapy's impact, as well as companion diagnostics to detect them. Ideally, the availability of those diagnostics matches up with the availability of the therapies they complement. "Biomarkers are like pizza delivery," Roubenoff notes. "You need it fresh, hot, and on time. It doesn't do anybody any good to have the biomarker developed five years after the drug."

But synchronizing drug and diagnostic development is easier said than done. To date, only a handful of targeted therapies have been approved simultaneously with a companion diagnostic. "What most people don't appreciate is that companion diagnostic development takes several years and adds an additional layer of complexity to what is already a pretty complex development process," says Pretorius. The resulting dilemma is known as precision medicine's chicken-and-egg problem. On the one hand, waiting to explore companion diagnostics risks holding up effective therapies. On the other, investing heavily in diagnostics early on is risky, as many drug candidates never make it to market. (While a diagnostic test may be useful on its own, it offers lower return on investment without the accompanying drug.)

To tackle this problem head-on, many companies are making diagnostics expertise a more integral part of drug discovery, whether through in-house development, acquisitions, or partnerships. In 2013, for example, pharmaceutical giant Eli Lilly signed a collaborative agreement with Qiagen, maker of the gefitinib diagnostic test and of a diagnostic for the cancer drug Erbitux (cetuximab), which Lilly developed in partnership with Bristol-Myers Squibb. And last year, AstraZeneca cut a deal with diagnostics company Abbott to develop a test to identify patients who might benefit from an asthma drug; AstraZeneca noted that it expects half of its drug launches to include companion diagnostics by 2020.

For companies built on the traditional blockbuster model of drug development, precision medicine presents a very different commercial landscape.

The result of these partnerships is that pharma can adopt a more integrated, evi-

dence-based approach to drug development. "We have been able to drive the biomarker process earlier into the drug discovery paradigm," says Roubenoff. At Novartis, whose collaborators include biomarker discovery company SomaLogic, "we start looking for the biomarkers basically at the time that we start finalizing the drug candidate," he adds. "There's a lot of pre-work that wasn't really on the agenda 10 or 15 years ago that's now become pretty routine."

The FDA is also now working to help smooth the transition to synchronous development, in part by clarifying its expectations. In 2014, the agency issued guidance aimed at "stimulating collaboration" between diagnostics and drug companies, and last year, the agency published updated guidelines on how companies can merge drug and diagnostic pipelines to increase the likelihood of simultaneous approval. But there's still a way to go, Pretorius says. "I firmly believe regulators in general are trying to come to terms as quickly as possible with how to deal with it," he says. "It's just so new, I don't think as a society we've got our arms completely around it yet."

Dealing with data

Before a drug and its companion diagnostic can be approved, both must pass muster for efficacy and safety. But testing a precision therapy differs markedly from testing blockbuster drugs. Patients can no longer be drawn from the population at large, but must be carefully selected—perhaps based on their DNA—to match the aims of the trial. "There's a school of thought in the industry that randomized clinical trials have served their purpose," says Pretorius. Newer, stratified approaches to clinical testing are becoming increasingly common, with the promise of reducing costs by increasing the probability of success. (See "Clinical Matchmaker," *The Scientist*, June 2015.)

This approach presents its own set of hurdles in terms of both trial design and statistical analysis. To draw meaningful conclusions, researchers need more clinical data, including genomic and potentially other omics information from every patient involved. "The sheer volume of data associated with precision medicine is itself a significant challenge," says Pretorius. "Collecting, managing, interpreting these mega–data sets is more difficult than most people appreciate."

The idea that a single person, single group, or single discipline has the answers to solve a problem like personalized medicine is now appreciated as not being true. It's in the nature of the problem.

—Peter Bergethon, Pfizer

One way that pharma is addressing this issue is through sharing data and analyses. "For genomics, virtually all companies are now taking an open approach," says David Goldstein, director of Columbia University's Institute for Genomic Medicine, which recently entered a $30 million alliance with drug developer Biogen Idec. This openness perhaps "reflects an awareness in companies that they don't have the ability to do it all," he adds. The government is getting involved, too. In 2015, the FDA launched a cloud-based portal called precisionFDA to foster a collaborative approach to developing the tools needed to evaluate DNA sequence data. Participants already include healthcare providers, academic medical centers, pharmaceutical companies, and patients.

Right now, the main obstacles to data sharing are technological, says Michael Blum, director of the Center for Digital Health Innovation (CDHI) at the University of California, San Francisco. He and his team are working towards "an information commons [to] describe not only millions of individuals, but life science, molecular, and omics data," he explains. In addition to being able to host massive data sets, such platforms must be designed to ensure that personal medical data are secure. "The model for sharing the data with industry is still being developed," he says.

But even as the tools for sharing and processing such large volumes of data evolve, researchers continue to up the ante, developing new medical devices that can record patient data regularly, even outside the clinic. With wearable technology, "you can now collect heart rate and blood pressure data on an ongoing basis, instead of when you happen to be in the office and checking it," says Blum. "That really changes the paradigm of what we learn."

Looking further ahead, Pfizer has teamed up with IBM to launch the Blue Sky project, which aims to develop technologies such as Internet-connected motion sensors and other devices to monitor patients in their homes and upload the data in real time. The technologies are being evaluated in trials with Parkinson's patients, with the view that the almost continuous data series could allow researchers to track the effect of a treatment and pick out relevant variation in patient responses. "You'd be identifying the noise, rather than hoping it gets washed out in a population study," explains Pfizer's Bergethon. "That's the precision medicine approach." Such technologies could also "allow you to do faster, less expensive trials," he adds.

Like companion diagnostics, these new digital solutions to data handling are likely to involve collaboration among diverse companies if they are to become a regular part of precision medicine in the future. "I think that we will wind up with companies partnering with the guys who are good at doing the different pieces," says Roubenoff. "Everything is now moving, or has moved, toward an integrated approach that requires biomarkers, requires consideration of digital endpoints, and requires that the core aspect of the chemical or biological medicine has a lot more around it than was the case even a few years ago."

If successful, it's an approach that ultimately promises to benefit patients—the motivation for advancing precision medicine in the first place, Roubenoff adds. After all, he says, "we're not really interested in giving people drugs that don't work." ■

Fraught Cuisine

Most humans may be revolted by cannibalism, but it turns out that the practice is not all that uncommon among animals.

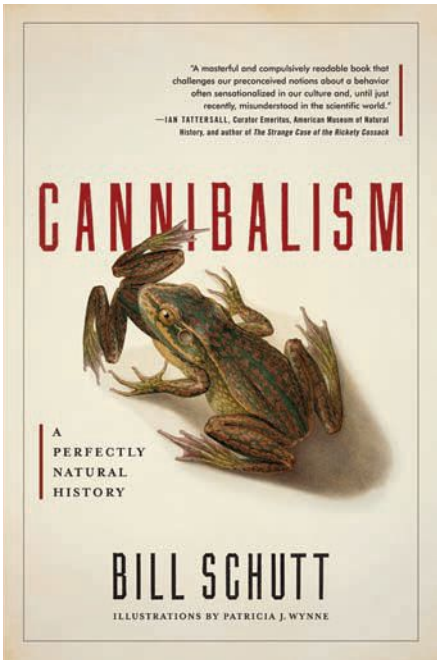
BY BILL SCHUTT

The golden hamster (*Mesocricetus auratus*) is a popular children's pet, but these cuddly fuzz balls are also known to display some nightmarish behavior in captivity—namely, cannibalism. Although it might be difficult for a parent to explain this to a child traumatized by the sight of a brood of "pinkies" being eaten by their mom, or the discovery of a half-consumed corpse of an adult hamster, the behavior is a typical response to a myriad of stress-related conditions. These stem from major differences between the animal's natural habitat (subterranean burrows in a dry desert environment) and captive conditions, where pets are typically housed in fish tanks carpeted in damp wood shavings, or in trendy modular contraptions in which see-through plastic tubes link "rooms" to each other. Add this to the fact that adult hamsters in the wild are solitary, highly territorial, and only emerge from their burrows for short periods at dawn and dusk (to avoid their natural enemies—dogs and cats), and you have the recipe for stressed-out pets that will cannibalize their siblings and even their own pups.

Until relatively recently, scientists believed that the cannibalism seen in species such as hamsters, black widow spiders, and praying mantises was a product of either the stresses associated with captivity or a lack of alternative forms of nutrition (think the Donner Party). In the 1970s and 80s, however, researchers such as Laurel Fox and Gary Polis learned that not only was cannibalism widespread in nature, it was related to completely natural phenomena, including sexual selection and variable environmental conditions. In my new book *Cannibalism: A Perfectly Natural History*, I explored the practice across the animal kingdom with an eye toward why it occurs. Viewing the phenomenon through the lens of modern biology, it became possible to examine some of the most famous examples of human cannibalism in a new light—as perfectly natural behavior. One such form of cannibalism-related behavior can be considered the ultimate parental care. A day after her spiderlings hatch, the mother black lace-weaver spider (*Amaurobius ferox*) lays a clutch of trophic eggs, whose only role is to serve as food for her hungry babies. Three days later, after the trophic eggs have been consumed, the spiderlings are too large for their mother to care for, but they are still in dire need of nutrition. In response, the mother spider calls the babies to her by drumming on their web and presses her body down into the gathering crowd. The ravenous spiderlings swarm over their mother's body. Then they eat her alive, draining her bodily fluids and leaving behind a husk-like corpse.

Cannibalism as parental care also extends into the vertebrates, and a fascinating example occurs in the caecilians—a small order of legless amphibians. In 2006, caecilian experts were studying an African species that researchers had previously observed guarding its hatchlings. On numerous occasions, scientists had observed a female sitting motionless while her newly hatched brood slithered energetically over her body. On closer inspection, they saw that the babies were pressing their heads against the female's body, then pulling away with her skin clamped tightly between their jaws—peeling off the outer layer of their mother's skin like a grape's, then eating it. Scientists now know that these bouts of "dermatophagy" reoccur regularly and that the mother's uniquely fat-laden epidermis (which endures multiple peelings) serves as the young caecilians' sole source of nutrition for up to several weeks. It also explained why the babies increase significantly in size while mothers suffer a concurrent decrease in body mass. In short, dermatophagy is a great way to fatten up the kids, but for moms on the receiving end of their gruesome attentions, the price is steep.

As I explored the phenomenon of cannibalism from a biological and zoological perspective, both in my own research and as a book author, I encountered dozens of similarly interesting stories—each leading me to the same conclusion: eating one's own kind is completely natural behavior for thousands of species, perhaps even for humans. ■



Algonquin Books, February 2017

Bill Schutt is a professor of biology at Long Island University-Post and serves as research associate in residence at the American Museum of Natural History. Read an excerpt of Cannibalism: A Perfectly Natural History *at the-scientist.com.*

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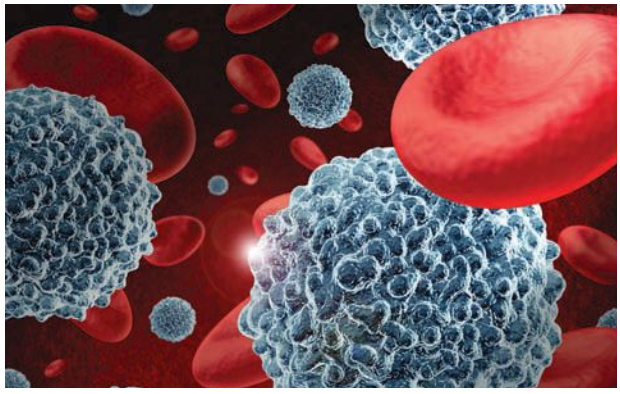

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Aging and Mechanisms of Aging-Related Disease

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Populations are aging rapidly worldwide, particularly in Asia, driving a strong interest in aging/longevity research. This Keystone Symposia meeting will capture the cutting-edge front of this exciting field of science, covering essential aspects of aging/longevity research, including critical signaling pathways and regulators, inter-tissue communication, stem cells, stress and damage responses, cellular senescence, physiological rhythms, human genetics and mental well-being (happiness). Because aging is a systemic phenomenon, it is important to address various layers of the aging/longevity-controlling hierarchy, particularly focusing on metabolic regulation, including mitochondria, NAD+, oxidative stress, inflammation, protein homeostasis, autophagy and many other age-associated pathophysiologies. The outcome of these studies needs to be translated to resolve social and economic issues caused by rapidly aging societies. Novel therapeutic and preventive interventions have been explored and developed as a growing attempt to meet the unmet needs of our aging societies, and these new aspects of aging/longevity research and the gaps in knowledge between the basic science and practical applications will also be covered in the meeting. There is a growing body of evidence that our modern lifestyle, such as the heavy use of blue light in smart phones and tablet computers, affects physiological rhythms and metabolism, promoting age-associated diseases such as obesity, diabetes, cancer and depression. Therefore, it is now time to think differently about what we can do to deal with all these problems in light of recent progress in this exciting field of science.

Session Topics:
- Signal Transduction I – Evolutionarily Conserved Players
- Workshop 1: Cutting-Edge Front of Aging/Longevity Science
- Signal Transduction II – Mitochondria
- Stem Cell Aging and Humoral Factors
- Intertissue Communication and Rhythm
- Cellular Senescence
- Stress, Damage and Epigenetic Changes
- Age-Associated Complications
- Interventions for Aging and Longevity

plus three workshops

KEYNOTE SPEAKERS
Johan Auwerx
Thomas A. Rando

CONFIRMED SPEAKERS
(as of January 4, 2017):
Rajendra S. Apte
Rochelle Buffenstein
Dongsheng Cai
Ana Maria Cuervo
Leonard P. Guarente
Marcia C. Haigis
Jing-Dong Jackie Han
Jan H. J. Hoeijmakers
Shin-ichiro Imai
Heinrich Jasper
Takashi Kadowaki
Matt Kaeberlein
Joan Mannick
Tohru Minamino
Noboru Mizushima
Eisuke Nishida
Emi Nishimura
Hideyuki Okano
Satchidananda Panda
Tomas Prolla
Michael Ristow
John Sedivy
Yousin Suh
Kazuo Tsubota
Jan M. van Deursen
Masashi Yanagisawa

Abstract Deadline: February 15, 2017
Discounted Registration Deadline: March 15, 2017

Note: Short talks on the program will be chosen from abstracts submitted by the abstract deadline.

Meeting Hashtag: #KSaging
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On the Ascent of Sap, 1895

BY BEN ANDREW HENRY

The uppermost branches of a tree might sway several hundred feet in the air, yet they will receive a constant supply of water sucked out of the soil below. In the late 19th century, the world's botanists were mired in fierce debate over this astonishing hydraulic feat, divided over whether and how trees expended energy to lift water against the force of gravity.

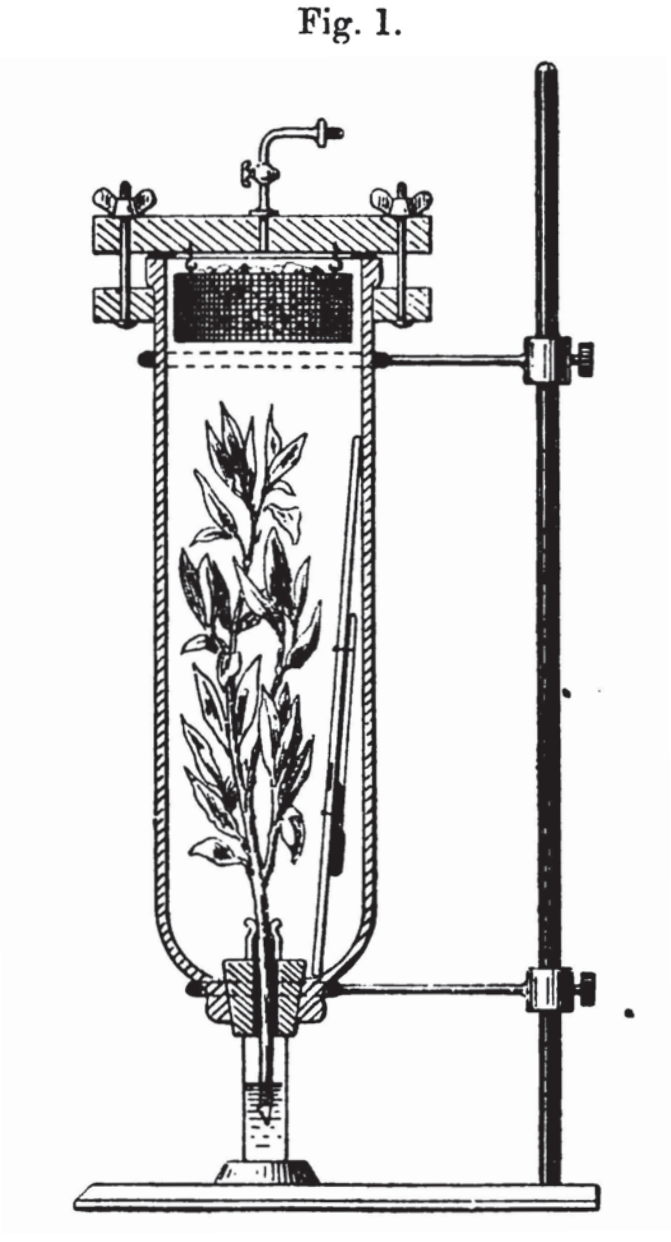
While theories and counter-theories flew, two Irish scientists, one of them a renowned physicist named John Joly and the other a young botanist named Henry Dixon, decided to test the strength of a tree's water-lifting capabilities.

Inside of a tree, water courses from root to leaf through tiny conduits collectively called xylem, and almost all water not consumed by cellular processes evaporates, or transpires, through pores on the undersides of leaves. In a paper published in 1895 (*Philos Trans R Soc B*, 186:563-76), Dixon and Joly reported the results of an experiment to push back against that upward flow.

The pair encased a maple branch in a thick glass tube, sealed except for the end of the branch protruding down into a vial of water. With a pump, they raised the air pressure inside the tube to twice that of the atmosphere in an attempt to stop the water's ascent. Yet, liquid in the vial steadily dropped over the course of an hour, evidence that the plant kept on transpiring. More air was pumped in, and at triple the normal atmospheric pressure, the branch still pulled up water. The same proved true with branches of sycamore and lime. Before Dixon and Joly could drive the pressure any higher, the glass tube shattered—"fortunately doing no harm," they wrote, "but putting a stop to further experiments."

A second, more technical experiment provided a crucial piece of explanatory evidence in the form of a new property of water. In a series of manipulations, Dixon and Joly showed that water in a tube could be placed under tension—the way pulling on a rope places it under tension. The two researchers, drawing together pieces of a number of earlier theories, proposed in full what would be known to posterity as the cohesion-tension theory. Inside the vasculature of a tree, they argued, water forms slender threads that stretch from roots to leaves. Transpiration—a cooling process enabled by the warmth of the sun—tugs at the end of the thread and pulls water upward.

Dixon and Joly did not get the last word on the matter. According to a history of cohesion-tension theory by Harvey Brown of the University of Oxford, Francis Darwin, son of Charles and an accomplished botanist, dismissively wrote that believing in the ability of water to bear tension "is to some of us equivalent to believing in ropes of sand" (*Phys Perspect*, 15:320-58, 2013). Even today, despite sophisticated experiments proving this peculiar property, and an understanding of the polar bonds between water molecules that account for it, xylem researcher Melvin Tyree says the idea sticks in the craw of engineers and even some dedicated plant biologists. "Fluids, by definition, are not supposed to have tensile properties." ■



HEAVY LIFTING: Among the theories put forth by 19th-century scientists to explain the movement of water up a tree was the idea that pressure gradients inside leaves sucked water upward. Others insisted cellular conduits must function like pumps. Dixon and Joly, in 1895, discredited both of those proposals. They watched a branch move water against triple the normal atmospheric pressure and posited that tensile properties of water enable trees to lift the fluid without exerting any effort whatsoever.

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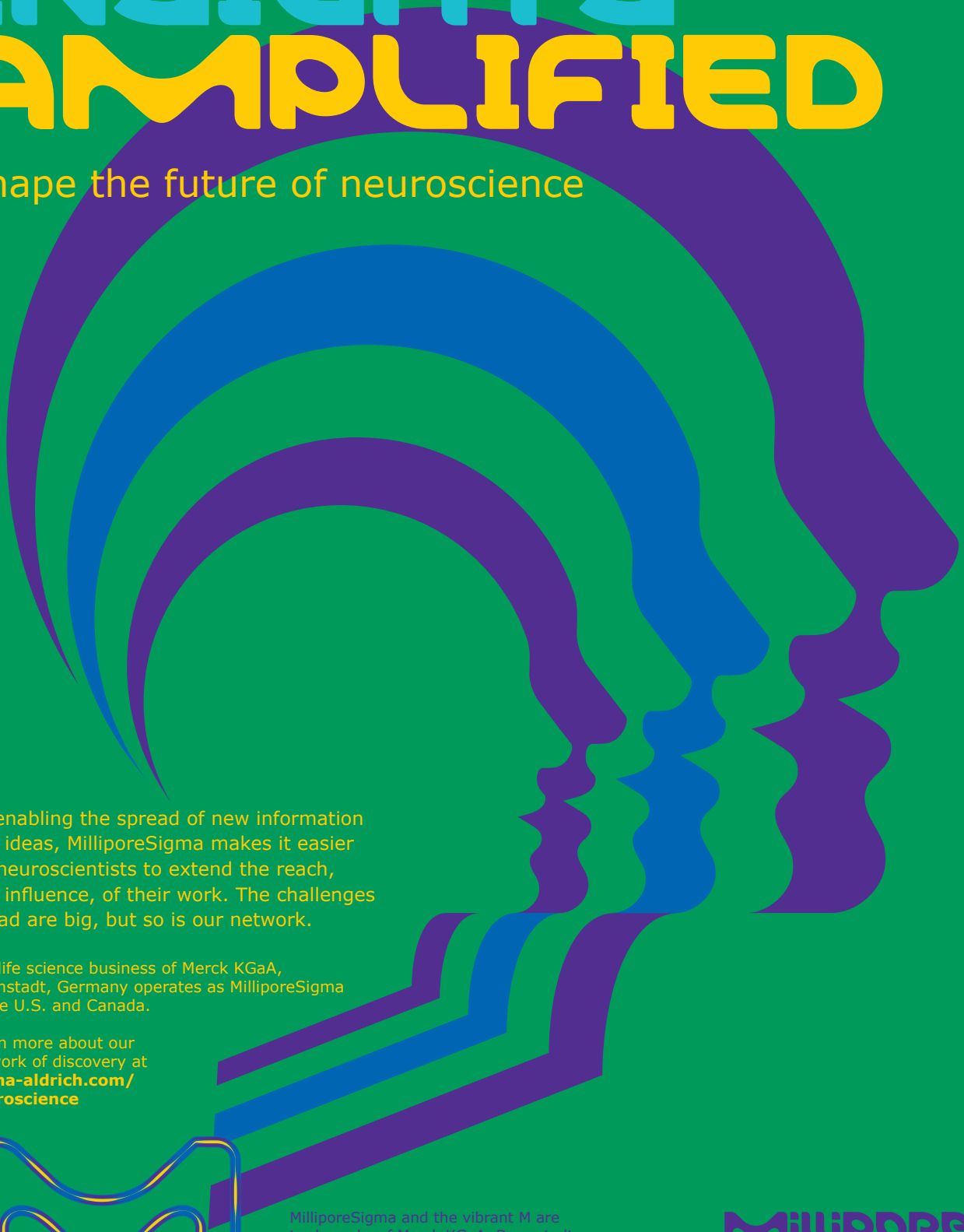

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